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Biogeography and Habitat-Use Behavior of Feral Horses in Western and Northern Canada

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Abstract

Current estimates of feral horse populations in western and northern sub-arctic Canada indicate that ~900 animals occur in Alberta, ~200 are present in British Columbia, and an additional 200-300 are found in Yukon Territory. These animals occupy areas characterized by coniferous forest cover and relatively cold climate. Studies in western Alberta showed that under favorable conditions the foal and yearling cohorts may together constitute 35% of the population, indicating a potential for rapid population growth; however, starvation and high levels of predation during severe winters are thought to be limiting factors. The Alberta studies are used to illustrate various aspects of habitat-use behavior (home range size, habitat selection, snow relationships, use of shelter, food habits, drinking, use of mineral licks); comparative aspects of habitat-use by feral horses in other areas of the northern coniferous forest in Canada are discussed. Reported land-use conflicts involving feral horses include competition for forage between horses and cattle, competition between horses and wild ungulates, and grazing and trampling damage on spring ranges.

Introduction

Although the present North American population of feral horses is concentrated in desert and semi-desert areas of the western United States, numbers also occur in western and sub-arctic northern Canada. These horses are the descendants of strays or escapees from resident Indian tribes (who had acquired horses by the mid-1700's), and from mining, ranching, and forestry activities that accompanied settlement during the last century. The most recent additions have been from unmanaged Indian herds, and from stock maintained by guides and outfitters in remote areas (Salter and Hudson, 1978a).

It can be inferred from the known distribution of these animals (McKnight, 1959, 1964) that they are found in areas characterized by predominantly coniferous forest cover and relatively cold climate, but detailed ecological studies have only recently been carried out (Storrar et al., 1977; Salter, 1978a). The purpose of this paper is to summarize available information on distribution, numbers, and habitat relationships (use of vegetation types, use of space, food habits, land-use conflicts) of feral horses in western and northern Canada.

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Distribution and Numbers

According to questionnaire surveys and interviews of Forest Service and wildlife management personnel conducted during 1975-79, there are now in the order of 1400 feral horses in British Columbia, Alberta, and the Yukon Territory. (This is a gross estimate only; it is based on the 'best guess' of field personnel, but most regional population estimates are not based on any standard survey methodology.) Fewer than 200 feral horses were reported in British Columbia, but the number there may be much higher as no information is available from a large area in the northwestern part of the province. Nine hundred feral horses were reported in Alberta, and an additional 200-300 in Yukon Territory. Substantial numbers of free-ranging Indian and outfitter horses were reported for both provinces--600 and 200, respectively, in British Columbia, and about 700 and 100, respectively, in Alberta. Eight hundred free-ranging outfitter horses were reported to be present in the Yukon. Herds in these categories are often difficult to distinguish from feral stock, and in some areas may continue to contribute to feral horse numbers.

Known numbers of truly feral (unowned) horses are presently concentrated along the eastern slope of the Rocky Mountains, where at least 700 horses are present between the Bow and North Saskatchewan Rivers (Figure 1). This foothill area is characterized by forest cover of lodgepole pine (Pinus contorta), with smaller stands of white spruce (Picea glauca), black spruce (P. mariana), alpine fir (Abies lasiocarpa), aspen poplar (Populus tremuloides), balsam poplar (P. balsamifera), and mixed woods. Meadow and shrub vegetation types cover ~20% of the area. Drainage is through numerous permanent streams. Climate of the area is classified as cool and subhumid (Table 1); during most winters, much of the snowfall is periodically removed by warm west winds.

An additional 100 or more animals occur in the Cariboo-Chilcotin area of British Columbia. Forest cover in this flat to gently rolling region is dominated by lodgepole pine; small mesic meadows surrounded by aspen poplar are characteristic. Climate is characterized by warm, dry summers and cold winters.

The main concentration of feral horses in Yukon Territory is in the Nordenskiold River area, a region characterized by extensive forest cover dominated by white spruce, with smaller areas of lodgepole pine forest and black spruce muskeg. Open habitats include burns, south-facing grassy slopes, and sedge fens. The area has short, cool summers and cold winters; daily temperatures average above $0^{\circ}C$ only during May-September.

Population Trends

Areas of concentration have remained about the same over the last two decades or more, at least in Alberta and British Columbia (McKnight, 1959, 1964), although total numbers of feral horses may have declined somewhat. McKnight reported between 1000 and 2000 feral horses in the southern half of British Columbia and from 1500-2000 animals in Alberta,

but it should be recognized that these are very rough estimates and the figures may have included some Indian and guide horses. McKnight did not report population estimates for the Yukon Territory.

Current trends in regional population levels appear to be due at least in part to level of management. In British Columbia, where populations are generally considered to be stable or decreasing, there has been a concerted effort to control numbers of free-ranging horses through roundups and shooting since the 1920's, and this effort continues to date (Storrar et al.,1977; Salter and Hudson,1978a). Numbers of free-ranging horses in Alberta were controlled through extensive roundups until the early 1970's; controls on feral horse populations have not been initiated since that time, and numbers are considered to be increasing in some foothill areas (Salter and Hudson, 1978a). Government controls on feral horse numbers have apparently never been instituted in the Yukon; it is not known whether populations there are presently increasing or decreasing.

Feral horse populations in western and northern Canada likely undergo natural fluctuations, although detailed studies of population dynamics have not been conducted. A study conducted in western Alberta (Salter, 1978a) showed that during mild winters, and in the absence of significant predation, there may be nearly complete over-winter survival of the foal cohort. During a year of intensive study foals and yearlings comprised 18% and 17%, respectively, of the population; this suggests a potential for rapid population growth. However, local residents and management personnel reported that abortions and high mortality rates due to starvation occur during winters with deep snow and abnormally cold temperatures, thus limiting local populations. Predation by cougars and wolves is reportedly also a source of mortality, particularly during severe winters.

Habitat-Use

Quantitative studies of habitat-use by feral horses have been conducted in western Alberta (Salter, 1978a) and in the Chilcotin region of British Columbia (Storrar et al., 1977). Limited qualitative information on habitat-use was obtained during questionnaire surveys and interviews referred to above.

Use of Space

During the study conducted in western Alberta the horse population was organized year-round into two basic herd types--harem groups and bachelor stallion groups. Herds occupied overlapping home ranges of less than 15 km^2; all home ranges included both non-forested (meadow and shrub) and forested habitat.

Habitat Selection

Pellet group counts, dietary data, and sightings of horses indicated that feral horses in western Alberta made use of virtually all available habitats. Pellet groups occurred on 85% of plots examined during summer (Table 2); they did not occur in any one habitat more frequently than

expected but on the basis of total counts certain meadow and shrub types (dwarf birch thicket, mixed shrub meadow, dwarf birch meadow) and adjacent forest types (black spruce woods) appeared to be most heavily utilized on a year-long basis. Poplar woods were heavily utilized during winter; use of this limited habitat appeared to be related to its proximity to concurrently used south-facing grassy slopes, and possibly to reduced snow depth due to southerly exposure. However, only habitats that were limited in areal extent or that had sparse forage growth were clearly under-utilized during winter. Habitat occupancy during spring was related to stage of forage growth; previously grazed meadows and disturbed areas were the first to green up and were heavily used for feeding. Horses also fed under forest cover during spring. The pattern of use of both forested and meadow habitats continued through summer, fall, and early winter.

Pellet group counts conducted in the Chilcotin area indicated preferential use of sedge meadows and poplar forests by feral horses, but seasonal use could not be determined (Storrar et al., 1977). From aerial sightings, sedge meadows and south-facing grassy slopes appear to be among the preferred habitats of feral horses in the Yukon (D. Russell, pers. comm.) although degree and season of use of various types has not been quantified.

Snow Relationships

Snow cover is more or less complete during several months of the year in western and northern Canada, and as noted above, inability of feral horses to obtain adequate forage during deep snow years may be a mortality factor. During the study in western Alberta, horses appeared well able to cope with snow depths (average ~30 cm), encountered during mid- to late winter, and pawed snow as deep as 60 cm away from underlying forage. Although observed at all snow depths, pawing was resorted to more frequently in deep as compared to shallow snow cover (Table 3). Each pawing bout tended to involve more strokes in deep as compared to shallow snow, suggesting greater energy expenditure. Horses also were able to feed in shallow snow without pawing. In these situations, snow was 'plowed' away by pushing the muzzle through the snow at a selected spot, and subsequent snow clearing was accomplished by thrusting the muzzle forward while feeding. It appeared that this technique also was used to enlarge or clear snow from craters already created by pawing; it was used without pawing only at the shallowest snow depths (Table 4).

In addition to behavioral adaptations for feeding in snow, horses took advantage of reduced snow depths at tree bases in woods, and on hummocks and around shrub bases in open areas. Steep south-facing slopes remained snow-free or had reduced snow depth throughout the winter and also were exploited as feeding habitat.

Multiple tracks or trails were found in several habitat types; although trails appeared to be developed primarily in deep snow areas this relationship was not quantitatively examined.

Use of Shelter

Coniferous habitats found in western Alberta appeared to have high shelter value, but due to a low incidence of observations on the

use of shelter its importance could not be quantified. Winters during the study were exceptionally mild, however, and it is possible that shelter might be an important determinant of habitat selection during more severe periods. Conversely, meadow habitats, especially those with south-facing orientations, likely afforded favorable microclimates during fair weather and this may have been a factor in their utilization.

During the summer period horses were observed deep within wooded areas on a number of occasions, and frequently made use of shade at forest-meadow edges (including cattle salt-lick areas).

Food habits

Feral horses in western Alberta subsisted primarily on gramineous plants throughout the year (Table 5). Sedges, hairy wild rye, and fescues were the most important species; seasonal shifts in relative importance of each in the diet was probably related to both palatability and availability factors (Salter and Hudson, 1979).

Drinking

Areas frequented by feral horses in western and northern Canada are generally characterized by adequate sources of water, and it is unlikely that water supply is a major determinant of dispersion or habitat-use. Water was readily available from numerous streams during the study in western Alberta, and consequently horses did not gather at specific sites to drink as reported for more arid regions. Water requirements in winter were probably met largely by ingestion of snow.

Use of Mineral Licks

Feral horses, like other ungulates, make periodic visits to mineral licks. Horses in western Alberta ingested quantities of soil at both natural mineral licks and cattle salt licks throughout the year. Chemical analyses of soil, feces, and vegetation indicated that soil-eating likely was related to sodium deficiency (Salter and Pluth, in press).

Land-Use Conflicts

Land-use conflicts reported by management personnel during interviews and in the questionnaire surveys were competition for forage between horses and cattle, competition between horses and wild ungulates, and grazing and trampling damage on spring ranges. Reported problems were concentrated in areas with large horse populations, particularly where cattle-grazing is an important form of land use. Thus, feral horses in the central part of the Alberta foothills were reported to be competing with elk in winter and with cattle in summer, and to be overgrazing cattle allotments prior to the turn-out date (Salter, 1978b). In the Cariboo-Chilcotin area of central British Columbia, early season use of open hillsides was reported to result in range damage and forage competition (Salter and Hudson, 1978a). By contrast, feral horse populations elsewhere in the Alberta foothills, and in the northern part of the province, were not considered to represent serious land-use problems.

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Studies conducted in western Alberta (Salter and Hudson, in press) provided additional insight into potential land-use conflicts. These studies documented some range damage as a result of grazing and trampling by horses in spring, and suggested the possibility of forage competition between feral horses and elk during winter and spring, dependin on local population levels. Winter diets of horses and elk were 51% similar; both species fed in dry grassland habitat during winter and spring The studies also showed that horses and cattle chose much the same diet (66% similarity during summer) and preferred some of the same habitats, resulting in overgrazing of dry grassland sites by fall. There was little indication of competition between horses and either deer or moose; horses ate little browse and habitat preferences were essentially different. Absence of competition between horses and moose also was suggested by the study in the Chilcotin area of British Columbia (Storrar et al., 1977).

Conclusions

The distribution of feral horses in western and northern Canada reflects both historic and current patterns of land-use--they are found in areas where, in the past, large numbers were allowed to stray or escape, and where present levels of management permit the existence of viable populations. They are currently confined largely to wilderness or semi-wilderness areas, where intensive management is difficult or significant land-use conflicts do not occur.

Present areas of concentration are characterized by coniferous forest cover and by relatively severe climate. Meadow (non-forested) vegetation types within the coniferous forest appear to be preferred habitats, although use is also made of forested types. Although forage selection has been studied in only one area of western Alberta, it is surmised (from observations on habitat preference) that feral horses depend primarily on gramineous plants throughout their range in western and northern Canada. Horses are able to feed in snow by pawing and by selecting feeding areas on the basis of snow depth, but the extent to which snow depth is a factor limiting mobility, habitat-use, and ultimately survival is not known. Availability of water is not considered to be a limiting factor.

Population dynamics of feral horse populations in western and northern Canada are not well understood. Limited quantitative evidence gathered in western Alberta suggested potential for rapid population growth, at least during mild winters and in the absence of predation and control of numbers through management. However, reports from management personnel and local residents indicated that mortality due to starvation may be high during severe winters--such die-offs have been reported from both British Columbia and Alberta, and in the absence of quantitative data over-winter mortality is considered to be the primary natural check on population growth.

Feral horses are thought to constitute land-use problems within parts of their range in western and northern Canada, but this has been adequately quantified in only one small area of western Alberta. At present, numbers of feral horses are being actively controlled in southern British Columbia in order to alleviate or prevent range-use

conflicts. Although round-ups have not been conducted in Alberta for several years, potential range-use conflicts in the foothills are being monitored and other types of free-ranging horses (primarily those owned by outfitters) are being controlled. Similarly, management personnel are attempting to collate information on numbers and distribution of feral and free-ranging outfitter horses in the Yukon, and to determine the extent of land-use conflicts. Assuming continued monitoring and control of feral horse numbers, populations in most areas of western and northern Canada are likely to decrease or remain relatively static in future.

Acknowledgements

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Table 1. Selected climatic characteristics of areas occupied by feral horses in western and northern Canada.[1]

	Mean daily maximum (°C)		Mean daily minimum (°C)		Mean annual snowfall (cm)
	Jan	Jul	Jan	Jul	
Western Alberta	-2	21	-19	4	218
Cariboo-Chilcotin	-3	23	-13	6	144
Southern Yukon	-15	20	-28	5	129

[1]From long-term measurements at Red Deer Ranger Station (western Alberta), Tatlayoko Lake (Cariboo-Chilcotin) and Haines Junction (southern Yukon). Data in Environment Canada 1973a, 1973b.

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Table 2. Seasonal distribution of horse sign by habitat type in western Alberta.

Habitat type[1]	Percent of total[2]		Mean number[3] all age pellet groups per plot
	Winter sign (n=185)	Any age pellet groups (n=417)	
Dwarf birch meadow	6.5 (5.3)	2.4 (2.4)	8.4
Mixed shrub meadow	11.4 (8.5)	7.0 (6.3)	8.3
Willow thicket	3.8 (2.6)	1.0 (1.6)	2.8
Dry grassland	9.7 (7.5)	4.8 (4.1)	4.4
Dwarf birch thicket	4.3 (3.2)	2.6 (2.2)	8.9
Mesic pine woods	0.5 (1.2)	... ...	...
Dry pine woods	6.0$^-$ (14.4)	19.2 (20.4)	3.2
Black spruce woods	3.8 (3.0)	2.9 (2.4)	6.5
White spruce woods	2.2$^-$ (7.1)	3.8 (4.3)	3.2
Poplar woods	7.6$^+$ (4.2)	4.3 (3.9)	4.9
Mixed woods	14.0 (12.8)	18.4 (16.9)	4.2
Pine/alder thicket	27.0 (26.8)	31.4 (32.9)	3.2
Disturbed	0.5 (0.4)	0.7 (1.0)	4.0
Forested watercourse	2.7 (1.8)	1.4 (1.4)	3.3
Total χ^2	29.0	5.0	

[1]Other habitats each comprising less than 0.5% of total area examined were sedge meadow, silver-berry thicket and alpine fir woods. Descriptions of habitat types are in Salter 1978a and Salter and Hudson 1978b.

[2]Based on presence/absence of tracks, feeding craters or pellet groups in 493 circular 15 m^2 plots examined Jan-Mar 1976 (winter sign) and of pellet groups in 490 circular 15 m^2 plots examined Jul-Aug 1976 (any age pellet groups). Percent of total plots sampled are in parentheses. Habitat types contributing 10% or more to significant total χ^2 values are underlined and marked + or - to signify preference or avoidance. Values of χ^2 at P=0.05 are 26.3 (winter) and 21.0 (any age).

[3]Based on counts conducted Jul-Aug 1976.

Table 3. Frequency of pawing by foraging horses in relation to snow depth, western Alberta.

Snow depth (cm)	Number of 5 min observations	Mean number of pawing bouts/ 5 min ± 1 s.d.	Mean number of strokes/pawing bout ± 1 s.d.
10	16	1.4 ± 2.0	5.4 ± 1.8
40-50	9	9.1 ± 3.8	9.7 ± 3.6

Table 4. Number of herd sightings during which pawing was used by foraging horses in relation to snow depth, western Alberta.

Number of observations involving:	Snow depth (cm)					
	1-10	11-20	21-30	31-40	40+	Total
Pawing	10	7	4	5	2	28
No pawing	6	2	0	0	0	8

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Table 5. Major components of feral horse annual diets[1] in western Alberta.

Species	Percent composition
Grasses, sedges and rushes	93
Sedge (*Carex* spp.)	(34)
Hairy wild rye (*Elymus innovatus*)	(27)
Fescue (*Festuca* spp.)	(20)
Forbs	1
Browse	4
Miscellaneous (*Equisetum* spp., lichen, moss)	3

[1]Based on fecal fragments analysis of 12 monthly samples obtained during Jan-Dec 1976. Each sample was composited from subsamples of 50 fresh fecal groups.

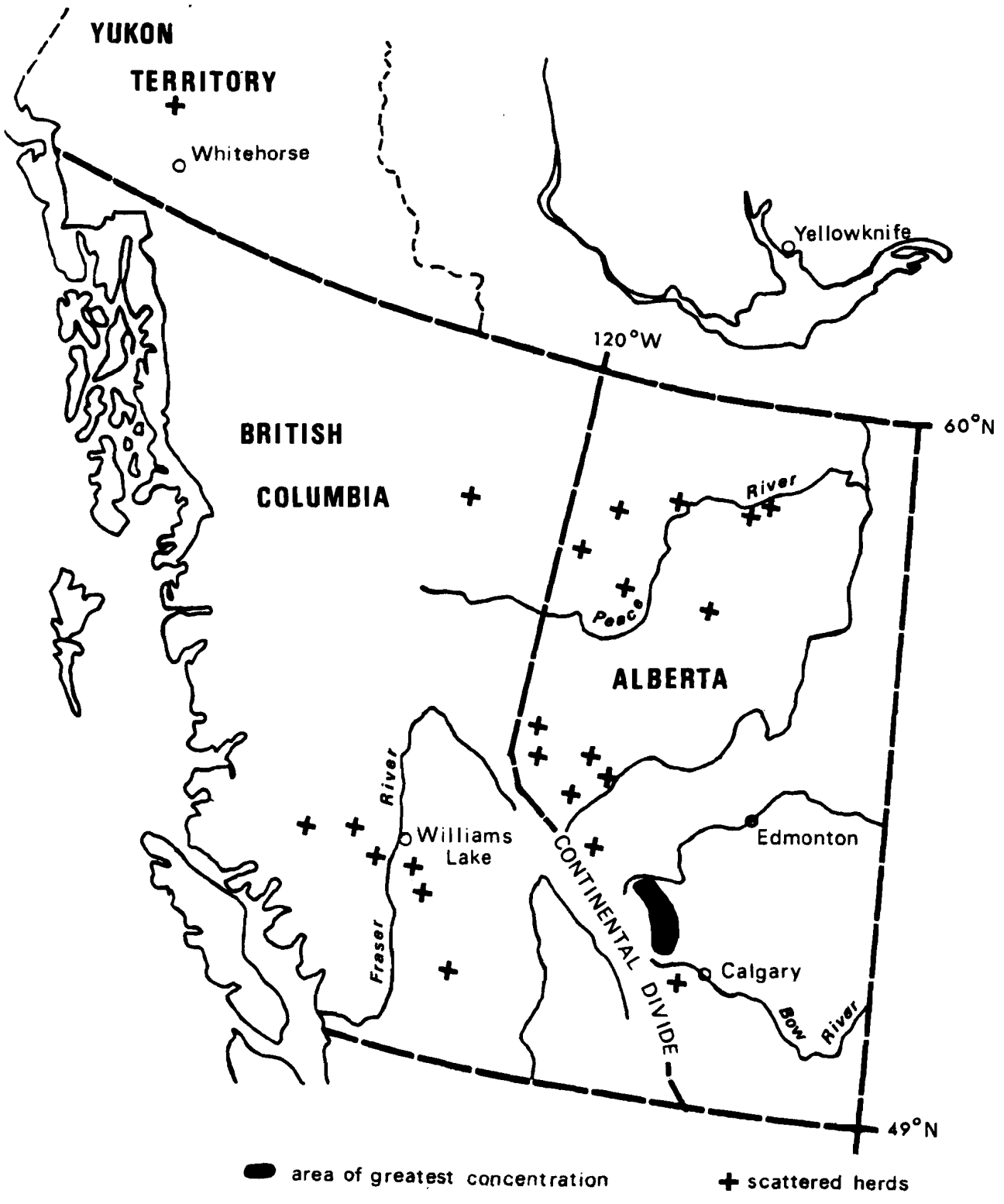


Figure 1. Documented locations of feral horse populations in western and northern Canada. Feral horses may also occur in the northwestern third of British Columbia, in southern and central Yukon, and in the southwestern corner of the Northwest Territories, but the ownership status of horses occurring in these areas is unclear. Data from questionnaire surveys and interviews of Forest Service and wildlife management personnel, 1975-79.

The Effect of Space-Use Patterns of Reintroduced Asiatic Wild Ass on Effective Population Size

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Abstract: *Empirical data on behavior, such as space-use patterns, are important to the success of animal reintroductions. We studied space-use patterns in a growing population of Asiatic wild ass (*Equus hemionus*) reintroduced into the Ramon erosion cirque in the Negev desert, Israel. Between 1988 and 1995 we used direct observation to determine the location and association of males and females. All adult females and dominant males were individually recognized. Home ranges of dominant males overlapped little, suggesting that in this population males are territorial. After the first release of males and females into the wild, only one territory was established, and it covered most of the 20,000 ha of the cirque. After 6 years the number of male territories increased as the number of males in the population increased, and average territory size decreased. Male territories were near permanent and ephemeral water sources, but the water sources were at the peripheries of the territories and were not centers of activity. When there was only one territorial male, female home ranges were almost entirely within the territory. As male territory size decreased, so did the spatial association of females with a single male. During the breeding season, males spent more time in close association with female groups, adopting what may temporarily appear to be a harem breeding strategy. Although demographic and environmental factors pose a greater threat to small populations, our data support the hypothesis that in small, reintroduced populations of territorial, polygynous species, effective population size (N_e) may be dangerously small. Our data suggest that this situation may last for several years until new males are recruited into the population. Thereafter, rapid male turnover and female use of several male territories may ameliorate this problem. We found no relationship between male turnover rate and female reproductive success. The establishment of more male territories is key to increasing N_e and should be the basis for planning reserves for territorial, polygynous species.*

Efecto de los Patrones de Uso del Espacio en el Tamaño Poblacional Efectivo en Asnos Asiáticos Silvestres Reintroducidos

Resumen: *Datos empíricos de conductas, tales como los patrones de uso del espacio, son importantes en el éxito de las reintroducciones de animales. Estudiamos los patrones de uso del espacio en una población en crecimiento de asnos asiáticos silvestres (*Equus hemionus*) reintroducidos en la depresión por erosión Ramon en el desierto de Negev, Israel. Utilizamos observaciones directas entre 1988 y 1995 para determinar la ubicación y asociación de hembras y machos. Las hembras adultas y los machos dominantes fueron reconocidos a nivel individual. Los rangos de hogar de los machos dominantes se sobrepusieron poco, sugiriendo que en esta población los machos son territoriales. Después de la primera liberación de machos y hembras en el ambiente silvestre, solo un territorio fue establecido, y cubrió la mayoría de las 20,000 Ha de la depresión. Después de seis años, el número de territorios de los machos se incrementó al incrementarse el número de machos en la población y el promedio del tamaño territorial disminuyó. Los territorios de los machos estuvieron cercanos a fuentes de agua permanentes y efímeras; sin embargo, las fuentes de agua estuvieron en las periferias de los territorios y no fueron los centros de actividad. Cuando solo hubo un macho territorial, los*

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rangos de hogar de las hembras estuvieron casi en su totalidad dentro del territorio. Cuando el tamaño del territorio de los machos disminuyó, el tamaño de la asociación espacial de las hembras con un solo macho disminuyó también. Durante la temporada de reproducción los machos pasaron más tiempo en cercana asociación con los grupos de hembras, adoptando lo que temporalmente aparenta ser una estrategia reproductiva de harén. A pesar de que los factores demográficos y ambientales representan una amenaza mayor para las poblaciones pequeñas, nuestros datos sustentan la hipótesis de que, en poblaciones reintroducidas pequeñas, de especies polígamas territoriales, el tamaño poblacional efectivo (N_e) puede ser peligrosamente pequeño. Nuestros datos sugieren que esta situación podría durar por muchos años hasta que nuevos machos sean reclutados hacia la población. Es por ello que una tasa de incorporación rápida de machos y el uso de varios territorios de machos por hembras podría aminorar este problema. No encontramos una relación entre la tasa de incorporación de machos y el éxito reproductivo de las hembras. El establecimiento de más territorios de machos es clave para el incremento de N_e y debería ser la base de la planeación de reservas para especies polígamas territoriales.

Introduction

Although reintroductions are an important part of world conservation efforts (World Conservation Union 1998), knowledge of the biology of reintroduced populations remains limited. Successfully reintroduced populations are initially small and have a positive growth rate, but most studies in conservation biology have been restricted to populations of either rare but stable species or declining species. Furthermore, although the volume of theoretical and empirical data concerning the deterministic and stochastic factors threatening small populations is impressive (Allee 1931; Shaffer 1981), little is known about how these factors are affected by and interact with the behavior and sociobiology of the species (Clemmons & Buchholz 1997; Sutherland 1998). Wildlife reintroduction efforts are generally concentrated on preparation and implementation (Ralls et al. 1992), with little attention to post-release monitoring (Beck et al. 1994). Consequently, empirical data are lacking on behavioral aspects, such as space-use patterns, considered important to the success of a reintroduction (Durant & Mace 1994; Beissinger 1997).

Knowledge of behavior patterns may be especially important in reintroduced social, polygynous species, in which a few dominant males obtain most of the breeding opportunities. In such cases, the demographic and genetic factors that play a critical role in the dynamics of small populations may be strongly affected by changes in behavior and space-use patterns that occur as the population increases. For example, in reintroductions where only a few animals are released, a single male may outcompete all other males for access to females. Consequently, a single male will mate with all females, making the genetically effective population size (N_e) considerably smaller than the actual number of animals released (Nunney 1993). If post-release reproductive success is low (Saltz & Rubenstein 1995; Novellie et al. 1996) or if the dominant male has low fertility, many years may pass before that male is replaced, exposing the population to increased demographic and genetic stochasticity.

We monitored the space-use patterns of reintroduced Asiatic wild ass (*Equus hemionus*) from the time the herd was established in 1983 until 1995. We describe the development over time of male home ranges and reproductive strategies and corresponding female movement patterns and consider our findings in the context of the conservation of small but growing (reintroduced) populations.

Animals and Study Area

The Asiatic wild ass is a mid-sized equid (approximately 200 kg) inhabiting arid environments. Once common throughout western Asia, the wild ass has been extirpated throughout much of its range with the advent of modern firearms. There are several subspecies (for review see Saltz & Rubenstein 1995). The subspecies endemic to the Middle East (*E. h. hemipus*) became extinct at the beginning of the twentieth century (Groves 1986). In 1982, the Israel Nature Reserves Authority began reintroducing Asiatic wild asses from a permanent breeding core founded in 1968 from six animals from the Persian subspecies (*E. h. onager*) and five animals from the Turkemen subspecies (*E. h. kulan*). The core was established shortly after these adjacent populations were separated into two subspecies (recent genetic studies suggest that this separation is erroneous) (A. Oakenfull, personal communication).

The site selected for reintroduction in Israel was Makhtesh Ramon, a nature reserve established within an unusual anticlinal erosion cirque (Fig. 1) in the Negev highlands (lat 30°35′N, long 34°45′E). The area of the cirque is about 20,000 ha. It is elongated, running 40 km southwest to northeast, with a maximum width of 10 km. It is drained by the Ramon dry riverbed, which exits on the eastern side of the southern rim. The north rim of the cirque consists of a sheer 200-m drop. The cirque floor tilts downward toward the northeast, so the rim elevation ranges between 800 and 900 m and the cirque

floor ranges from approximately 800 m at the southwest edge to about 400 m in the northeast basin. The southern rim is less steep, with an elevation of about 510 m, and it has several openings. Annual rainfall ranges from about 95 mm in the southwest basin to 40 mm in the northeast basin and is restricted to the fall and winter (Ward et al. 1993). A single permanent water source, En-Saharonim, exists in the Ramon riverbed near its exit from the cirque. Several natural and artificial (from mining activity) ephemeral ponds exist in the center of the cirque and sometimes hold water from winter floods until July. In addition, an artificial water source was constructed for the reintroduced animals 1 km west of En-Saharonim.

Asiatic wild asses are polygynous. Male spatial organization and breeding strategy are currently unclear, but males of other equid species are either territorial or protect a harem, depending on the social and physical environment (Rubenstein 1986). If female assemblages are temporary but their movements are predictable, males will defend a territory. Territorial males that defend an exclusive home range are often found alone, patrolling their territory, and are not necessarily associated with a specific group of females (Maher & Lott 1995). Permanent-membership equid societies evolve when resources are uniformly distributed and feeding competition is low (Rubenstein 1986). When females live in permanent-membership groups, males will form a long-term association with and defend a specific group of females, or harem, against other males. In such harem-based societies, male home ranges may overlap with other males and their harems. Feh et al. (1994) concluded that Mongolian Asiatic wild asses (*E. b. luteus*) have a harem-based society, but they live in arid environments where resources are limited, movements are predictable, and females live in open-membership groups (Klingel 1977). Thus, males are expected to be territorial, especially when the population is small (Rubenstein 1986).

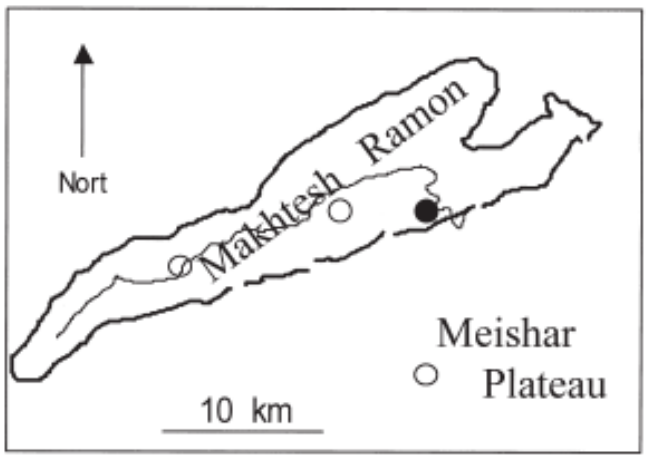


Figure 1. The Makhtesh Ramon study area. The thick line delineates the cliffs of the cirque, and the thin line is the Ramon dry riverbed. Open circles are ephemeral water sources; the filled circle is the En-Saharonim spring.

Females usually attain sexual maturity at age 2 and give birth for the first time at age 3 (Groves 1974). A single foal is born after 11 months of gestation. Females may give birth in consecutive years (Zhirnov & Ilyinsky 1986). Parturition is seasonal, with most births occurring in May and June. Post-partum estrus usually occurs within a few weeks after parturition. Based on our observations, juvenile females remain with their mother for 2–3 years. Dominant males expel yearling males from the maternal herd, who then join bachelor herds. Little is know about the dynamics of bachelor herds.

Methods

Between 1982 and 1987, 28 animals (14 males and 14 females) were released in Makhtesh Ramon. The first release in 1982 included only five males. All of these animals dispersed immediately after release, and none survived (Saltz & Rubenstein 1995). In 1983 two males and six females were released. The males were 3 and 4 years old and were named Randa and Gandolf, respectively. This release succeeded (females remained in Makhtesh Ramon and produced offspring) and was followed by a release of two males (3 and 4 years old) and five females in 1984. The last release in this area was in 1987 and included five males (four 4-year-olds and one 3-year-old) and three females. Except for two females, aged 6 and 17, that died shortly after their release, all females were between 2 and 5 years old. Until 1989, reproductive success was low and population growth was slow (Saltz & Rubenstein 1995). Thereafter, reproductive success increased, and the population was estimated at over 100 animals in 1997. Currently, the reintroduced population has had little effect on the vegetation in the cirque (Saltz et al. 1999).

Of the animals reintroduced between 1982 and 1984, only one female from the 1983 release was radiocollared. In 1987, all animals were numbered with freeze marks. Wild-born animals were not marked. All females and all unmarked reintroduced males were readily recognizable by natural markings. Female foals remained with their mother until age 2 or 3; during this period natural markings, such as scars, are accrued. Regular monitoring of the population and careful updating of pictorial identification charts enabled identification. This was not the case for male foals; because they were expelled from the herd by the dominant male by the time they were 1 year old, few if any identifying scars were noted before expulsion. Without close association with their mothers, it was almost impossible to identify individual males when they returned as dominant males (except those that had

early scars or birthmarks). Thus, the genealogy of adult males born in the wild was, in most cases, unknown.

From 1982 to 1987 the Makhtesh Ramon ranger carried out post-release monitoring with periodic surveys to record the presence and reproductive success of all animals in the cirque. From 1988 to 1992 and in 1995 we monitored the herd intensively. In 1993 the population was surveyed and monitored for 10 days (in July and August), and in 1994 only reproductive success was noted.

We concentrated on movement patterns and association between males and females during the foaling and breeding season (April–September). Monitoring was done by systematically scanning the cirque 2–3 days per week from April to September. Each day, a different part of the cirque was randomly selected and scanned. Animals that we sighted were characterized according to group composition: (1) dominant males alone, a dominant male being defined as a male that appears to have regular and exclusive access to females and acts to maintain this exclusivity; (2) groups of females and young accompanied by a dominant male; (3) groups of females and young accompanied by one or more bachelor males (males that were sighted with females only once or twice); (4) groups of females and young unaccompanied by a dominant male or bachelor males; and (5) bachelor groups without females. Dominant and bachelor males are easily distinguishable. Dominant males are alert to human intruders (on foot or in a vehicle) but will generally stand their ground. They will turn and face the intruder until the human is within 50–100 m. Bachelors, on the other hand, roam in a group with other bachelors, are sensitive to human intruders, have large flight distances (>1 km), and will run at the first sight of humans.

Once animals were sighted, their location was recorded. Then, if the group was not a bachelor group, all animals were identified. Because of their large flight distances, we were unable to identify individuals in bachelor groups. We monitored female groups for at least 3 hours and collected behavioral data before searching for another group.

We analyzed the location data to determine dominant male home ranges, to assess whether males adopt a territorial or harem strategy, and to determine how female space-use patterns relate to male movements. Because female group composition changes on a daily basis, we calculated the home range of each individual separately. To ensure that locations were independent, we used only one location per animal per day.

To describe male home ranges, we used the Kernel estimator (Worton 1989) with the fixed-kernel option in the Ranges-V software. This is a robust technique that gives a good description of the animal's use intensity (utilization distribution) and center(s) of activity within its home range. These are depicted graphically by isopleths (isolines describing areas with equal location density). The Kernel estimator, however, requires a large sample size. We calculated the home range of each dominant male from all locations during the study. If a major shift in the home range of a specific male was evident during the study, we carried out a separate analysis for each period.

To assess female space-use patterns in relation to those of males, we calculated the amount of overlap between each female and each dominant male for each of the foaling/breeding seasons. Because of the differences in sample size available for each male in each foaling/breeding season and the relative unimportance of the utilization distribution for this analysis, we used the more general and more robust bivariate normal technique (Jennrich & Turner 1969) to depict the home range of males. The bivariate normal calculates a home range by assuming that the animal's location in space is distributed normally along two axes (x and y) with a single center of activity. With Ranges-V software, we then determined the 90% density distribution line for each male and the fraction of sightings of each female that fell within it. We used a 90% density distribution to minimize home-range inflation by outliers. We considered the relative use of male home ranges by a female as the fraction of all locations obtained for that female which fell within the male's home range. We then categorized the overlap between each possible male-female pair as low, 1–25%; moderate, 25–50%; high, 50–75%; or very high, 75–100% (pairs with no overlap were excluded).

We assessed shifts of females among male home ranges by comparing changes in the overlap of female locations and male home ranges between years. For each female we determined the male with which she had the maximum overlap in a specific season, based on the number of her locations in each male's home range. A female was considered to shift between males if her maximum overlap changed from one male to another.

We tested whether a change in the male that a particular female was most associated with (compared with the previous season) had an effect on the reproductive success of the female in the following season. We considered a change in association as either a takeover by a new dominant male or a shift of the female's home range causing a change in the identity of the male with whom she had the greatest overlap.

Results

Male Space-Use Patterns

Shortly after the 1983 release, Gandolf expelled Randa and appeared to establish a home range on the east side of the cirque near the site of release. Over the next year, Gandolf's movements and those of the females expanded to include most of the cirque. The males released in 1984 and in 1987 disappeared shortly after their release and

were not seen again. (A. Cohen, local ranger, personal communication).

In the summer of 1988, when the intensive study began, Gandolf was still the only dominant male in the cirque, with a center of activity adjacent to the northern rim in the center of the cirque (Fig. 2a). At the end of July 1989, a second male (Napoleon) took over the eastern side of the cirque, and Gandolf's home range and center of activity shifted westward (Fig. 2b). In early spring of 1991, Napoleon disappeared after losing his home range to a new male named Triang. At the same time, a fourth male named HT established a home range in the western part of the cirque, taking over the western side of Gandolf's home range (Fig 2c). Consequently, Gandolf's home range became even smaller. A fourth home range was established in late 1991 by a male (Hector) born in Makhtesh Ramon in 1989 on the Meishar plain about 10 km south of the cirque. In 1992 two new

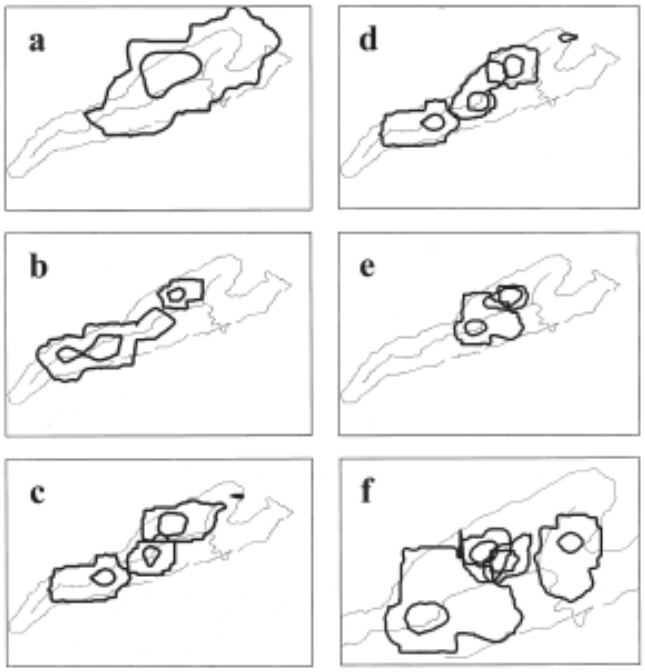


Figure 2. Male home ranges of the Asiatic wild ass as depicted by the Kernel estimator (heavy lines) superimposed on the Makhtesh Ramon study area (light lines). Isopleths (see text) are from the inside-out 30% and 90%. Home ranges are (a) Gandolf until 1989; (b) 1990, Gandolf (west) and Napoleon (east); (c) 1991, HT (west), Gandolf (center), and Triang (east); (d) 1992, HT (west), Gorby (center), and Cut (east and including isolated circle at northeast corner of the cirque); (e) 1993, HT moved to the center of the cirque between Gorby and Cut (data are insufficient to present Cut's home range); (f) 1995 close-up and the center and east end of the cirque, Gorby (center), Put (east), and between them Questa (center-north) and Richard (center-south).

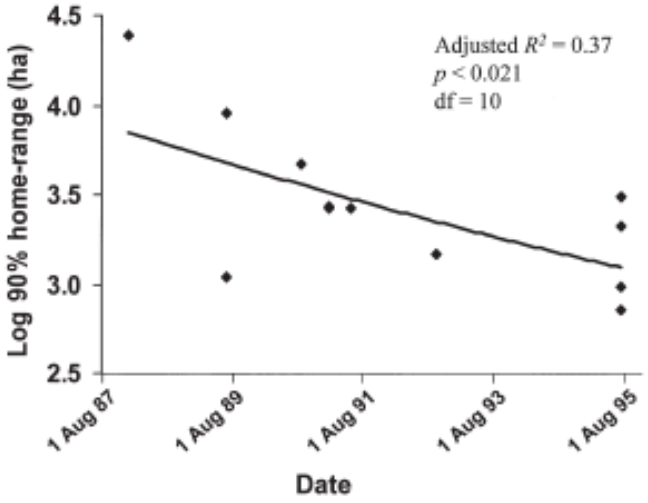


Figure 3. Male home-range size in hectares, calculated by the 90% isopleth using the Kernel method, as a function of date following release. Each point is the home range of one male as calculated at the time he was first sighted. If a major shift occurred in the home range with the appearance of another male, home-range size was recalculated and the second value was used. Regression line based on linear regression after log transformation.

males, Gorby and Cut, replaced Gandolf and Triang, respectively (Fig 2d). Gorby initially appeared at the northeast corner of Gandolf's home range and, after several weeks, took over entirely. In 1993 HT shifted his home range to the center of the cirque between Gorby and Cut (Fig 2e). In 1995 Gorby maintained his home range, but HT and Cut disappeared and three new males took their place: Richard, Questa, and Put (Fig. 2f). Overall, male 90% home range size declined significantly ($p < 0.021$, linear regression after log transformation), from 24,700 ha while Gandolf was the only dominant male in the cirque in 1988–1989 to 1,720 $\pm$ 1090 ha ($n = 4$) in 1995 (Fig. 3).

The fate of the reintroduced males that did not hold territories and territorial males that were deposed is unknown. None of the freeze-marked males released in 1987 ever held territories. Shortly after they were displaced, deposed territorial males were often sighted once or twice at the periphery of their home ranges, and then they disappeared.

Dominant males were often found either patrolling, resting at high points where part of the territory could be well observed, or following females. Occasionally, two dominant males would be found standing and facing each other along the boundary line between their respective home ranges. The movement patterns of dominant males varied with time of year. During the height of the breeding season (June), dominant males were often sighted with females (Fig. 4). In contrast, in earlier and

later months males were generally sighted alone (p = 0.032, second-degree polynomial regression), often while patrolling their home range.

Although water is probably the most critical resource in this region, individual male home ranges were not set up around water sources. Most water sources were located at the periphery of the home ranges and were used by several dominant males (Figs. 1 & 2). Certain males traversed other males' home ranges to get to water. We have two observations of such males being located by the resident male and chased away. Males were rarely found in the vicinity of water and were never sighted with the females near the water.

Bachelor males avoided entering existing dominant-male home ranges and were sighted mostly on peripheries and as far as 70 km from the cirque. Bachelor herds usually had 2–20 animals and were rarely sighted within male home ranges (<10 sightings over the entire study period). We sighted bachelors in association with females only once, and the dominant male was not present. Sightings of lone bachelors were rare. In most cases these bachelors were subadult males recently displaced by a territorial male. Bachelors were never sighted together with dominant males.

Male Recruitment

Reproductive success was low during the first several years after the reintroduction began, and progeny sex ratio was male-skewed (Saltz & Rubenstein 1995) until 1992. Thus, between 1985 and 1995 a total of 47 male foals were born, of which 43 survived to 1 year. In contrast, 37 females were born during this period, 20 of them between 1993 and 1995.

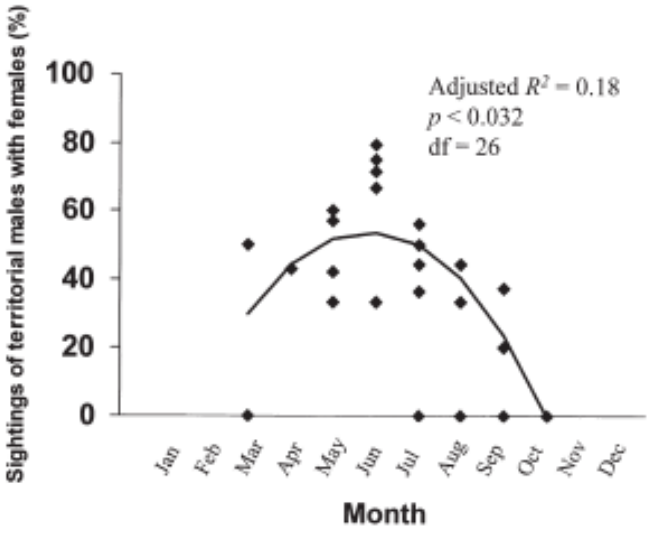


Figure 4. The association of territorial males with females as determined by the proportion of male sightings in which the male was accompanied by females. Each point is the percentage of all sightings per male per month. Only cases in which a male was sighted four times or more in 1 month are included. Regression line is a second-degree polynomial in which each point is weighted by the number of sightings.

During the breeding season, dominant males continually harassed yearlings, eventually forcing them to leave their mothers and the female group. Thereafter, the yearlings roamed the peripheries of dominant-male home ranges and joined bachelor groups. Because bachelors were rarely sighted, we were unable to identify them individually and consequently have no data on their survival during this period. We were unable to determine at what age bachelors return to challenge the dominant males.

Male-Female Home-Range Overlap and Association

Between April and September, female movements were dictated by water sources (unpublished data). During this time, lactating females moved between grazing areas and water sources on a daily basis. Females tended to associate with conspecifics that had overlapping home ranges, with fission and fusion occurring on a daily basis. Based on the presence of dominant males in the cirque (Figs 2a–f), we analyzed female space-use patterns in six different periods: up to July 1989, July 1989 until the end of the summer of 1990, and spring and summer of 1991, 1992, 1993, and 1995.

When Gandolf was the only dominant male in the cirque, most female sightings (96% to 100%) fell within his home range. In 1990 when Napoleon took over part of Gandolf's territory, overlap was bimodally distributed, with the intermediate overlaps (25–75%) being less frequent (Fig. 5). Eight of 12 females had a high overlap (>72%) with Gandolf, and 4 exhibited a high overlap with Napoleon. Over time this distribution changed, with most females having a maximum overlap of <50% with any one male (χ^2 = 29.1, p = 0.004, df = 12). The reproductive success of females that experienced a change in dominant male was 0.66 foal per female (n = 33), compared with 0.64 foals per female (n = 56) among females experiencing no change (p = 0.82, χ^2 = 0.052, df = 1).

On several occasions males were sighted trying to herd females. This was done when females were moving to or from water and were approaching the edge of the male's home range. Males attempted to herd the females back toward the center of their home range by placing themselves between the females and the outer edge of the home range and lifting their heads in a threatening gesture. These attempts were never successful, and the females simply bypassed the male.

Discussion

Successful reintroductions offer a unique opportunity to study an important aspect of conservation—the biology

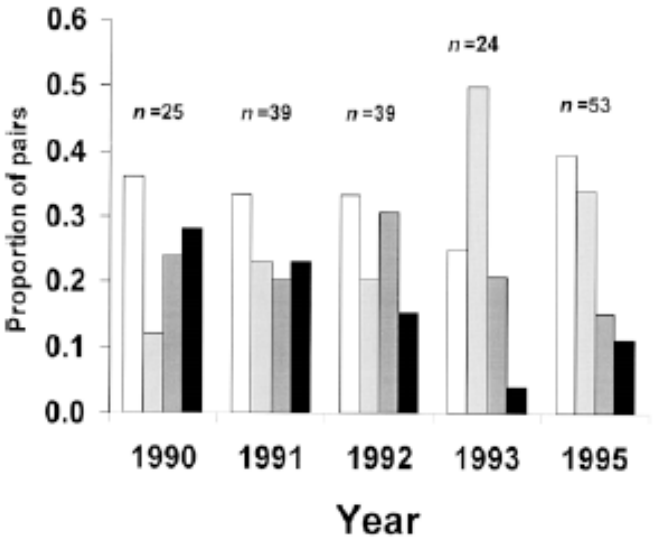


Figure 5. Proportion of all male-females pairs with overlapping home ranges in a given year that fall into each category (see text for details on calculation of percent overlap). Overlap categories include low (1–25%), white; moderate (25–50%), light gray; high (50–75%), dark gray; and very high (75–100%), black. Numbers above the bars of each year are the total number of overlapping pairs.

of small, increasing populations (Sarrazin & Barbault 1996). Our results indicate that in such populations space-use patterns may change dramatically over a relatively short period of time, during which there are only modest changes in the absolute size of the population.

Male Asiatic wild asses reintroduced into Makhtesh Ramon cirque exhibit exclusive use of their home range with respect to other males. Although sightings of actual combat and active defense were few, the expulsion of juvenile males from the home range and the general avoidance of these home ranges by bachelor groups and other males suggest that these areas are also defended. We conclude that male Asiatic wild asses in our study population are territorial.

As the number of males controlling territories increases, females may either (1) exhibit high fidelity to a specific male or (2) roam freely between different male home ranges. If females exhibit fidelity to a specific male, the proportion of female sightings that overlap with male home ranges should be distributed bimodally—each female exhibiting high overlap with one male and low overlap with all other neighboring males—with no intermediate overlaps. If females do not exhibit fidelity to a specific male, overlaps would be mostly in the middle and low ranges. In our study, as the number of territories increased, female fidelity to a single male decreased. Furthermore, female groups fissioned and fused on a daily basis. Thus, we conclude that Asiatic wild asses in our study population do not protect harems.

In contrast, Feh et al. (1994) concluded that Mongolian Asiatic wild asses (*E. h. luteus*) exhibit a harem-type behavior. The discrepancy may be real and may result from different conditions in Mongolia, such as higher densities or a different distribution of resources, that would render territories undefendable. Feh et al. (1994) did not individually identify males, and their observations were made between June and August, when females are in estrus. Our results indicate that during this period territorial males spend more time with female groups. This is not a harem protection strategy, however, but simply a male strategy of following estrus females in his territory to enhance his mating probability.

For several reasons, it is unlikely that any of the unmarked, reintroduced males other than Gandolf had held territories. First, in a reintroduction carried out in the Paran riverbed 50 km south of Makhtesh Ramon (Sinai 1994), the dominant male in the reintroduced group was displaced immediately by a 4-year-old male born in Makhtesh Ramon. This suggests the existence of a "resident advantage" (Krebs 1982). Second, displaced territorial males never returned to hold a territory, suggesting that, once displaced as adults, males do not attain territorial-male status again. Third, two territorial males born in the wild and identifiable as adults were young (≤4 years old) when they became territorial. Thus, the three unmarked males reintroduced in 1983 and 1984 would have suffered from three disadvantages: they were displaced as adults, they were non-native, and they were relatively old (≥8 years old) when Gandolf began losing parts of his territory. The failure of the five freeze-marked males released in 1987 to secure territories lends further support to this hypothesis.

The increase in the number of territory-holding males and the associated decline in territory size as the number of males in the population increased both indicate that intruder pressure is important in shaping the spatial organization of males. Similar results were found in other vertebrate species (Stamps 1994). Surveys we conducted in 1996–1999 indicate that the process is continuing as the population continues to grow. In 1996 and 1997, new territorial males were sighted in the southeastern lobe of the cirque, the southern portion of Hector's territory in the Meishar plateau, and the west side of the cirque originally occupied by HT. In 1998 and 1999, six and nine males (respectively) exhibiting territorial behavior were sighted in various parts of the cirque. The males were sighted alone or with female groups and in different geographic regions of the cirque.

Effective Population Size

The social system of a species is expected to have considerable effects on effective population size (Primack 1993). The presence of a single territorial male in the reintroduced population during the first 6 years of the re-

introduction greatly reduces N_e. In species with nonoverlapping generations, if one male does all the breeding N_e can not exceed 4, regardless of population size ($N_e = 4r(1 - r)N$, where r is the proportion of males and N is total population size; Nunney 1993). As generation time increases, however, N_e approaches $N/2$.

Asiatic wild asses have a relatively long generation time (7.4 years; Saltz & Rubenstein 1995). Thus, we used the equations developed by Nunney (1993) for calculating N_e in polygynous species with nonrandom mating and overlapping generations. In 1989 there were 12 breeding animals with only 1 male. Because of the nonnormal age structure, reproductive success was male-skewed (2:1; Saltz & Rubenstein 1995), and we assumed that male survival was similar to that of females (0.9/year). Based on equation 18a from Nunney (1993), we calculate that $N_e = 4.1$. Although N_e can exceed 4 with only one breeding male, the contribution of additional females is marginal. For example, doubling the number of females to 22 would increase N_e to only 6.3.

In 1995 there were 40 breeding animals with five territorial males, and age structure was more normal; as a result, sex ratio at birth was approximately 1:1 (unpublished data). If we assume that the female survival rate remained unchanged and the adult male survival rate declined because of increased competition for territories to somewhere between 0.8–0.3/year, then according to equation 17b from Nunney (1993) N_e would be 19.0 for a male survival rate of 0.8/year and 25.0 for male survival rate of 0.3/year (Fig. 6).

Although other stochastic factors are considered a greater threat to small populations (Nunney & Campbell 1993), the low N_e in relation to total population size contributes significantly to the loss of genetic variability in the reintroduced population and, if possible, should be minimized by active management. Appropriate management depends on identifying the causes of the problem. We postulate that the extended presence of a single breeding male in the Makhtesh Ramon population was the result of four factors:

(1) A small number of animals was released in 1983 (2 males and 5 females), enabling Gandolf to take over the entire herd and maintain control over it with little effort (i.e., only one competitor and few females to protect).
(2) In later releases, Gandolf already had the "resident advantage" and, because the population was still small, he was able to maintain his dominance.
(3) Due to low reproductive success in the first 4 years of the reintroduction (Saltz & Rubenstein 1995), few males were recruited, so competition for territories and intruder pressure remained low.
(4) Asiatic wild asses are fully grown only at age 3, so young males are not expected to challenge territorial males before they are at least 3 years old. The first males were born in the wild in 1985. Thus, no native-born males could challenge Gandolf before 1988.

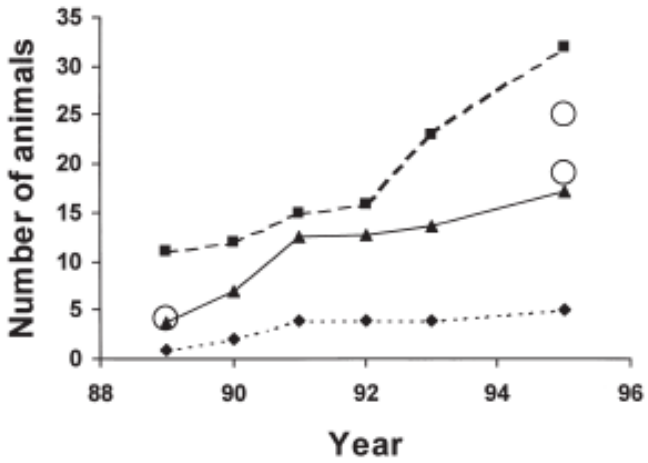


Figure 6. Number by year of adult females (squares), territorial males (diamonds), and genetically effective population size (N_e) based on nonoverlapping generation (triangles). Circles represent N_e calculated for territorial polygynous species with overlapping generations (in 1989 assuming a difference in recruitment between the sexes and in 1995 assuming differences in survival between the sexes, with female survival being 0.9/year and male survival being 0.8/year [lower circle] and 0.3/year [upper circle]).

Given these conditions, several possible actions can be taken to increase the number of breeding males. Managers may be able to artificially increase male turnover by removing the dominant male. We found no correlation between male turnover and reproductive success, but the question remains of whether the possible cost—reduced reproductive success as a result of male turnover (Berger 1983)—outweighs the benefits of an increased male turnover rate.

The simultaneous or sequential release of animals to different areas will result in at least one territory in each release site. This technique also has the advantage of reducing the effect of environmental stochasticity, but it would greatly increase the cost of reintroduction because of the need to erect several habituation enclosures. In the case of sequential releases, we recommend that new release sites be outside the existing range of the previously released populations to prevent the displacement of new males by resident offspring of the dominant males from the previous releases. The release sites, however, should be located to enable eventual genetic flow among them.

Carrying out a single release of a large number of males and females will make the defense of a large territory costly and could lead to the establishment of several territories. The disadvantages of this method are (1) the

absence of knowledge regarding the number of animals needed to ensure that more than one territory is established and (2) the monetary and logistical effort required for such large releases.

Releasing a herd with many male yearlings creates a large pool of potential future challengers. Although these yearlings will be displaced immediately upon release, they are expected to return within 2 years to challenge those males holding a territory.

Because of the advantage of resident males and the low probability of displaced adult males attaining territorial status, we believe that repeated releases of adult males in the same area, as conducted in this reintroduction, are not feasible. The release of more females may also have limited advantages because, as long as the number of released females is small enough for a single male to control all of them, N_e will increase little.

Reserve Design

In populations where few males breed with many females, an increase in the number of females has a limited impact on N_e (Nunney 1993). Consequently, in polygynous species the number of males is key to achieving a genetically viable population. In the case of territorial polygynous species, more males may mean more territories. Thus, reserve design for such species must account for territoriality and territory characteristics.

Water is a critical resource in deserts and is an important determinant in the space-use patterns of Asiatic wild asses. During lactation, mothers must drink daily (personal observations) and can travel up to 20 km in each direction for this purpose, crossing several male territories in the process. Females spend only a few minutes at a watering site, presumably to minimize the risk of predation. Because water sources in this region are point sites serving many other animals, including predators and other conspecifics, and because females spend little time in their vicinity, the cost of defending them relative to the benefit may be unacceptably high. Thus, to enhance fitness, males are expected to set up territories in the vicinity of the water source or within a certain radius from water along female travel routes, but not to defend the water source itself. In our study, water sources were always on the periphery of several male territories and were shared by these males and by males in territories farther away. We conclude that although desert water sources are limited and critical to the existence of Asiatic wild ass populations, a considerable number of male territories and female home ranges can be established around a single water source. A similar pattern was found in the feral ass population of Death Valley (Moehlman 1974) and in Grevy's zebra (*Equus grevyii*; Ginsberg 1988; Rowen 1992). A single water source supporting a population within a radius of 20 km translates into an area of 120,000 ha (area $= \pi r^2 \times 100$). If average territory size is 2000 ha, then a single water source can support a maximum of 60 territories, depending on other characteristics of the habitat and terrain. If we assume, based on the number of territorial males and adult females in the Ramon cirque, that the average number of females controlled by a single male is seven, then N_e for this population is roughly $60 \times 4 = 240$. If a minimum viable population should have an effective size of 5000 (Lande 1995), then at least 24 such reserves are required to ensure the population's long-term existence.

Behavior and social structure are seldom considered in population viability analyses. Growing reintroduced populations offer an excellent opportunity to study the effects of behavior and social structure on population viability. Our study demonstrates that the social structure of Asiatic wild asses is an important component of its viability, mostly in terms of N_e. Space-use patterns and behavior may change rapidly as small populations increase, and with season. Consequently, detailed, long-term knowledge is required to correctly characterize these patterns. These data can then be used to specify the reserve characteristics necessary to support a viable population.

Acknowledgments

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The effects of induced sterility on the territorial behaviour and survival of foxes

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Summary

1. This paper reports on the behavioural effects of surgical sterilization when used to simulate immunocontraception in free-ranging female foxes *Vulpes vulpes*.
2. During 3 years of trapping, 348 male and female foxes were fitted with transmitters in two treatment (females sterilized) and two untreated areas.
3. Radio-tracking indicated that sterile and fertile vixens maintained similar-sized territories during the breeding season, but that sterile females were possibly more likely to share their territories with each other.
4. There were no consistent differences in survival or dispersal between sterile and fertile females.
5. Outcomes from the study suggest that immunocontraception in free-living foxes is feasible.

Key-words: dispersal, immunocontraception, movement, pest management, ranges, *Vulpes vulpes*.

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Introduction

The introduced European red fox *Vulpes vulpes* L. has long been recognized as a serious threat to populations of Australian wildlife (Finlayson 1961; Kinnear, Onus & Bromilow 1988; Priddel 1989; Friend 1990; Short & Milkovits 1990). Native fauna did not evolve with the fox and hence have few predation avoidance strategies, a problem further compounded by habitat fragmentation since European settlement. The fox is also increasingly perceived as a significant predator of livestock, although studies to determine the extent of this impact have been highly variable (Saunders *et al.* 1995; Greentree *et al.* 2000).

Historically, a variety of management strategies has been employed to try and reduce the impact of foxes. These have included bounty systems, shooting, trapping, den destruction and poisoning. The most common fox control strategy used today is poisoning with 1080 bait, an activity carried out over large tracts of Australia (Saunders *et al.* 1995). Evaluations of effectiveness for this strategy are limited, although Tyndale-Biscoe (1994) suggested that poisoning is ineffective in the long term because of the intrinsic capacity of foxes to rapidly replace those that are killed. There are additional concerns over the long-term use of lethal control measures, covering issues such as animal welfare, non-target risk and bait shyness. Consequently, it is highly desirable that suitable alternatives or supplements to lethal baiting are made available.

With recent research into the molecular basis of fertilization, immunocontraception (preventing normal recognition between sperm and egg, thus impeding fertilization) has been suggested as a potential strategy for the suppression of reproduction in free-living pest animals (Tyndale-Biscoe 1994). To be effective in reducing breeding success to a level that achieves population control, such a strategy by necessity is targeted at females (Bomford 1990).

Foxes are monoestrus, largely monogamous, mostly live as pairs or sometimes in family groups, and maintain territories (Saunders *et al.* 1995). The number of non-breeding vixens in any population is variable, being greatest where populations are subject to low levels of control and least where mortality rates are high (Corbet & Harris 1991). Sterilization of dominant females has been shown theoretically to reduce the productivity of a target population (Caughley, Pech & Grice 1992). One of the basic premises of immunocontraception is that, although pregnancy is prevented, normal endocrine

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function and reproductive behaviour of treated individuals remains unimpaired. For the fox this should result in the retention of the fundamental attributes of sociality, such as territorial integrity and mating systems (Newsome 1995). Without this tactical advantage, compensatory behaviour could render immunocontraception useless as a fox management strategy. Other possible forms of compensation that may occur within a treated population include greater post-natal survival of cubs produced by the remaining fertile females, and in turn a greater contribution by these cubs to the rate of increase in subsequent years. Reduced survivorship of sterile females resulting in their replacement by resident (non-territorial) or dispersing fertile females might also negate any treatment effect.

In this paper, we report on the effects of surgical sterilization on female foxes. This approach has previously been suggested as a viable means of simulating fertility control for pest animals (Kennelly & Converse 1993). Population control through immunocontraception seeks to reduce the average rate of population increase (r) by suppressing the birth rate. To achieve this may require levels of sterility of between 65% and 80% (Pech *et al.* 1997; Hone 1999), a difficult target to simulate and maintain in free-living fox populations when using such a labour-intensive method as surgical sterilization. Our main objective was therefore to determine if the normal territorial behaviour of these sterilized foxes was maintained and if any other compensatory mechanisms became apparent in the process.

Materials and methods

STUDY SITES

Research was conducted at four sites on the Great Dividing Range in central New South Wales, Australia (Fig. 1). Two sites (sites 1 and 2) were situated on the eastern and western foreshores of Lake Burrendong (32°42′ S, 149°10′ E). The third study site (site 3) was situated at Larras Lee (33°17′ S, 149°06′ E) and the fourth site (site 4) at Murringo (34°18′ S, 148°32′ E). The two Burrendong sites were separated by a large enough body of water that we considered them to represent two distinct fox populations (later confirmed by radio-tracking studies).

Site topography ranged from hilly to undulating lowland, with elevations from 450 m to 800 m. The climate is typically temperate, with warm to hot summers and cool to cold winters. Temperatures range from a mean maximum of 32 °C in summer to a mean minimum of 0 °C in winter. Rainfall is generally reliable and

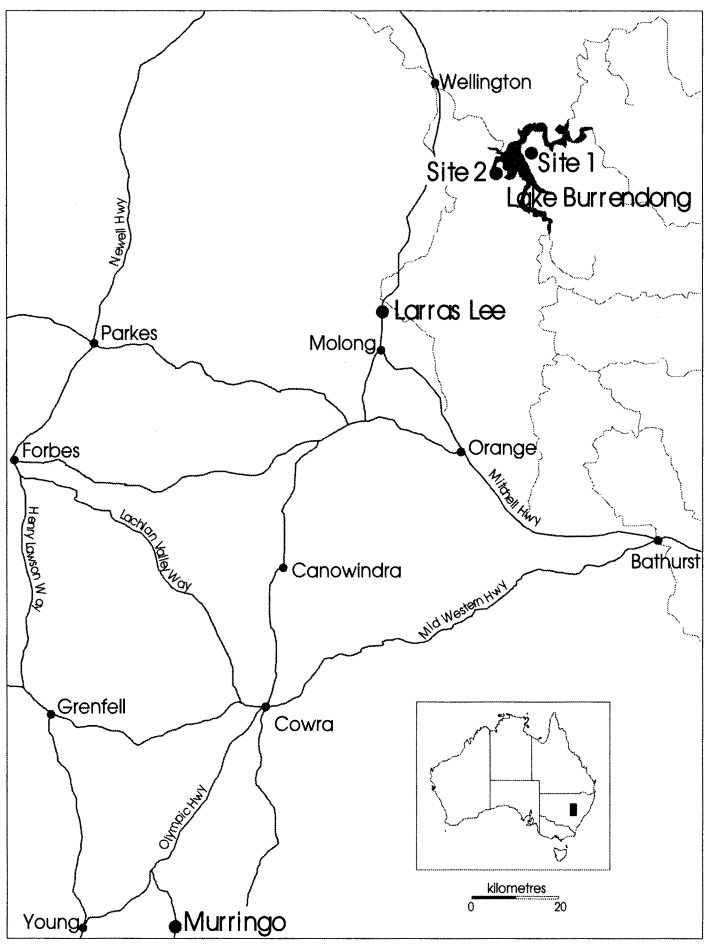


Fig. 1. Location of study sites.

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Fig. 2. Cumulative rainfall deficiency graphs for Burrendong, Molong and Young.

slightly seasonal, with a winter–spring dominance. Average annual rainfall across the region is within the range of 550–750 mm (Australian Bureau of Meteorology 1988). Rainfall for the period leading up to and during the study for the three site localities is presented as cumulative rainfall deficiency graphs (Foley 1957) in Fig. 2. Cumulative deviation of monthly rainfall from the mean of that month provided a description of rainfall trends at the sites using averaged data from the nearest weather stations (Lake Burrendong, Molong and Young).

The Burrendong sites on the Macquarie River consisted of dam foreshores that are managed for water catchment. This includes the use of grazing stock. The other sites were purely agricultural enterprises concentrating on the production of merino wool, prime fat lambs, beef cattle and winter cereal crops. Most of the grazing country has been cleared of native vegetation and, at Larras Lee and Murringo, pastures are improved with legume and *Phalaris* spp. Remnant vegetation consists of dry sclerophyll forest and woodland.

Apart from occasional recreational hunting, no fox control had been undertaken at any of these sites within the 10 years prior to project commencement.

FOX BEHAVIOUR

The project involved the study of fox behaviour with and without imposed sterility. Sites 2 and 4 (Burrendong 2 and Murringo) were selected for study of intact populations, while at sites 1 and 3 (Burrendong 1 and Larras Lee) vixens were caught and surgically sterilized by tubal ligation at a nearby veterinary clinic.

Rates of increase

Spotlight counts were conducted at all four sites along set transects (Burrendong 1, 23·8 km; Burrendong 2, 21·5 km; Larras Lee, 19·1 km; Murringo, 18·9 km) for three consecutive nights and at 3-monthly intervals. A four-wheel drive vehicle travelled at 5–10 km h^{-1} along set tracks at each site. An observer used a 100-W spotlight and counted all foxes seen on both sides of the track. The mean number of foxes per kilometre of transect in each session was then used as a seasonal index of relative fox abundance. Counts were conducted every season between July 1994 and October 1997 (Fig. 3).

Annual rates of increase were calculated using spotlight counts (foxes seen per transect kilometre) for each session. The log-transformed fox densities (Y) were then modelled using a linear mixed-effects model:

$$Y = \text{mean} + \text{season} + \text{day} + \text{site} + \text{treatment} \times \text{day} + \textit{site} \times \textit{season} + \textit{error}$$

where season, site and treatment (with and without imposed sterility) are factors with four, four and two levels, respectively, and day is a quantitative variable. Terms in italics were fitted as random effects. The error structure was an exponential correlation model (Diggle, Liang & Zeger 1994) fitted separately for each site. The models were fitted using ASREML (Gilmour *et al.* 1999). The purpose of this analysis was to determine if there was a significant difference in the rates of increase between the treatment sites (females sterilized) and the control sites (females intact).

Live capture and handling

Foxes were caught using Victor Soft-Catch™ traps (Woodstream Corporation, Lititz, PA, USA). These were set just below ground level and tethered to a 50-cm steel retaining peg that was driven 2–5 cm below ground level. The traps were set at irregular intervals along trails, farm roads, fence holes and at locations considered to be in suitable fox-trapping habitat. Single trap sets

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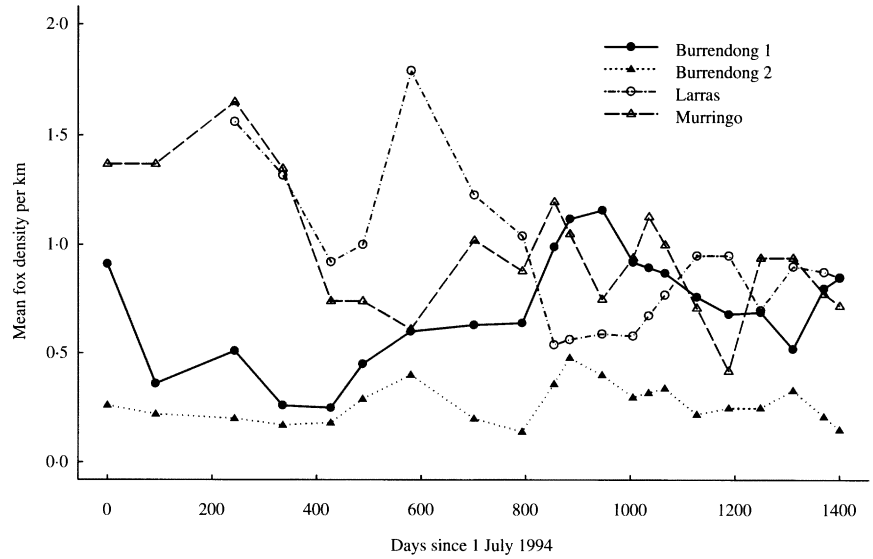


Fig. 3. Spotlight counts of foxes (mean density per transect kilometre) for the four study sites.

were baited with meat (rabbit, sheep, kangaroo) or lure (fox urine, fox faeces, synthetic fermented egg) or both. Multiple trap sets of between two and six traps were occasionally established around animal carcasses (sheep or kangaroo). Details of trapping procedures and results are described in Kay *et al.* (2000).

Trapping was restricted to a period prior to the onset of breeding (January–July) and was conducted in 1994, 1995 and 1996 at the Burrendong sites and in 1995 and 1996 at the Larras Lee and Murringo sites. Upon capture, foxes were anaesthetized with a mixture of ketamine hydrochloride (0·2 ml 5 kg^{-1}) and xylazine hydrochloride (0·1 ml 5 kg^{-1}). Each fox was marked with an individual tag number, and sex, weight and morphometrics were recorded. Foxes were categorized as juvenile or adult using the tooth eruption and wear technique described by Harris (1978). Any severely injured animals or those suffering from mange were killed immediately. At sites 1 and 3, healthy, adult, female foxes were transported to the veterinary clinic for sterilization. Each fox was given an intramuscular injection of penicillin as a post-operative prophylactic. Foxes were released at their point of capture only after recovery from anaesthesia (minimum of 12 h). Males and intact (non-sterilized) females were released sooner.

Sham operations

Early in this experiment it was considered necessary to ensure that the process of surgical sterilization did not have an adverse effect on the survival of study animals. At site 2, where no sterility was imposed, female foxes were trapped, handled and transported as they would have been for the purposes of sterilization. Sham operations were conducted that were identical to the sterilization procedure except that the fallopian tubes were left intact. During 1994 and 1995 we determined the fate of all sham-operated foxes fitted with transmitters. Only those that died of natural causes (excluding those shot, poisoned, killed on roads, etc.) were included in the analysis. Using a GLM procedure in S-Plus 2000 (1999), a logistic model was used to test for differences in the proportions dying. The model included effects for site, male vs. female, intact female vs. sterile female and sham vs. sterile female. This allowed a test of the latter effect after removing the earlier terms in the model.

Telemetry

Trapped foxes were fitted with a transmitter collar (150–151 MHz; Sirtrack™ Ltd, Havelock North, New Zealand) and released at their site of capture. Expected operational life of the collars was 60 months with a pulse rate of 60 beats min^{-1}. The weight of the collar and transmitter was approximately 140 g and only adult animals were fitted with collars. Radio-tracking was conducted from semi-permanent receiving towers located at suitable high points throughout the study sites. Tower locations were positioned accurately from known survey points and differential global positioning systems (GPS). Each receiving tower consisted of a weather-proof shed housing a 6-m high rotating mast fitted with either a single nine-element or twin five-element yagi antennae. Bearings to fox collars were determined using either a null–peak system or single arrays (Amlaner 1980).

Simultaneous radio-tracking was undertaken on an hourly basis for 6 consecutive hours following sunset and for four consecutive nights. These sessions were conducted at least once a month for the 6 months (July–December) incorporating the breeding season. Each fox could potentially be radio-tracked 144 times during this 6-month period. The majority of locations were determined from the triangulation of bearings from the fixed towers. The number of towers at each site depended on the topography, which in turn determined

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the coverage of foxes. At Burrendong four towers were sufficient to cover both sides of the dam (sites 1 and 2) and ensure that locations on foxes were nearly always determined from a minimum of three towers. Similarly, five towers were necessary at Larras Lee and four at Murringo. Locations were also derived opportunistically from the towers or by walking down foxes during daylight with hand-held antennae and taking locations with a GPS.

The accuracy of the fixed-tower system was determined at each site through the use of 'dummy' collars, which were placed at random locations and tracked each session. The locations, as determined by GPS, were regularly changed and were only known by one operator. These collars were fixed to 2-l plastic bottles filled with saline solution to mimic a fox fitted with a collar.

Telemetry data analysis

Bearings from towers were fixed as locations using Locate II© (Nams 1992). As a first step, obviously erroneous locations were discarded from the data set. These were identified from the size of the error ellipse (Nams 1992) and the lack of three or more convergent bearings. All reasonable locations were then entered into Ranges V© (Kenwood & Hodder 1996) for home range analysis. Home range areas were determined using the minimum convex polygon (MCP) method (Mohr 1947; Southwood 1966). This method produces a home range measure that does not explicitly involve the assumption of data independence (Swihart & Slade 1985). It is also a method that has been shown to give a realistic representation of a fox home range (Saunders *et al.* 1993). In our study it was necessary to understand intraspecific patterns of range use within and between sites. Core areas are more useful for this purpose than ranges represented by peripheral contours (Harris *et al.* 1990). Consequently, we used 95% and 60% MCP when comparing the home ranges of sterile and intact vixens. A logarithmic transformation was performed on the home range data, which were then analysed using standard linear mixed modelling procedures. The two variables, 60% MCP and 95% MCP, were analysed separately.

The initial model fitted in each case was:

$$Y = \text{mean} + \text{site} + \text{sex} + \text{year} + \text{site} \times \text{sex} + \text{site} \times \text{year} + \text{sex} \times \text{site} + \text{site} \times \text{sex} \times \text{year} + \textit{fox} + \textit{error}$$

where Y denotes the logarithm of the dependent variable (home range), sex denotes the three categories (male, intact female and sterile female).

Fox is included as a random effect associated with each animal and *error* is assumed to be distributed normally with zero mean and constant variance.

Least-square means for 60% MCP and 95% MCP across significantly different categories were then calculated. These means were obtained using the result that if $\log(X)$ is distributed normally with mean μ and variance σ^2, then the mean of X equals $\exp(\mu + \sigma^2/2)$. Standard errors of the estimated means were based on first-order Taylor series approximations.

Dispersal

Collared foxes that had moved off a study site were searched for opportunistically. This was usually done from a vehicle using the network of roads surrounding a study site. Some flights were also conducted for aerial tracking using the methods described in Saunders & Kay (1996). Every trapped fox was fitted with a numbered ear-tag that also included a contact telephone number. This resulted in some hunter returns from foxes collected further away from the study sites. Because the search for dispersing animals was biased, i.e. mostly adjacent to a study site, and with some hunter returns producing exceptionally long distances of travel, measurements of dispersal could only be indicative and not necessarily representative of the population.

The most important differences to be measured related to the probabilities of dispersal for sterile and intact females. Thus, the most consistent effort was concentrated in searching for dispersing animals at Burrendong (site 1) and Larras Lee (site 3). An analysis was performed on the proportions of collared foxes from these sites that were known to have dispersed compared with those that remained on site for sufficient time to presume that they were non-dispersers. Data for the two sites were combined.

The difficulty with this analysis is that a collar can fail or animals can move off site at a rate such that they are never again located. Animals that remained alive on the study site for a minimum of 12 months and were never located off-site (apart from short-term forays) were classed as non-dispersers. Animals that were found alive or dead completely off-site were classed as dispersers. Animals that died within 1 month of release were excluded from the analysis on the basis that their behaviour may have been potentially affected by some post-trapping trauma.

Survivorship

To determine if compensation in survivorship was occurring, we examined, using analysis of variance, the length of time that foxes survived (in days) after being fitted with a transmitter and, in the case of selected females, after they were sterilized. As with analysis of sham operations, we only used animals whose fate was able to be determined and excluded any interventions such as poisoning, shooting and road kills.

Results

During the 3 years of the study at all sites, a total of 348 foxes was trapped from 45 000 trap nights and fitted

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Table 1. Survivorship of foxes at Burrendong sites (1 and 2) in 1994 and 1995

Site	Sex category	Total	Died
Burrendong (1)	Intact female	6	1 (17%)
Burrendong (1)	Sterilized female	20	3 (15%)
Burrendong (1)	Male	26	3 (12%)
Burrendong (2)	Sham female	14	3 (21%)
Burrendong (2)	Male	22	1 (5%)

with radio-transmitters. As a consequence of emigration, death, collar failure or poor data sets, only 45% of foxes originally fitted with collars could be used in subsequent home range analysis.

SHAM OPERATIONS

Sham operations were conducted on 14 females at site 2 in 1994. All mortalities known to be caused by natural factors were recorded at both Burrendong sites (1 and 2) for all categories of fox throughout 1994 and 1995. Details of these foxes are presented in Table 1.

No site and sex category comparison was significant, including sham vs. sterile ($P = 0{\cdot}43$). Consequently, we concluded that surgical sterilization did not have any adverse effects on fox survival and that no further sham comparisons were necessary.

RATE OF INCREASE

After omitting the non-significant terms treatment × day and *site* × *season*, the final model used to examine rates of increase was:

$$Y = \text{mean} + \text{season} + \text{day} + \text{site} + \textit{error}$$

with Y = log density and the error structure as given in the Methods. Of primary interest is the coefficient of day, as this corresponds to the rate of increase (r) as defined by Caughley (1977). There was a significant ($P < 0{\cdot}05$) decline in fox density with day but there was no significant difference in the decline rate between the treatment and control groups. That is, the level of surgical sterilization of trapped females was not sufficient to have a significant effect on rate of increase.

Table 2. Foxes used in home range analysis

Site	Year	Male	Intact female	Sterile female	Total
Burrendong (1)	1995	9	5	7	21
	1996	6	8	10	24
Burrendong (2)	1995	11	8	–	19
	1996	7	5	–	12
Larras Lee (3)	1995	10	4	9	23
	1996	3	6	12	21
Murringo (4)	1995	6	11	–	17
	1996	7	11	–	18
Total		59	58	38	155

HOME RANGE ANALYSIS

Over all sites and years, the data from 155 foxes were used to determine ranging behaviour during the breeding season (July–December). This involved 15 545 accepted telemetry locations at an average of 100 (± 65 SD) fixes per fox. Details of these foxes are provided in Table 2. Bearing error across all sites ($n = 953$) was 3·49° (± 2·4° SD).

Omitting terms from the initial model that were not significant at $P < 0{\cdot}05$ left the following:

$$\log(\text{X60MCP}) = \text{mean} + \text{site} + \textit{fox} + \textit{error}$$
$$\log(\text{X95MCP}) = \text{mean} + \text{year} + \text{site} \times \text{sex} + \textit{fox} + \textit{error}$$

That is, for fixed effects, only site was significant for the 60% MCP, while for the 95% MCP there was a significant site × sex interaction as well as a uniform (on the log scale) year effect over all site and sex combinations.

Least-square means across significantly different categories for the two variables, 60% MCP and 95% MCP, averaged over years for the latter variable are presented in Table 3. This table also gives the least significant difference (LSD) rankings. From this table we can see that the sex × site interaction for 95% MCP was mostly due to the low range for intact females at the Murringo site. Without this effect it appears that, biologically, the home ranges of intact females and sterile females were similar. If site is considered as a random effect and site × sex effects, after removing the main effect for sex, are also considered random, then there are no significant differences (at the 0·05 level) between the three categories (males, intact females and sterile females).

Table 3. Estimated means home ranges (km^2) averaged over years (and over sexes for 60% MCP). Also given are standard errors of means in parentheses and LSD ranks for the 60% MCP and 95% MCP means separately. Means differing by more than twice the standard error of their difference are represented by different letters

		95% MCP		
Site	60% MCP	Male	Sterile female	Intact female
Murringo	0·82 (0·10)a	3·64 (0·67)b		1·86 (0·26)a
Larras	0·98 (0·11)ab	3·09 (0·57)b	4·37 (0·63)b	5·23 (1·12)bc
Burrendong 1	1·22 (0·13)b	3·97 (0·68)b	4·75 (0·75)bc	7·23 (1·33)c
Burrendong 2	1·26 (0·16)b	4·28 (0·67)b		4·38 (0·82)bc

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Table 4. The number of collared foxes at Larras Lee and Burrendong 1 classified as residents on site or that had dispersed. Mean duration that each fox was tracked after fitting with collars is presented in parentheses (months)

Released with collar	Stayed on site > 12 months	Dispersed	Fate not known
Female sterile (n = 54)	23 (25)	9 (28)	22
Female intact (n = 33)	9 (29)	10 (16)	14
Male (n = 66)	18 (25)	18 (13)	30

SURVIVORSHIP

Analyses of days of survival (log-transformed) by analysis of variance indicated that the six site × sex combinations (sterile females, intact females and males) did not differ significantly (P = 0·6). The mean numbers of days of survival for the 56 animals was 343 days, with a standard deviation of 273 days.

DISPERSAL

Hunter returns produced some extreme examples of dispersal from the animal's point of release. The longest of these was a straight line distance of 300 km south-west of the Murringo site. Another animal was shot 260 km north of the Burrendong site only 1 month after being released. However, these represent outliers, with the majority of animals only moving relatively short distances away from the study sites. Search effort was concentrated around the Burrendong (1) and Larras Lee (3) sites in an attempt to observe any differences in behaviour between intact and sterile females. Details for these sites combined are presented in Table 4.

Assuming that the foxes in the category 'fate not known' are missing at random, i.e. the proportion of dispersers amongst these is the same as all animals within the corresponding sex category, the estimates of the proportion of dispersers within each sex category are sterile female 28%, intact female 53% and male 73%. These proportions are not significantly different (P > 0·05) based on a binomial model. This analysis could be questioned because animals with an unknown fate are more likely to have dispersed (hence unable to be relocated) than stayed on site. If we now consider the extreme assumption that all animals with an unknown fate had dispersed, estimates of the proportion of dispersers within each sex category would be sterile female 57%, intact female 73% and male 73%. Again, these proportions are not significantly different (P > 0·05) based on a binomial model. The true situation probably lies somewhere between these two assumptions, indicating that sterile females are less likely to disperse than either intact females or males.

Discussion

The use of immunocontraception is highly appealing as a humane alternative to lethal forms of population control (Oogjes 1997). The strategy currently being developed is to target female foxes using a bait-delivered oral vaccine (Bradley, Hinds & Bird 1997), with attenuated *Salmonella typhimurium* as a candidate vaccine vector (de Jersey *et al.* 1999). This work remains in the developmental stage and to date the most popular means of predicting the outcomes of fertility control or immunocontraception for the management of foxes and other pest species has been through the use of theoretical models (Caughley, Pech & Grice 1992; Hone 1992; Saunders & Choquenot 1995; Barlow, Kean & Briggs 1997; Pech *et al.* 1997; Courchamp & Cornell 2000). The main problems with this process are the lack of reliable information on key population parameters and likely compensatory effects (Bomford 1990). Surgical sterilization is an alternative means of testing the concept of immunocontraception and has been used successfully in determining the level of infertility required to cause sustained reduction in the abundance of rabbits *Oryctolagus cuniculus* (Twigg & Williams 1999). Similarly, Ji, Clout & Sarre (2000) used it to test for behavioural effects of sterilization on possums and it is also the only means available to test for possible effects on the social behaviour of foxes (Robinson & Holland 1995). Newsome (1995) attempted to examine one form of compensatory behaviour (social control of breeding) using pen studies with surgically sterilized female foxes. His studies indicated no loss of dominance with sterilization and no compensatory breeding in their subordinates. In a field study using surgically sterilized foxes, Bubela (1995) found that in the first year sterile females maintained their home ranges and dominance and, when present, subordinate females did not breed. In the second year of the study, one sterilized female may have lost her position in the dominance hierarchy and allowed compensatory breeding in a previously subordinate female. While both of these studies yielded useful observations, throughout the majority of the fox distribution in Australia family groups consist of mated pairs, as dealt with in this study, and not complex hierarchical systems (Birchfield 1980; Saunders *et al.* 1995; Marlow *et al.* 2000; McIlroy, Saunders & Hinds 2001). Under these circumstances it is difficult to apply the assumptions of the various territorial hypotheses for foxes based on group living, namely the resource dispersion hypothesis (Macdonald 1983; Carr & Macdonald 1986), the constant territory size hypothesis (von Schantz 1984) and the territory inheritance hypothesis (Lindstrom 1986).

The home range of animals is generally considered to be proportional in area to the resources it contains (Harestad & Bunnell 1979; Lindstedt, Miller & Buskirk 1986), although many other physical and environmental attributes may be equally important (Stamps 1994).

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Various studies have confirmed that foxes, and other carnivores, living in habitats with abundant resources have relatively small home ranges and that territory size can be determined by metabolic needs (Gittleman & Harvey 1982; Saunders *et al.* 1993). The overall home range area (MCP) of foxes at all sites in our study was comparable with those observed in the only previous Australian study in temperate farmland habitats (Coman, Robinson & Beaumont 1991) and was also comparable to similar studies in the northern hemisphere (Tullar & Berchielli 1980; Zimen 1984; Cavallini 1992). Given that we concentrated radio-tracking effort mostly during winter and spring seasons, further comparisons with other longer term rural studies are not appropriate as home range size may vary outside these seasons.

The availability of food resources for foxes is likely to be most important during late summer and autumn, when climatically driven fluctuations in prey availability are greatest (Pech *et al.* 1997) and when female foxes need to accumulate maximum fat reserves in preparation for breeding (Winstanley, Buttemer & Saunders 1999). At all sites in the year prior to the commencement of this study (1994) there was a major drought during this critical period (Fig. 2). In 1995 and 1996, Murringo recovered from this drought while rainfall at Burrendong and Larras Lee continued to be depressed until the beginning of 1996. Between-site comparisons of core (60% MCP) and overall home range (95% MCP) (Table 3) suggest that Murringo foxes had the smaller territories while the remaining sites tended to be similar but larger. In our study, radio-tracking was concentrated on the period of the fox biological year, starting with mating (July) and ending with the independence of cubs from the natal den (December). Saunders *et al.* (1993) observed an increase in the distance travelled by females as a consequence of foraging for food on behalf of cubs, although this did not affect total home range size. If resources were in any way drought limited at the Burrendong and Larras Lee sites, female foxes may have needed to travel further afield to gather sufficient food to provide for their cubs. Although not significantly different, the predicted home ranges of intact foxes was always greater than those sterilized on the same site, which lends further support to this observation.

The above differences are subtle; however, they do tend to support the notion that the complexities in fox populations will change according to whether drought or plenty prevails (Newsome 1995).

Perhaps more importantly for possible compensatory behaviour under the effects of immunocontraception, there were no significant differences within sites in home range size at either 60% MCP or 95% MCP between sterile or intact females. Although intact female ranges were slightly larger, this was probably only associated with the added burden of provisioning of cubs as discussed above. From this observation we could arrive at the simplistic conclusion that a sterilized population would maintain normal territories but not have the increased demand on resources (and hence impact on prey abundance) associated with cub rearing. Females at all sites and years, regardless of reproductive status, were observed to maintain territories that were generally exclusive. Some exceptions (as observed in similar studies; Harris 1980) were created by mother–daughter relationships, brief forays outside traditional territorial boundaries, and areas of overlap created by possible errors in tracking locations (produced by geographical features, e.g. signal bounce). In 1995 we observed a deviation from this trend at Larras Lee in respect of sterile females where the extent of overlap was marked (Fig. 4). This may have been a manifestation of compensatory behaviour as a consequence of imposed sterility. The extent of territory 'stacking' could result in an increased population density of sterile females that may in turn favour reproductive females through greater resource availability in exclusive territories. Stacking may also result in no net change in predation pressure with a greater density of sterile females. That this trend was only apparent at one treatment site and in one year suggests that caution is needed in extrapolating this to a proven compensatory effect.

One form of compensation that may have a bearing on the use of immunocontraception is survivorship or longevity of treated females. Sterilization may mean that a female is able to survive longer without the stress of reproduction and provisioning of food for cubs. Survivorship of foxes in Australia has been shown to be affected by environmental conditions (Pech *et al.* 1992). It was fortunate in this study that environmental conditions at the two treatment sites (Burrendong 1 and Larras Lee) were similar so that differences in survivorship between intact and sterile females, for the duration of our study, could be more easily interpreted. What happens towards the end of the expected life span of foxes would require a longer study, although in a parallel demographic study of foxes only 20% of females live past the age of one (Saunders *et al.*, in press). No apparent compensation was occurring in this parameter, i.e. sterile females were not living longer. This was perhaps unexpected given the other observations about female territoriality. Whether or not this is a positive or negative outcome for immunocontraception is unclear. Intuitively, a population with a high proportion of sterile females maintaining stable territories and not producing cubs will have the least impact on prey species (excluding the possibility of territory stacking). The longer these females survive, the more stable will be the spatial organization and the less likely the immigration of new and fertile females into the population. Twigg *et al.* (2000) found that there was significant increased survivorship in sterilized female rabbits and concluded that this was advantageous as it would lead to a demographic shift towards older, sterile, females that maintain their position in the social hierarchy. Conversely, Jewell (1986) sterilized male

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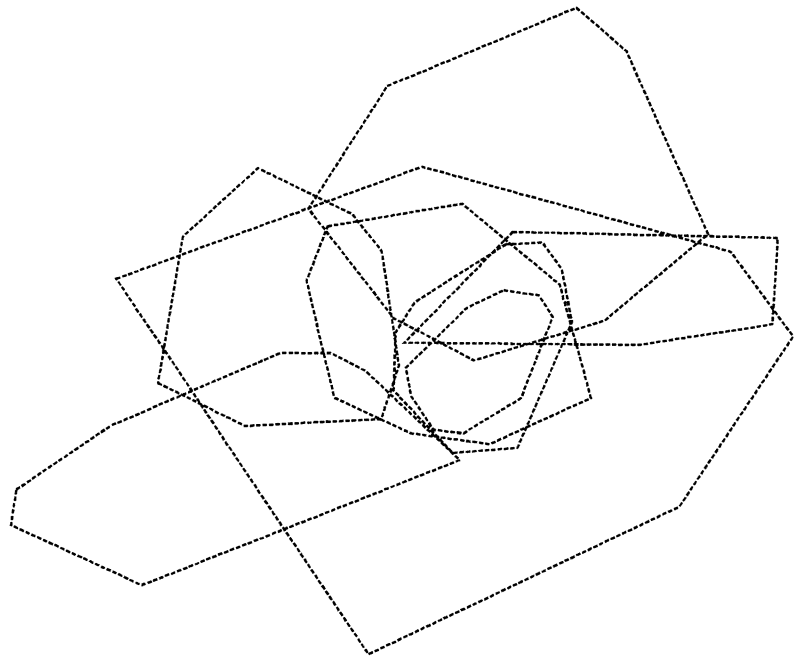


Fig. 4. Home range overlap (95% MCP) of sterilized vixens at Larras Lee in 1995.

Soay lambs *Ovis aries* and found that this too increased survivorship but also resulted in greater impact through increased grazing pressure. Tuyttens & Macdonald (1998) suggest that reduced mortality of sterilized animals will see the ratio of cubs to adults drop to the point where compensatory increases in fecundity are likely to become significant. However, Saunders & Choquenot (1995), based on a modelled population, estimated that to negate the effects of 75% sterility would require a 100% increase in both adult and juvenile survival and a doubling in the proportion of vixens breeding in their first year. The importance of survivorship in sterilized female foxes and the effect of this at the population level is obviously in need of further clarification. This may not be possible until other factors, such as optimum modes and frequency of delivery of sterility agents, are determined.

Like survivorship, dispersal (and migration) may also exert a significant influence on population parameters and the reduction of impact produced by an immunocontraceptive-treated fox population. In their study of factors affecting the dispersal of foxes, Harris & Trewhella (1988) found that approximately 30% of juvenile and adult female foxes dispersed in any one year. Such a mixing effect would soon negate the efficacy of a sterilizing agent, particularly if it were applied to site-specific fox populations. While not conclusive, the observations in this study suggest that sterile females are more likely to remain resident that intact females. More conclusive was the fact that sterilization did not result in a greater probability of dispersal.

Most of the findings from this study indicate that immunocontraception in free-living fox populations would be ecologically feasible. There are, of course, caveats, such as the absence of longer term effects on behaviour and minimization of recruitment into a treated population. There will also be many logistical considerations that may need to be overcome or at least accommodated. These potentially include integration with conventional (lethal) control operations, levels of bait uptake and rates of sero-conversion, cost-effectiveness, prey response and scale of operation necessary to minimize the effect of recruitment.

The ultimate proof of concept for immunocontraception will only be possible when a viable vaccine and vector are available for field testing. Studies such as the one reported here provide insight into the extent that compensatory behaviour might work against the efficacy of immunocontraception. They also provide the information needed on key parameters that will allow more precise theoretical modelling, the next best way of progressing the current debate on whether or not fertility control is a viable, humane, alternative to the lethal culling of carnivore populations.

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EDITOR'S MESSAGE

The Wildlife Profession's Duty in Achieving Science-Based Sustainable Management of Free-Roaming Equids

The special section in this issue of the *Journal of Wildlife Management* presents new science for application to sustainable management of free-roaming equids: feral horses (*Equus caballus*) and burros (*E. asinus*). The engagement of all stakeholders, particularly the wildlife biology community, is emerging as a critical need for management based on sound science.

HISTORICAL BACKGROUND

Equidae originated in North America approximately 10–20 million years ago (Hurlbert 1993, Kelekna 2009) and radiated across the steppes of Eurasia to Africa with multiple extinction and recolonization events facilitated by movement across the Bering Strait. Ancient equids included a broad assemblage of species, exhibiting the greatest species radiation of all North American ungulates (Bravo-Cuevas and Jiménez-Hidalgo 2019). Eventually, all equid species went extinct in North America 10,000–12,000 years ago during the late-Pleistocene epoch, likely due to a combination of climate and environmental change, disease, and the arrival of human hunters (Buck and Bard 2007, Olsen 2016). Horses survived on the steppes of Eurasia and continued to evolve, ultimately being domesticated for milk and meat about 5,500 years ago (Olsen 2016). Early cultures had an intimate association with horses, in which horse and human survival were closely intertwined (Levine 1999). Horse domestication is an important component of human history and significant in humans' emotional attachment to horses. Horses enabled cultures to disperse and advance agriculture, transportation, industry, commerce, and warfare (Olsen 2016). Domestication and artificial selection by humans for certain traits over thousands of years led to horses that were optimized for certain size, color, and reproductive characteristics (Librado et al. 2016). All domesticated and feral horses today differ genetically and phenotypically from their non-domesticated ancestors (Fages et al. 2019). Today's domestic horses are also morphologically different from their only extant wild relative, the Przewalski's horse (*Equus ferus przewalskii*; Groves 1994).

Domestic horses were first brought to North America in 1493 by European explorers and colonists (Haines 1938, Dobie 1952, Csurhes et al. 2009, Olsen 2016). Burros (also called donkeys) were domesticated from African wild ass (*E. africanus*) in Northeast Africa over 5,000 years ago and likely brought to North America about the same time as domestic horses (McKnight 1958; Beja-Pereira et al. 2004; Kimura et al. 2011, 2013; Olsen 2016). Some of these domesticated horses and burros escaped or were intentionally released by Native Americans, European settlers, the military, and others (Dobie 1952, Young and Sparks 2002). With the advent of the industrial age and a rapidly urbanizing and mechanized society, coupled with the relatively high cost of equid care and land, the demand for horses and burros by the military and individual interest in equid ownership declined (Garrott 2018, Scasta et al. 2018), resulting in an increase in intentional releases in the mid-twentieth century. As free-ranging horses became more visible across western rangelands, they were captured by mustangers and others who sold them for slaughter, re-sale, or other economic purposes (Danvir 2018). But individuals in the animal rights movement in the United States questioned the treatment of horses (and other animals; Singer 1975), eventually leading to passage of legislation to protect them. In 1971 the United States Congress passed the Wild Free-Roaming Horse and Burro Act (WFRHBA) and the Bureau of Land Management (BLM) and United States Forest Service (USFS) assumed statutory obligation to manage and protect free-roaming equids (wild horses and burros) in designated management areas (Public Law 92-195). Although all free-roaming equids in the United States are considered feral (wild descendants of domesticated animals; The Wildlife Society 2020), only the subset designated by the WFRHBA have federal legal protection. These include descendants of unclaimed, unbranded, free-roaming horses and burros present on BLM and USFS lands in 1971 when the Act was signed. The WFRHBA does not apply to most feral equids that inhabit tribal, state, private, and other federal lands.

Similar to North America, domesticated equids were introduced to South America and Australia where they also became feral. In the United States, horses and burros are the first and only feral animals that are federally protected (Public Law 92-195). Other countries such as Argentina and Australia have also enacted legislation to manage free-roaming equids in balance with other land uses (Western Australia Government 2016, Scorolli 2018).

The classification of domesticated equids as feral is biologically accurate but is nonetheless contested by some. Kirkpatrick and Fazio (2010) argued that free-roaming equids on designated public lands are a public resource, and because society already pays for the forage they use through taxes, the term feral has been incorrectly applied to them to favor domestic livestock permittees. Kirkpatrick and Fazio (2010)

suggested all free-roaming equids should be legally recognized as re-introduced native wildlife. Danvir (2018) countered that if feral horses were legally re-designated as wildlife, they would also be subject to hunting by the same state management authorities that regulate wild ungulate game species.

WORLDWIDE DISTRIBUTION AND ABUNDANCE

Feral equids are on every continent except Antarctica. In most places they have effectively few or no predators, circumstances requiring the need for management to control population growth and protect limited resources, similar to management of other large ungulates. Domestic horses, like other domesticated ungulates, have undergone thousands of years of artificial selection for high reproductive performance, meaning that feral populations have the potential to grow rapidly because females will sacrifice their own body condition for survival of their offspring (Grange et al. 2009) and mortality rates of horses tend to be low for multiple reasons (Garrott 2018, Boyce and McLoughlin 2021). The annual finite rate of increase for feral equids in Australia ranges as high as 1.36 (Dawson and Hone 2012), a remarkable rate of increase (36%) for a large ungulate with only 1 offspring per year. As a consequence, their density-dependent patterns differ from the classic Eberhardt and Siniff (1977) population model (Grange et al. 2009). This high reproductive rate coupled with high survival of horses is similar to management challenges posed by white-tailed deer (*Odocoileus virginianus*) in the eastern United States (Curtis 2020). We estimate conservatively the national population in the United States to be almost 300,000 horses across multiple land jurisdictions, with more free-ranging horses found on sovereign tribal lands today than on BLM and USFS lands (Table 1). In Australia there were an estimated 400,000 feral horses and over 5 million feral donkeys in 2011 (Commonwealth of Australia 2011), with large populations also found in South America. Population sizes of feral equids are staggering in comparison to global populations remaining of native wild equid species (Figure 1). Of the 7 extant wild equids (plains zebra [*E. quagga*], mountain zebra [*E. zebra*], Grevy's zebra [*E. grevyi*], kiang [*E. kiang*], Asiatic wild ass [*E. hemionus*], African wild ass, and Przewalski's horse) only 2 are not considered under threat of extinction (plains zebra and kiang; Moehlman et al. 2016). Population sizes of almost all wild native equids are declining due to anthropogenic effects such as poaching, habitat conversion, livestock monopolizing water sources, and drought, whereas population sizes of feral equids are growing (National Research Council 2013, Moehlman et al. 2016, Garrott 2018). The contrast is particularly stark for the closest wild relatives of domestic horses and burros; there are approximately 600 African wild asses (i.e., non-domesticated, non-feral) remaining in the world, and Przewalski's horses only remain extant due to captive breeding in zoos and subsequent reintroduction after becoming extinct in the wild (King et al. 2015).

PUBLIC OPINION AND POLICY

In the United States, the intent of the WFRHBA was to ensure healthy wild horse and burro populations in ecological balance with other multiple-uses on designated public lands. The WFRHBA as amended by the Public Rangelands Improvement Act of 1978 (Public Law 95-514) required the BLM to determine appropriate management levels (AMLs) for wild horses and burros on designated public lands. But currently, overabundant free-roaming equids are affecting rangelands, degrading ecosystem function, and reducing forage and water available for other wildlife (National Research Council 2013). The BLM and USFS have the authority to gather excess horses and burros in areas where populations exceed AML and where horse and burro health is compromised (Hendrickson 2018). But actions to manage free-roaming equids, particularly in the

Table 1. Population estimates of free-roaming (on range) and off range feral horses on different land jurisdictions in the United States, compiled May 2021, obtained from various sources for each land management jurisdiction.

Land jurisdiction or entity	Horse estimate	Date(s) of estimate	Source(s)
Tribal Nations	117,384	2020	T. Francisco, U.S. Forest Service (USFS), oral communication; Beever et al. (2018); Wallace et al. (2021)
Bureau of Land Management (BLM) on range	79,568	2019	BLM (2020)
BLM off range (in holding for public adoption)	52,832	21 Mar 2021	BLM (2021)
National Park Service (NPS)	1,606	2011, 2014, 2020, 2021	Oral communication with 20 NPS units; Powers (2014); NPS (2020)
Fish and Wildlife Service	150	2020	U.S. Fish and Wildlife Service (2021)
Forest Service	9,600	2020	T. Drotar, USFS Wild Horse and Burro Program Lead, oral communication
Department of Defense	1,645	2020	Oral communication via phone calls to military installations
State, municipality, and other (AZ, NM, NV, FL, KY, CO)	14,450	2020, 2021	J. French, Humboldt County NV Commissioner, oral communication; Paynes Prairie State Park FL; B. C. Lubow, Colorado State University, unpublished data; appalachianhorseproject.org (accessed 15 Jun 2021)
Total	277,235		

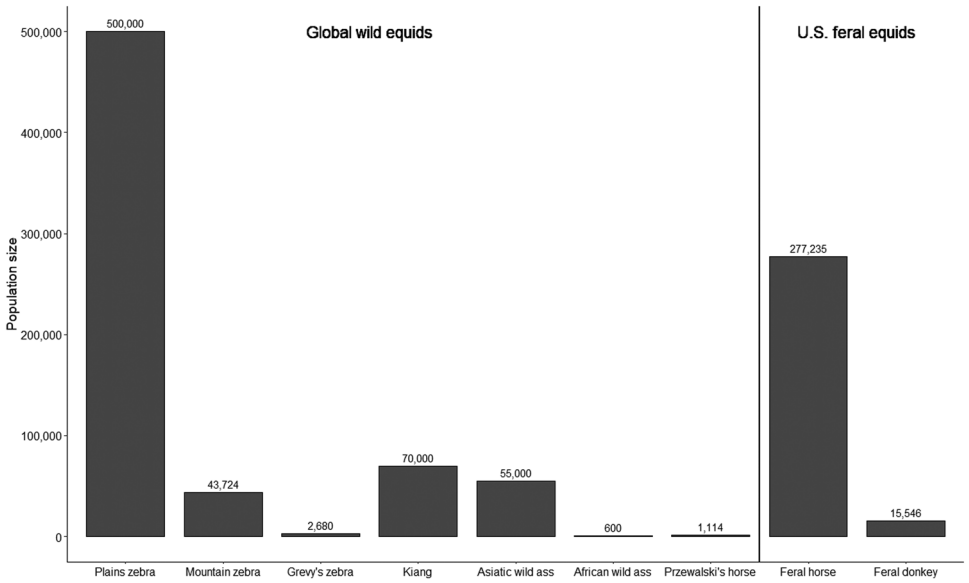


Figure 1. Population estimates of the 7 species of wild equids globally, and feral equids in the United States only. Population sizes are given above the bar for each species: plains zebra (King and Moehlman 2016), mountain zebra (Gosling et al. 2019), Grevy's zebra (Rubenstein et al. 2016), kiang (Shah et al. 2015), Asiatic wild ass (Kaczensky et al. 2020), African wild ass (Moehlman et al. 2015), Przewalski's horse (Rademacher et al. 2020), feral horse (Table 1), feral donkey (BLM 2020). Population estimates of wild equids are likely an over-estimate due to decreasing populations; population estimates of feral equids are a minimum count at best.

United States, are frequently impeded by litigation from groups who disagree with management decisions (Norris 2018). Once gathered, if animals are not adopted or sold under applicable legal limitations, the agencies must care for them for the remainder of their lives. In fiscal year 2020, BLM spent $57 million (62% of the $91.2 million spent by the BLM wild horse and burro program) to care for animals in holding facilities. The BLM acknowledges that public and congressional support will be required to achieve appropriate management levels (BLM 2018). Contemporary free-roaming equid management policies, which include administrative and political constraints on management (Norris 2018) suggest that horses and burros may be valued above all other animals in our society. Similar dynamics are emerging in other continents and countries (Nimmo et al. 2011, Scorolli 2018). In 2018, the New South Wales Threatened Species Scientific Committee, established under the Biodiversity Conservation Act (New South Wales Government 2016) identified habitat degradation and loss caused by feral horses as a conservation threat affecting hundreds of endemic species and millions of hectares of native habitats. Despite these documented negative ecological effects, public stakeholders maintain high regard for free-roaming equids (Nimmo et al. 2011, Frey 2021). Thus, as governments pursue free-roaming equid management options, success may hinge on actions that also develop, implement, and evaluate proactive public outreach programs that inform the public about tradeoffs or consequences that result from failure to deploy all management tools identified and available.

To achieve the sustainable management of free-roaming equids in balance with other societal needs and desires, managers and governments need to actively engage stakeholders in the decision-making process (Hewitt and Messmer 1997, National Research Council 2013). Recent coalition-building efforts facilitated by the Free-roaming Equid and Ecosystem Sustainability Network (FREES; https://extension.usu.edu/freesnetwork/, accessed 15 Jun 2021) provide new insights and approaches to re-engage diverse stakeholders in seeking solutions to difficult management issues. The FREES network is committed to facilitating open dialogue and building positive relationships that engage partners in collective actions to promote the health of free-roaming equids, western rangeland ecosystem health and sustainability, and principles of multiple-use. All FREES participants are encouraged to seek opportunities to work with others who might have different perspectives or experiences regarding the management of free-roaming equids and lands they inhabit.

MESSAGE TO WILDLIFE PROFESSIONALS

Wildlife professionals are uniquely qualified to contribute scientific expertise and information that could improve free-roaming equid management. Population dynamics, resource conflicts, and ecological interactions are a few topics in which our skills overlap with management needs. The human-dimensions aspects of sustainable management of feral equids are even less understood than ecological or biological issues (Scasta et al. 2018), requiring the attention of social science. Thus, we encourage all wildlife professionals working in areas with free-roaming equids to take an interest in their populations, habitat use, ecological effects, and interactions with other species. Much research remains to be done on horse and burro ecology and interactions with sympatric birds, mammals, and herpetofauna. It is necessary for wildlife professionals to include equids with observations and studies of native species, rather than ignore them as

non-wildlife, so we can understand their role in the ecosystem and scientifically assist with their management for the benefit of their habitats and the public. This is essential for sustainable management of these equids, which have statutory protection, and for the sympatric fauna and ecosystems they all inhabit. Wildlife professionals are poised to improve feral equid management by applying scientific research, translating research into informed management recommendations, and helping bridge the social and biological sciences.

In this special section, authors present a broad range of research on feral equids, including estimating space use and population size (Gedir et al. 2021, King et al. 2021, Schoenecker et al. 2021), population control in the absence of co-evolved predators (Andreasen et al. 2021, Boyce and McLoughlin 2021, Hinchcliffe et al. 2021), effects of feral equids on wildlife habitat and sympatric species (Burdick et al. 2021, Clancy et al. 2021, Coates et al. 2021, Hennig et al. 2021, Scorolli 2021, Stoner et al. 2021), and a promising new equid fertility control method (Holyoak et al. 2021). Although these papers provide techniques or approaches to manage feral equids, their application will vary by population, political jurisdiction, and country. We hope the studies in this special section are a step toward sustainability for this challenging and difficult wildlife management issue.

DISCLAIMER

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of Colorado State University or Utah State University. This article has been peer reviewed and approved for publication consistent with USGS Fundamental Science Practices (http://pubs.usgs.gov/circ/1367/).

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ORIGINAL CONTRIBUTION

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Effect of ovariectomy on bone histology and plasma parameters of bone metabolism in nulliparous and multiparous sows

Einfluß der Ovariektomie auf Knochenhistologie und Plasmaparameter des Knochenstoffwechsels bei nulliparen und multiparen Sauen

Summary To investigate the suitability of the pig as animal model for postmenopausal osteoporosis, effects of ovariectomy (OVX) on bone metabolism and histology were studied in two groups of sows (9 months, nulliparous or 35 months, multiparous). A standard diet of about 1.5 % calcium (Ca) was fed till sacrifice at either 12 or 20 months post OVX when mineral content and histology were studied in representative bone specimens of proximal tibia, iliac crest and lumbar vertebrae. At 4, 8, 12, and 18 months post OVX, total and bone-specific alkaline phosphatase (AP_t, AP_b) calcidiol, calcitriol and parathyroid hormone (PTH) were measured in plasma.

In young sows OVX did not significantly affect plasma variables except for calcitriol, which was higher at 4 months post OVX. No significant differences between OVX or control animals were observed in the variables of bone chemical and histological analyses, neither 12 nor 20 months post OVX.

In multiparous sows OVX significantly increased PTH plasma concentrations at 8 months post OVX and plasma calcitriol, AP_t and AP_b at 12 months post OVX. All effects were moderate and transient. OVX did not significantly affect the variables of bone chemical and histological analyses neither 12 nor 20 months post OVX.

Although undoubtedly the clinical-chemical changes observed were not accompanied by any histomorphometric signs of osteopenia/osteoporosis, it must be left to future experiments as to whether this resulted from the ample calcium supply provided. This possibility is supported by recent observations showing that porcine osteopenia could be induced by OVX in animals maintained on only 0.75 % dietary calcium but not on higher (0.9 %) Ca regimens (33).

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Zusammenfassung In der vorliegenden Untersuchung sollte die Eignung des ovariektomierten Schweins als Modell für die postmenopausale Osteoporose beschrieben werden. Hierzu wurde der Einfluß der Ovariektomie (OVX) auf Plasmaparameter des Knochenstoffwechsels und auf knochenhistologische Parameter bei zwei Gruppen von Sauen (9 Monate alt, Nullipara oder 35 Monate alt, Multipara) untersucht. Eine Standarddiät für Schweine mit einem Calciumgehalt von 1,5 % wurde verabreicht. Gesamt- und knochenspezifische alkalische Phosphatase (AP_t, AP_b) Calcidiol, Calcitriol und Parathormon (PTH) wurden im Plasma 4, 8, 12 und 18 Monate nach OVX bestimmt. Der Mineralgehalt sowie histologische Parameter wurden in repräsentativen Knochenproben 12 Monate nach OVX und am Ende des Versuchs 18 Monate nach OVX gemessen.

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Bei jungen Sauen führte die Ovariektomie zu keinen signifikanten Veränderungen der Plasmaparameter mit Ausnahme höherer Konzentrationen des Calcitriols 4 Monate nach OVX. Es wurden weder 12 noch 20 Monate nach OVX signifikante Unterschiede zwischen OVX- und Kontrolltieren in den Parametern der chemischen oder histologischen Knochenanalyse beobachtet.

Bei multiparen Sauen waren die Plasmakonzentrationen des PTH gegenüber den Kontrolltieren 8 Monate nach OVX signifikant erhöht. Die Konzentrationen des Calcitriols sowie die Aktivität der AP_t und AP_b lagen 12 Monate nach OVX signifikant über den Werten der intakten Tiere. Alle Effekte waren moderat. Weder 12 Monate noch 20 Monate nach OVX kam es zu signifikanten Veränderungen in den Parametern der chemischen oder histologischen Knochenanalyse.

Obwohl die beobachteten Veränderungen in den klinisch-chemischen Plasmaparametern zweifellos keine histomorphometrischen Anzeichen einer Osteopenie/Osteoporose zur Folge hatten, bleibt es zukünftigen Untersuchungen überlassen nachzuprüfen, ob das Ausbleiben des Osteopenie ein Ergebnis der in diesem Experiment hohen Calciumzufuhr war. Eine solche Möglichkeit sollte aufgrund der Beobachtung in Betracht gezogen werden, daß Osteopenie beim Schwein nach OVX eintritt, wenn eine Diät mit geringerer Calciumzufuhr (0,75 % im Vergleich zu 0,9 %) verabreicht wurde (33).

Key words Ovariectomy – pig – bone histology – calcitriol – parathyroid hormone – alkaline phosphatase

Schlüsselwörter Ovariektomie – Schwein – Knochenhistologie – Calcitriol – Parathormon – alkalische Phosphatase

Abbreviation index AP_t = total alkaline phosphatase · AP_b = bone specific alkaline phosphatase *BV/TV* = bone volume/tissue volume · *CV* = coefficient of variation · *OVX* = ovariectomy *PTH* = parathyroid hormone *RIA* = radioimmunoassay *TBPf* = trabecular bone pattern factor · $25(OH)D_3$ = calcidiol $1,25\ (OH)_2D_3$ = calcitriol

Introduction

Osteoporosis is a multifactorial disease of the skeleton with considerable prevalence in Western society and increasing relevance for developing countries. Postmenopausal osteoporosis – a specific form of this disease – has been related to estrogen deficiency (39). Symptoms of osteoporosis develop over a long time period. Controlled long-term experiments in humans to elucidate the etiology or possibilities for prevention and therapy of osteoporosis are difficult and often impossible to perform. Therefore one has to rely on suitable animal models.

Ovariectomy has been established as a method to simulate the hormonal status of postmenopausal women. In rats, ovariectomy resulted in changes of bone mass and bone structure comparable to postmenopausal women (49, 12). Still, there are basic differences in bone metabolism between both species. It has been accepted for a long time that in rats there is no epiphyseal closure although in a more recent study this has been questioned (15). Furthermore, in the rat, in contrast to humans, bone modeling prevails; almost no intracortical remodeling has been observed (13, 14). Whereas remodeling is characterized by a coupling of osteoblast and osteoclast activities, modeling implies the independence of osteoblast and osteoclast activities (14).

Other species-specific differences, e.g. no reduction of circulating osteocalcin following Vitamin K antagonist administration in rats (36) in contrast to humans (47) demonstrate some limitations of the rat as animal model in the field of bone metabolism.

With respect to bone metabolism pigs are closer to humans than rats are. Miniature pigs have been used as models to investigate bone mineralization in the neonatal state (40) and remodeling has been described (48, 26). Moreover, the general anatomy and physiology of the pig is very similar to humans (6) and in many respects, especially in nutritional physiology, the pig or miniature pig is regarded as a very suitable animal model with regard to humans.

This experiment was performed to investigate the suitability of the ovariectomized pig as a model for postmenopausal osteoporosis in women. Both gilts (young, nulliparous) and old multiparous sows were ovariectomized and compared with intact control animals. Clinical-chemical parameters relevant to bone metabolism, and chemical composition and histological structure of bone were analyzed. In analogy to the human postmenopausal state bone loss and a rise in AP_b were to be expected.

Material and methods

Animals and sampling

Two different groups of pigs (German Landrace) were used: group one consisting of 14 young sows with an average age of 9 months, group two consisting of 14 old sows with an average age of 35 months. The young sows were fertile but had never been pregnant (nulliparous). The number of pregnancies of old sows (multiparous) varied between 3 and 7. The mean body weights at slaughter were 230 kg and 300 kg for young and old sows, respectively (Table 1).

Table 1 Basic data of sows

	Nulliparous sows		Multiparous sows	
	OVX[1]	C[2]	OVX	C
Age[3] (months)	9 ± 0	9 ± 0	30.4 ± 2.3	39.7 ± 3.8
Litters (n)	–	–	3.8 ± 0.4*	6.1 ± 0.7
Body weight[4] (kg)	226 ± 20	232 ± 15	294 ± 12	318 ± 12
Uterus weight (g)	104 ± 9***	974 ± 105	221 ± 27***	1 404 ± 172

Mean ± SEM, n = 7; [1] ovariectomized; [2] control group; [3] at timepoint of ovariectomy; [4] at slaughter;
* $p \leq 0.05$; *** $p \leq 0.001$ significantly different from control group

Estrogen deficiency was induced by bilateral ovariectomy (OVX) in 7 animals per group at time zero. Permission according to the national law for animal welfare was obtained from the authorities. Four animals per group were sacrified 12 months, and the remaining 20 animals 20 months after OVX. Blood samples were taken after an overnight fast and always at the same time 4, 8, 12, and 18 months after OVX by puncturing the vena cava cranialis to follow the time-course of parameters related to bone- or calcium metabolism such as calcium (Ca), phosphate, total alkaline phosphatase (AP_t), bone-specific alkaline phosphatase (AP_b), parathyroid hormone (PTH), calcidiol ($25(OH)D_3$), and calcitriol ($1,25(OH)_2D_3$).

At slaughter, the uterus was removed and weighed to provide a measure of estrogen production. Representative bone specimens were taken for chemical and histological analyses: *Iliac crest* 1 cm below the spina ileaca anterior superior, a transiliacal specimen of 15–20 mm x 5–10 mm; *proximal tibia* about 5 cm distal from the eminentia intercondylaria, a specimen of 5–15 mm medial, and first and second *lumbar vertebra*, sagittal, a specimen of 5–10 mm central.

Diet

The animals were fed 2.5–3 kg of a standard diet for breeding sows, based on barley and soy, containing 1.5 % calcium and 0.9 % phosphorous. The composition was as follows (g/kg): barley: 500; oats: 60; wheat: 150; alfalfa green flour: 60; protein concentrate: 100; soy concentrate: 80; dry yeast: 20; mineral and vitamin premixture: 30. Free access to tap water, containing 20.2 mg calcium/L, was given. Water intake was about 8 L/d, as observed in sows in similar housing conditions.

Analytical methods

Plasma calcium was analyzed directly by atomic absorption spectroscopy (Perkin Elmer 1100) using an air/acetylene-flame at 2 300 °C after dilution with a 0.5 % lanthanium chloride solution. Phosphate was estimated in EDTA plasma as inorganic phosphorous with an automatic analyzer (Cobas Bio, Hoffmann La Roche, Basel, Switzerland) using a test kit (Boehringer-Mannheim, Germany). PTH was determined in EDTA plasma using a radioimmunoassay (RIA) (Amersham Buchler, Braunschweig, Germany). The kit contained an antiserum of guinea pigs directed against the C-terminal end, and a goat-anti-guinea pig-globulin as precipitation reagent. Values for sensitivity, intra- and inter-assay variability were 15.4 pg/L, 6.5 % coefficient of variation (CV) and 7.2 % CV, respectively. Calcidiol was measured in EDTA plasma by RIA (Immundiagnostik, Bensheim, Germany) after acetonitrile precipitation. Values for sensitivity, intra- and inter-assay variability were 2.5 nmol/L, 9.8 % CV and 14 % CV, respectively. Calcitriol was measured in EDTA plasma by RIA (Immundiagnostik, Bensheim, Germany). For this process the plasma was cleaned by Extrelut extraction. Subsequently, a separation from other vitamin-D metabolites was achieved by HPLC (41). Values for intra- and inter-assay variability were 12 % CV and 17 % CV, respectively. AP_t and AP_b were determined in heparinized plasma either directly or as the difference after lectin precipitation, respectively. The kit (Boehringer-Mannheim, Germany) was based on a kinetic color reaction. Values for intra- and inter-assay variability were 1.55 % CV and 2.5 % CV, respectively.

The *chemical composition of bone* was analyzed in representative samples of iliac crest, tibia and 1st lumbar vertebra. Bone specimens were dried for 4 h at 105 °C to determine dry matter, and consequently ashed in a muffle furnace at 450 °C overnight. The ash was dissolved in 20 % (v/v) hydrochloric acid. Ca was analyzed by atomic absorption spectroscopy using an air/acetylene-flame at 2 300 °C (Perkin Elmer 1100) after dilution 1:1 000 with 0.5 % lanthanium chloride solution. Inorganic phosphorous was estimated with an automatic analyzer (Cobas Bio, Hoffmann La Roche, Basel, Switzerland) using a test kit (Boehringer-Mannheim, Germany).

For *histological analyses*, defined bone specimens of the 2nd lumbar vertebra, the iliac crest, and the proximal tibia were cut with a diamond saw, fixed in buffered formalin (4 %) and embedded in epon. The embedded material was cut with a microtome to 5 µm-thick sections.

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These were stained with a modification of van Kossa technique which results in a black staining of mineralized tissue and a red staining of osteoid. The method was chosen because it allows an automatic image analysis by means of a video-based automatic image analysis system (IBAS II; Kontron, München, Germany) after a 10-fold magnification. Bone volume/tissue volume (BV/TV) and connectivity of trabeculae, i.e., trabecular bone pattern factor (TBPf) were analyzed. For calculating TBPf the spongiosa was assessed for bone area (A) and bone perimeter (P). The TBPf is the ratio of two measurements, before (1) and after (2) dilatation: TBPf (mm^{-1}) = (P1-P2)/(A1-A2). This value can be positive, if mainly convex forms prevail, i.e., isolated trabeculae are predominating. A negative value indicates mainly concave forms, i.e., a good connectivity between trabeculae. The method has been described in detail (19).

Statistics

The statistical evaluation was based on analysis of variance using the program "Statgraphics" (Vers. 5.0). Age, ovariectomy and the interaction of both were taken into account as fixed effects. The significance of differences was tested using an unpaired, two-tailed *t*-test (for AP, a one-tailed *t*-test was used). Since the time point of necropsy (12 months or 20 months) had no effect on bone parameters, analysis of variance for chemical and histological bone parameters (BV/TV and TBPf) was done on pooled samples of both time points. Means and standard error of the mean (SEM) are given throughout.

Results

In Table 1 some basic parameters of the experimental groups are given. Significant differences between the ovariectomized and the intact sows were observed in uterus weight, indicating a complete OVX.

In Figs. 1 and 2 the time-courses of clinical plasma parameters of multiparous old sows are given. Ca and phosphate plasma levels are nonspecific parameters regarding primary osteoporosis. Usually they remain constant unless a secondary form of osteoporosis occurs. In the present experiment OVX did not affect plasma calcium and phosphate plasma concentrations in old sows. Calcidiol tended to be elevated following OVX. However, plasma calcitriol and alkaline phosphatase (AP_b and AP_t) were significantly increased at 12 months post OVX, and PTH at 8 months post OVX.

In nulliparous sows OVX did not affect plasma parameters, except for calcitriol, which was significantly higher 4 months post OVX, and PTH which tended to be higher most of the time compared to control animals (Figs. 3 and 4). Compared to multiparous sows the age-dependent decrease in AP observed in growing minipigs

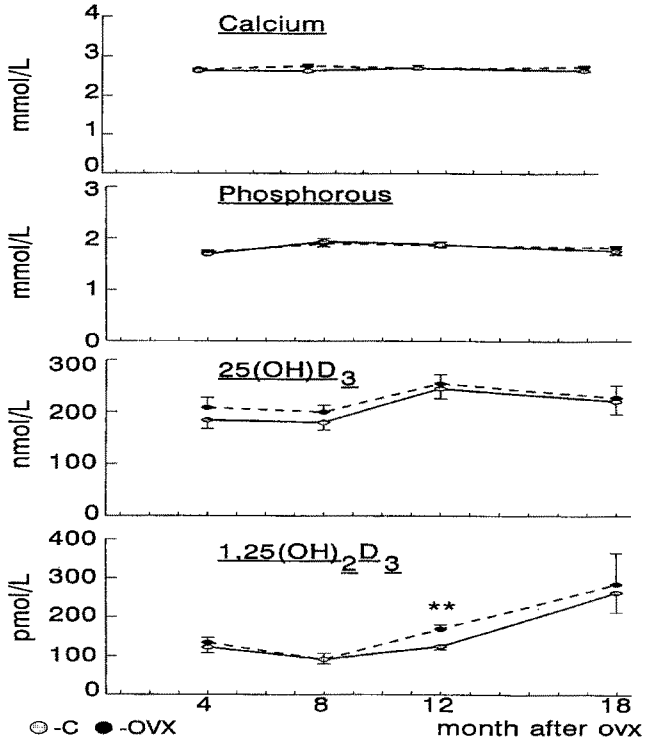


Fig. 1 Time-course of calcium, phosphorous, calcidiol, and calcitriol in plasma of ovariectomized multiparous sows. Mean and standard error of the mean, n = 7, resp. 5 at month 18; **: $p < 0.01$.

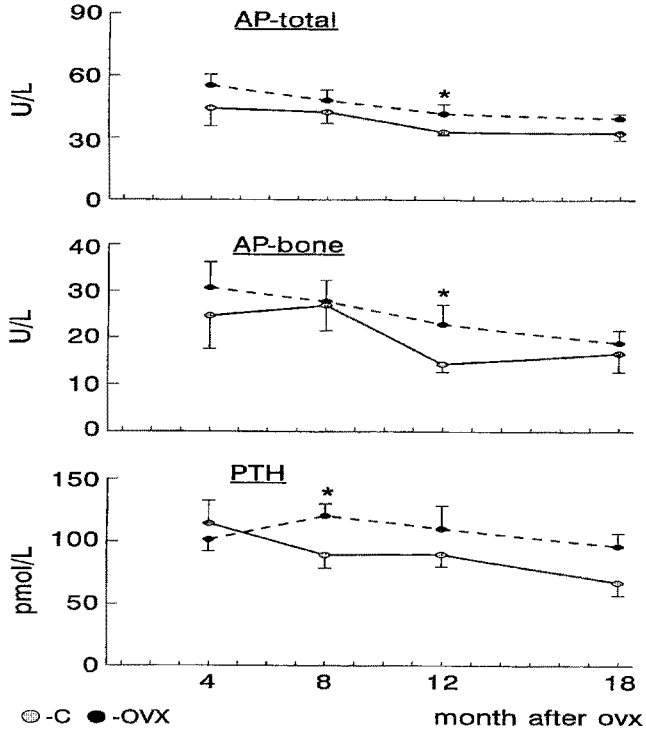


Fig. 2 Time-course of total alkaline phosphatase, bone specific alkaline phosphatase, and parathyroid hormone in plasma of ovariectomized multiparous sows. Mean and standard error of the mean; n = 7, resp. 5 at month 18; *: $p < 0.05$.

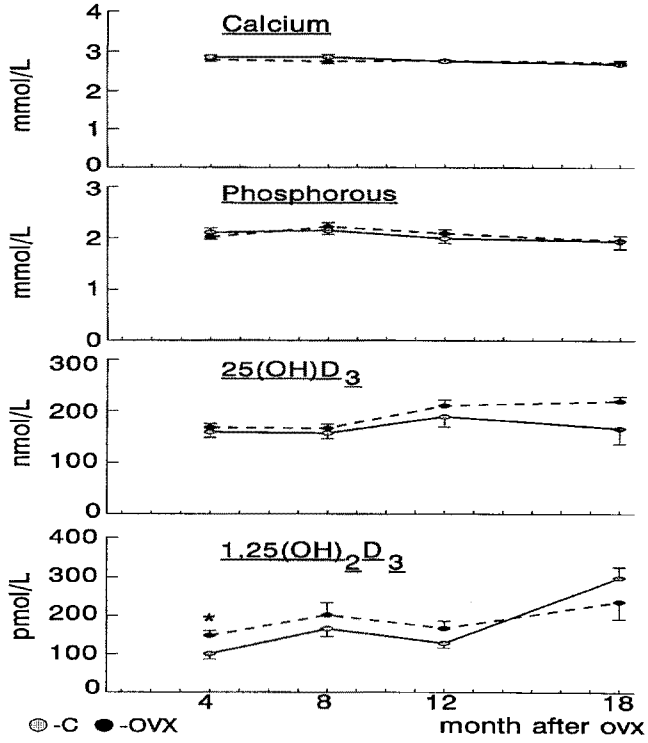


Fig. 3 Time-course of calcium, phosphorous, calcidiol, and calcitriol in plasma of ovariectomized young nulliparous sows. Mean and standard error of the mean; n = 7, resp. 5 at month 18;
*: $p < 0.05$.

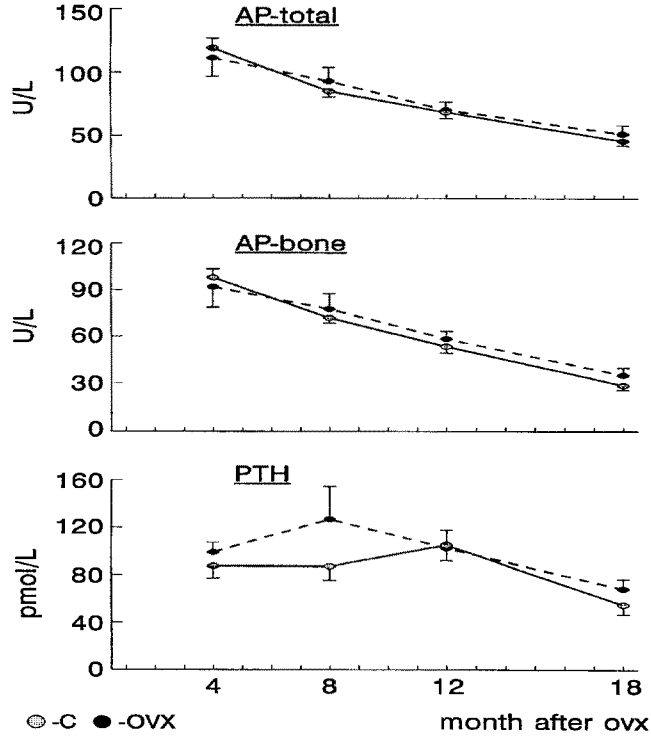


Fig. 4 Time-course of total alkaline phosphatase, bone specific alkaline phosphatase, and parathyroid hormone in plasma of ovariectomized young nulliparous sows. Mean and standard error of the mean; n = 7, resp. 5 at month 18.

(30) was more marked in these younger nulliparous animals following time according to their higher growing rate.

Multiparous sows tended to have less dry matter in the iliac crest and tibia following OVX as compared to intact animals. In total, however, OVX did not affect the parameters of bone density: Dry matter, ash, calcium and phosphorous as % of wet weight were not significantly different in iliac crest, tibia or lumbar vertebra compared to control animals. This was true for old multiparous (Table 2) and for young nulliparous sows (Table 3).

The results of the chemical analyses were supported by histological analyses. A normal architecture of spongiosa and trabecular connectivity was observed at the proximal tibia and ileac crest (not shown), and the 2nd lumbar vertebra (Fig. 5).

Signs for increased osteoclastic resorption were not observed and there was no increase in osteoid. Differences in structure of the 2nd lumbar vertebra between intact and OVX nulliparous and multiparous sows were not observed: The spongiosa in specimens of OVX animals showed a normal architecture (TBPf) and bone vol-

Table 2 Parameters of bone mass and bone density of iliac crest, proximal tibia and 1st lumbar vertebra of ovariectomized multiparous sows

	Ileac crest		Tibia		1st Lumbar vertebra	
Parameter	OVX[1)]	C[2)]	OVX	C	OVX	C
Dry matter (%)	77.6 ± 1.4	81.6 ± 1.0	88.6 ± 0.8	90.0 ± 0.7	77.2 ± 1.1	74.1 ± 1.0
Ash (% ww[3)])	37.0 ± 1.2	37.7 ± 2.2	43.5 ± 1.8	41.9 ± 0.8	42.8 ± 0.4	43.5 ± 1.6
Ca (mg/g ww)	137.4 ± 3.8	136.1 ± 8.3	152.2 ± 7.8	152.0 ± 3.1	146.7 ± 3.7	151.0 ± 3.5
P (mg/g ww)	57.5 ± 1.9	58.2 ± 3.8	69.6 ± 2.3	65.6 ± 1.2	68.1 ± 1.2	69.2 ± 1.8

Mean ± SEM; n = 7; [1)] ovariectomized; [2)] control group; [3)] wet weight

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Table 3 Parameters of bone mass and bone density of ileac crest, proximal tibia and 1st lumbar vertebra of ovariectomized nulliparous sows

	Ileac crest		Tibia		1st Lumbar vertebra	
Parameter	OVX[1]	C[2]	OVX	C	OVX	C
Dry matter (%)	74.8 ± 1.8	71.1 ± 1.0	90.7 ± 2.6	90.1 ± 2.1	72.1 ± 0.5	73.7 ± 1.8
Ash (% ww[3])	32.6 ± 2.1	31.8 ± 1.2	44.3 ± 3.5	42.9 ± 1.8	40.4 ± 0.5	42.0 ± 1.2
Ca (mg/g ww)	120.6 ± 7.6	116.2 ± 4.3	163.8 ± 14.2	160.3 ± 7.4	144.4 ± 2.5	144.8 ± 3.1
P (mg/g ww)	51.8 ± 3.4	50.0 ± 2.1	71.6 ± 5.2	78.0 ± 4.7	65.1 ± 1.0	67.4 ± 1.5

Mean ± SEM; n = 7; [1] ovariectomized; [2] controlgroup; [3] wet weight

ume (BV/TV) (Table 4). This was true 12 and 20 months post OVX. Signs of degeneration were visible in multiparous (M) vs nulliparous sows as an effect of age and/or multiple lactations.

Table 4 Bone volume (BV/TV)[1] and bone structure (TBPf)[2] of 2nd lumbar vertebra in sows following ovariectomy

	BV/TV	TBPf
	(%)	(mm^{-1})
N[3]/OVX[4]	31.88 ± 2.28	-1.44 ± 0.22
N/C[5]	35.46 ± 1.92	-1.54 ± 0.19
M[6]/OVX	30.55 ± 1.92	-1.33 ± 0.19
M/C	32.65 ± 1.92	-1.38 ± 0.19

Mean ± pooled SEM, n = 7 except group N/OVX where n = 5; [1] bone volume/tissue volume; [2] trabecular bone pattern factor, for details see "methods"; [3] nulliparous; [4] ovariectomized; [5] control; [6] multiparous. There were no significant differences between groups

Discussion

In the present experiment we investigated whether the ovariectomized sow is a suitable model for postmenopausal osteoporosis in women. The criteria selected were clinical-chemical plasma parameters and the chemical and histological analyses of representative bone specimens. A decrease in bone mass and – structure was to be expected accompanied with a rise in AP_b in case that a high turnover osteoporosis had occurred.

We observed that OVX had no significant effect on clinical-chemical plasma parameters in *nulliparous* sows, with the exception of elevated calcitriol concentrations 4 months after surgery. Moreover, chemical and histological analyses of bone did not reveal significant differences between OVX and control sows. Consequently, nulliparous sows appear to be less suitable as animal model than multiparous sows for human postmenopausal osteoporosis. At the moment we have no explanation for the lower sensitivity of nulliparous sows to OVX compared with multiparous sows. In this connection it is of note that Spencer has described a lactational osteoporosis

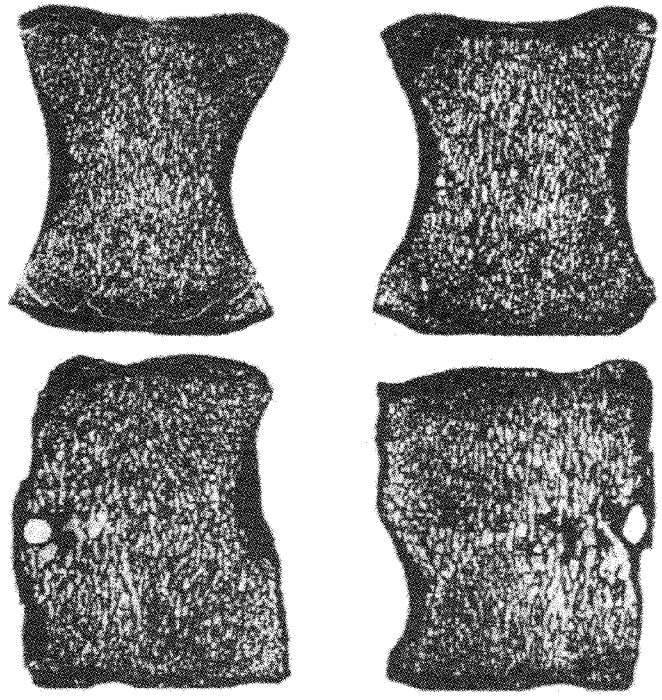


Fig. 5 Representative surface stained block grindings of undecalcified 2nd lumbar vertebra: Nulliparous sows (top) and multiparous sows (bottom); control (left), ovariectomized (right).

in pigs (44). In women, a correlation between the number of pregnancies or total time of breastfeeding and bone density is controversial (20, 22, 31).

In *multiparous* sows we observed a significant rise in plasma concentrations of PTH and calcitriol and in the activity of AP_t and AP_b as a consequence of OVX.

PTH stimulates the hydroxylation of calcidiol to calcitriol in the kidney. Furthermore, PTH stimulates osteoclast activity if it is administered in physiological doses (23) and therefore is regarded as indirect parameter of increased bone resorption, too (1). Elevated PTH concentrations have been observed by some authors in postmenopausal osteoporotic patients (46), and elderly people with hip fracture compared to healthy age-matched con-

trols (4), but not by others (17). In our experiment, PTH concentrations of multiparous sows were significantly increased 8 months after OVX and tended to remain higher for the rest of the experiment (Fig. 2). The chronological order of these changes could imply that OVX primarily stimulates PTH secretion in multiparous sows and as a consequence raises calcitriol concentration.

Calcitriol acts at different sites of calcium- or bone metabolism. Apart from stimulating renal calcium reabsorption and synthesis of calcium-binding protein in the intestinal mucosa, which means increasing calcium absorption (21), calcitriol also stimulates osteoclast activity (38, 28). In our experiment, multiparous old sows had significantly higher plasma concentrations of the biologically active metabolite $1,25(OH)_2D_3$, 12 months after OVX. The rise in calcitriol might also be related to estrogen reduction by OVX, since there is recent evidence that the calcitriol receptor of different organs is estrogen-regulated (10). In osteoporotic women conflicting results have been reported (16, 21).

It has been shown that patients with osteoporosis often suffer from a low vitamin-D status (2, 35). This could then result in a reduced calcium absorption rate contributing to osteoporosis. In our experiment there were no significant differences in plasma calcidiol between groups, indicating that no differences in vitamin D status caused the metabolic differences we observed following OVX.

Bone-specific alkaline phosphatase is a measure of osteoblast activity and positively correlated with the activity of these bone building cells. According to Delmas (9), increased activity of AP is an indicator of increased bone turnover. Total AP activity was elevated in old multiparous sows at all time points, significantly however only 12 months after OVX as compared to intact animals (Fig. 2). Bone-specific AP almost paralleled total AP. This finding agrees with AP activities in postmenopausal osteoporotic (37) or ovariectomized women (27, 43), but is in contrast to plasma AP concentrations of dogs following ovariectomy (29). The rise 12 months post OVX in the present study was of comparable magnitude as in women 12 months post OVX (45).

It is conceivable that the sum of the above changes in plasma concentrations of clinical-chemical parameters reflects an increase in bone turnover as a result of OVX. However, under these experimental conditions no significant changes either in bone mass or in histomorphometric parameters occurred. It is interesting to note that in one study reporting on premenopausal women following surgical ovariectomy, no significant decrease in bone mass was observed although plasma parameters of bone metabolism indicated a rise in bone turnover (43). No information on habitual or supplemented calcium intake of the patients was given.

At present, we are unable to answer our initial question with respect to the suitability of the ovariectomized pig as a model for postmenopausal osteoporosis in women. With regard to the chemical and histomorphometric bone analyses the pig may not appear to be a suitable model, since there was no osteopenia/osteoporosis following ovariectomy in our experiment, in contrast to primates (32), dogs (8), rats (49, 42) or even sheep (18).

However, the most probable explanation for our findings, namely a significant change in plasma parameters of bone metabolism indicating a rise in bone turnover but without symptoms of osteopenia, e.g. without decrease in bone mass or -structure may be the calcium content of the diet. The high intake of calcium, resulting from feeding a pig diet with 1.5 % Ca (this corresponds to 8 g Ca/70 kg body weight x day) may have prevented the demineralization of bone. The role of dietary calcium in preventing or postponing bone demineralization is controversial (34, 25). Differences in methodical approach (7) and difficulties assessing true calcium intake over a long period of time in investigations with humans (3) may contribute to these different data. In rats bone mineralization of ovariectomized animals on a high calcium diet (1.1 %) was not different from the intact control group on a diet containing 0.35 % calcium (5). We could confirm this positive correlation between calcium intake and bone development in growing and ovariectomized rats (24).

A recent study with ovariectomized minipigs (33) has shown that osteopenia also developed in this species, and that osteopenia was more pronounced in animals kept on a diet containing 0.75 % as compared to 0.9 % Ca in the diet. The observation that PTH did not rise following OVX (33) is in good agreement with our findings in nulliparous sows.

The calcium dependency of manifestation of osteoporosis as reported by Mosekilde et al. (33) can also be deduced from clinical observations: An intervention study in postmenopausal women has shown that a high calcium supply (1 or 2 g/d) reduced demineralization of bone. This dietary effect was more marked in late than in early postmenopausal women (11) indicating a higher susceptibility to dietary intervention at times not immediately following natural or artificial estrogen deficiency. In contrast to our experiment, all pigs were nulliparous and the time post OVX was only 6 months (33). Therefore a compensatory mineralization at longer times post OVX (i.e. 12–20 months) could have occurred in our study. In conclusion, the increased concentrations of plasma AP_b, PTH, and calcitriol leads us to hypothesize that ovariectomy initiated a rise in bone turnover in pigs. In this respect multiparous sows appear to be more sensitive than nulliparous animals. However, osteopenia may have been prevented by a high intake of calcium over a prolonged period of time.

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Satellite Article

Complications of castration

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Introduction

Castration is one of the most commonly performed surgical procedures of horses, and complications are the most common cause of malpractice claims against equine practitioners in the USA (Wilson and Quist 1992). In this issue (p 248), Boussauw and Wilderjans (1996) report an inguinal herniation occuring 12 days after a unilateral primary castration and on p 251, Fitch and Schumacher (1996) describe a case of infection of the spermatic cord.

To avoid castration complications, veterinarians should be aware of the types of complications that have been reported and should know the factors that predispose to their development and be able to recognise and resolve them.

Preoperative considerations

To minimise risks of complications of castration, the horse should be examined before surgery. The scrotum and inguinal canals should be palpated to detect the presence of inguinal herniation or the absence of one or both testes. Examination of foals for the presence of a congenital inguinal hernia prior to castration is especially important to avoid evisceration. Sedation usually permits the scrotum and inguinal areas of a fractious horse to be palpated safely and may facilitate palpation of an inguinally located testis. Discovery of inguinal herniation or cryptorchidism may influence the selection of anaesthesia and the technique of castration.

Castration can be performed as follows: 1) with the horse sedated and standing, after the spermatic cords and scrotum are anaesthetised or 2) while anaesthetised and positioned in dorsal or lateral recumbency. By performing castration with the horse standing, the risk of general anaesthesia is avoided and a clean, safe area to anaesthetise the horse is not essential. **For safety** of the surgeon and the horse, only docile stallions with well developed testes should be castrated while standing. Castrating small ponies while they are standing is mechanically difficult. If the surgeon intends to suture the scrotal incision, castration should be performed with the horse anaesthetised and recumbent.

Techniques of castration

Regardless of whether the horse is castrated while standing or anaesthetised and recumbent, the testes are removed using an open or closed technique. **A variation of the closed technique is the half-closed technique**.

Using the **open technique**, each testis is exteriorised through an incision into the parietal tunic (**Fig 1**). The ligament of the tail of the epididymis, which attaches the parietal tunic to the tail of the epididymis, is severed and the testis, its vasculature, epididymis and distal portion of the *vas deferens* are removed using emasculators. The parietal tunic and cremaster muscle remain with the horse.

Using the **closed technique**, the parietal tunic of each testis is freed from fascia, and the testis and parietal tunic that surrounds it are excised (**Fig 2**). Using this technique, a portion of the parietal tunic and cremaster muscle is removed. To perform the **half-closed technique**, each testis and its spermatic cord is freed from surrounding fascia (as in the closed technique), but before the spermatic cord and the cremaster muscle are severed, the parietal tunic is incised and the testis, its vasculature, epididymis and *ductus deferens* are exteriorised (**Fig 3**).

No controlled studies have been performed to demonstrate the superiority of one technique of castration over the others, but removing the parietal tunic may decrease the incidence of septic funiculitis and hydrocele, although these complications are relatively uncommon (Bishop *et al.* 1964). If the testis is affected by disease such as neoplasia or infection, the parietal tunic should be removed. Because the parietal tunic and cremaster muscle are included in the tissue crushed by the emasculators when using the closed or half-closed technique, haemostasis may not be as good as that achieved using the open technique. The open technique requires less dissection than does the closed technique and is, therefore, preferred by some veterinarians, especially when performing castration with the horse standing. The open technique allows the components of the spermatic cords to be examined before the cords are severed.

Scrotal incisions are usually left unsutured to heal by second intention but can be sutured to achieve healing by primary intention. If the scrotal incision is to be left unsutured, a generous portion of the bottom of the scrotum should be be removed to ensure good drainage so that excessive swelling is prevented (**Fig 4**). If the scrotum is incised over each testis, the incisions should be connected cranially and caudally and the portion of scrotum between the incisions removed.

Horses, whose scrotal incision is sutured, develop fewer complications, such as haemorrhage, infection, oedema and pain, but primary closure increases time of

anaesthesia and must be performed under strict aseptic conditions (Cox 1984; Barber 1985; Palmer and Passmore 1989). **Good haemostasis is a prerequisite to primary closure**. Primary closure of the scrotal wound may be indicated if vigorous exercise cannot be enforced following castration or if the spermatic cords are ligated, so that bacterial contamination of the ligature from an open wound is avoided.

Post operative treatment

Horses not previously immunised against tetanus should receive both tetanus toxoid and tetanus antitoxin. A horse previously immunised against tetanus should receive a tetanus toxoid booster if more than a year has passed since its last vaccination (Liefman 1981). Antimicrobial therapy is unnecessary if a clean technique is used and the horse is maintained in clean surroundings. Fly control is usually unnecessary if the horse's tail is long enough to reach the scrotum. To avoid haemorrhage, the horse's activity should be restricted for 24 h following castration but, after this time, the horse should be exercised vigorously to prevent preputial and scrotal swelling.

Complications

Excessive haemorrhage

One of the most common complications following castration is excessive haemorrhage and the usual source is the testicular artery (Cox 1987). The most common cause is use of an **emasculator that is in improper working order** or that is improperly applied.

Emasculators that are **too sharp** may cut the cord before it is properly crushed. Emasculators should be applied to the cord so that the crushing part is proximal to the cutting part and they should be applied perpendicular to the cord because transection of the cord other than at a right angle increases the diameter of the severed ends of the testicular vessels. Including scrotal skin in the emasculator's jaws not only traumatises the scrotum but also diminishes the effectiveness of the crush. When perfoming a closed or half-closed technique, large cords can be separated into 2 sections, one composed of the parietal tunic and cremaster muscle and the other of the testicular vessels and *ductus deferens*. **Each section is then crushed and transected separately**.

Blood from the scrotal wound following castration is normal, but if blood streams unabated for more than 15 min, haemorrhage should be considered excessive. To stop excessive haemorrhage, the cord can be grasped and recrushed with the emasculator, or a haemostat can be applied to the end of the cord and removed the following day. **A kidney clamp, because of its curved end, may be easier to apply than a haemostat**.

If the horse was castrated while standing, the spermatic cords and scrotum will still be locally desensitised, and recrushing the cord can be accomplished without anaesthetising the horse. If the the horse was castrated while anaesthetised, its scrotum and cord will be highly sensitive to manipulation and the horse may need, therefore, to be reanaesthetised. If the end of the cord cannot be grasped, haemorrhage can sometimes be controlled by packing the scrotum with gauze and temporarily closing it with sutures or towel clamps.

The author has administered dilute solutions of formalin (0.5–1.0%) i.v. to decrease haemorrhage caused by castration and observed marked reduction of bleeding. Diluted formalin has been administered i.v. to horses, with apparent safety, to treat *purpura haemorrhagica* (Roberts 1943), but there is no scientific evidence to support the efficacy or safety of i.v. administration to decrease haemorrhage. If measures to alleviate haemorrhage are unsuccessful, fluid therapy and blood transfusions may be necessary.

Preputial and scrotal oedema

Preputial and scrotal oedema is inevitable following castration and is usually greatest on the 4th or 5th day. Excessive oedema may occur if the scrotal wound becomes infected, especially if septic exudate becomes entrapped within the wound. A horse that develops excessive oedema can be treated by increasing the amount of exercise it receives and, if the scrotal wound is sealed, by opening the scrotal wound using scrotal massage or by inserting a gloved finger into the scrotal cavity. Administration of **nonsteriodal anti-inflammatory drugs** may decrease inflammation and increase the horse's tolerance to exercise. Lavage of the scrotum using a garden hose may be useful in keeping the scrotal wound from resealing.

Clostridial infection of the scrotal wound causes severe necrosis and toxaemia, but specific signs of clostridial infection vary according to the type of clostridial organism involved. Protection against infection by *Clostridium tetani* can be prevented by vaccination, but vaccination against other types of clostridial organisms is not routinely performed. Treatment of clostridial infection of the scrotal wound includes administration of high doses of penicillin and nonsteroidal anti-inflammatory drugs and radical debridement of infected tissue. Disease caused by *C. botulinum* and *C. tetani* can be treated with antitoxin (Muylle *et al.* 1975; Bernard *et al.* 1987).

Evisceration

Evisceration through the vaginal ring and open scrotal incision may follow castration if the horse has an inguinal hernia. **Evisceration can occur after several days, but most often occurs within the first few hours** (Hutchins and Rawlinson 1972). To avoid evisceration, the scrotum and inguinal regions, especially of the foal and of horses of breeds predisposed to congenital inguinal herniation, such as the Standardbred, should be palpated before castration to detect the presence of inguinal herniation.

If inguinal herniation is suspected, the vaginal rings should be examined per rectum or ultrasonographically. If the horse has an inguinal hernia, evisceration can be prevented by suturing the superficial

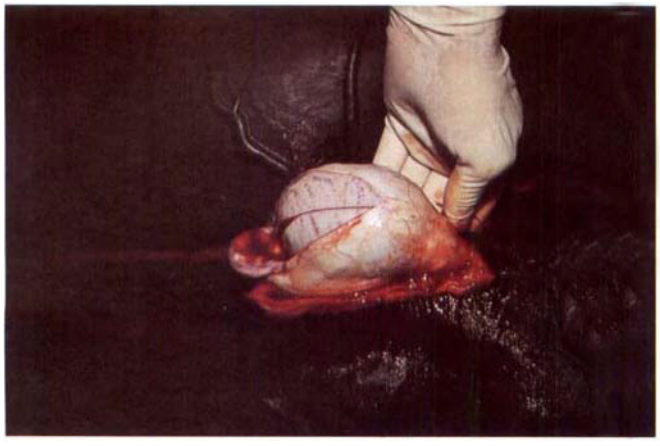


Fig 1: Using the open technique of castration, the parietal tunic has been incised to expose the testis, its vasculature, epididymis and distal portion of the* vas deferens, *which are removed using emasculators.



Fig 2: Using the closed technique of castration, the parietal tunic is not incised so that a portion of the parietal tunic and cremaster muscle, along with the testis, is removed.

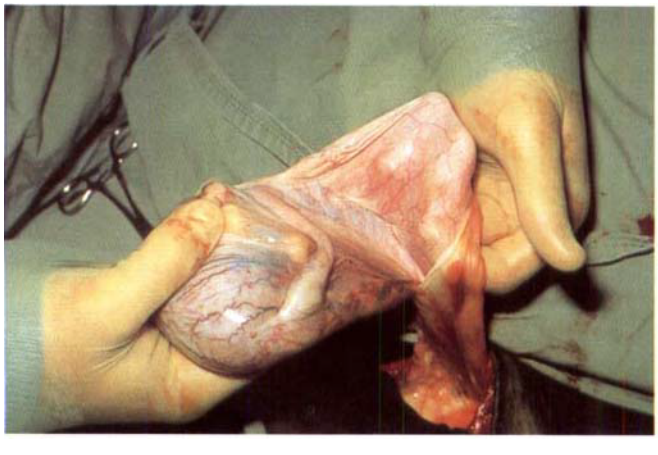


Fig 3: Using the half-closed technique of castration, each testis and its spermatic cord is isolated, but before the spermatic cord, its contents and cremaster muscle are excised, the parietal tunic is incised and the testis, its vasculature, epididymis and* ductus deferens *are exposed. *The surgical area is draped because the other testis was located abdominally.*



Fig 4: A large portion of the distal part of the scrotum should be be removed to permit good drainage.



Fig 5: Omentum protrudes from the scrotal wound following castration.



Fig 6: Chronic drainage from the scrotal incision following castration caused by bilateral septic funiculitis. *The horse is positioned in dorsal recumbency in preparation for surgery to remove the infected portion of the spermatic cords.*

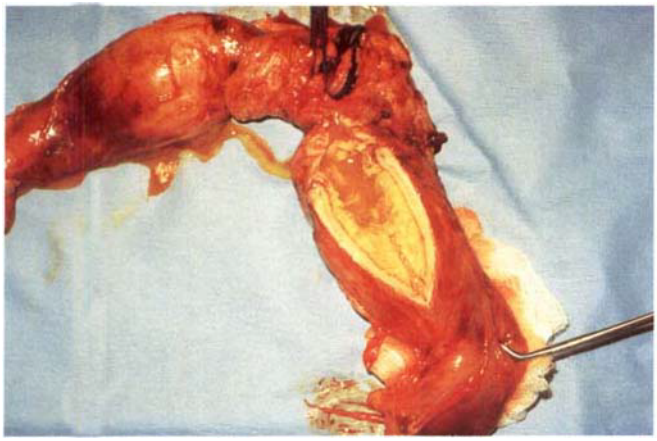


Fig 7: Bilateral, septic funiculitis following castration (see Figure 6). *Infected portions of the spermatic cords were removed. The parietal tunic of one cord has been opened.*



Fig 8: Bilateral hydroceles following castration causing horse's appearance to be that of an entire stallion.

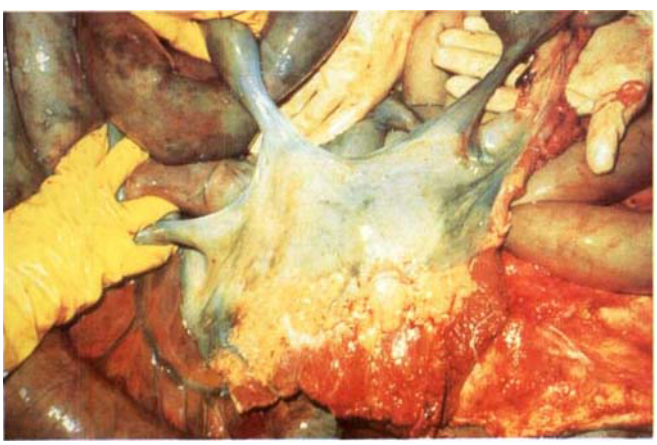


Fig 9: Adhesions, noted at necropsy, caused by septic peritonitis following castration.



Fig 10: Peritoneal lavage used to treat a horse suffering from septic peritonitis. *Ten litres of fluid, administered into the peritoneal cavity through a 32-French chest drain, are allowed to exit the abdomen by gravity.*



Fig 11: Penile paralysis and damage to the* corpus cavernosum *penis caused by administration of a tranquiliser to a stallion.

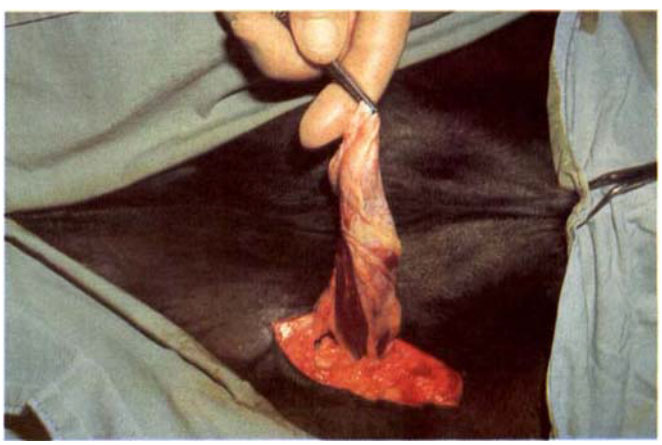


Fig 12: The epididymis is contained within the everted vaginal process of a partial abdominal cryptorchid stallion. *The tail of the epididymis may resemble a small testis to someone inexperienced in genital surgery.*

inguinal ring or by using the closed or half-closed technique and ligating the spermatic cord, including the parietal tunic, as proximal as possible with an absorbable suture. To avoid evisceration from inapparent inguinal herniation, the surgeon should consider perfoming a closed technique and ligating the cords when castrating horses under 3 months old.

A horse that has eviscerated following castration should be anaesthetised immediately and exposed intestine cleaned and replaced into the abdomen. To replace the intestine, dilation of the vaginal ring and traction on the intestine via celiotomy are usually necessary. Resection and anastomosis of devitalised and damaged intestine are usually best accomplished via celiotomy.

Re-evisceration can be prevented by packing the inguinal canal with gauze or, preferably, by suturing the superficial inguinal ring or by ligating the parietal tunic and its contents as far proximally as possible. The deep inguinal and vaginal rings are inaccessible for suturing, but the superficial inguinal ring can be easily sutured. Although intestine can still enter the inguinal canal, complications associated with suturing the superficial inguinal ring have not been reported. If the canal is packed with gauze to prevent re-evisceration, the vaginal ring should be palpated per rectum to ensure that the pack does not protrude into the abdominal cavity. Before the pack is removed, usually at 24–48 h, the vaginal ring should again be examined to ensure that intestine has not adhered to the pack.

Exit of omentum from the scrotal wound (**Fig 5**) is much less serious, and usually, the only treatment required is transection of exposed omentum, which usually can be done with the horse standing. The vaginal ring should be palpated *per rectum* to ensure that only omentum, and not intestine, has entered the inguinal canal. Because the presence of omentum in the scrotum has been reported to cause chronic scrotal swelling, some surgeons prefer to replace the omentum, following amputation of the exposed segment, into the abdomen with the horse anaesthetised (van der Velden and Rutgers 1990). If the omentum is replaced into the abdomen, the spermatic cord, including the parietal tunic, should be ligated, or the superficial inguinal ring should be sutured.

Septic funiculitis

Septic funiculitis (**infection of the spermatic cord**), is an uncommon complication of castration that can occur from extension of scrotal infection or, more probably, from a contaminated emasculator or ligature. Ligating the cords increases risk of septic funiculitis, if the scrotal incision is not closed primarily. Horses castrated using the open technique may be at more risk of developing septic funiculitis than are horses castrated using the closed or half-closed technique (Bishop *et al.* 1964). Clinical signs include pyrexia, lameness, and inguinal and scrotal swelling and drainage (**Fig 6**). Clinical signs may not appear for months or even years after castration. Treatment includes antimicrobial therapy and re-establishment of drainage. If infection is chronic, only excision of the infected portion of the spermatic cord will resolve the condition (**Fig 7**).

Hydrocele

A hydrocele, or vaginocele, is a painless, idiopathic accumulation of fluid in the vaginal cavity of stallions but can also occur in geldings months or years following castration (**Fig 8**). The swelling may resemble a scrotal testis or, even, scrotal hernia. Aspiration of the swelling produces a clear, amber fluid and by squeezing the scrotal sac, the fluid can often be reduced into the abdomen. Hydroceles occur uncommonly following castration but are most likely to occur in mules (White 1947). **The open method of castration predisposes to the condition**, because, using this technique, the parietal tunic is not removed. Treatment of a horse with a hydrocele is removal of the parietal tunic. This is best performed with the horse anesthetised and positioned in dorsal recumbency. The incision can be sutured or left to heal by second intention. Drainage, by aspiration or incision, only temporarily alleviates the condition.

Bacterial peritonitis

Because the vaginal and peritoneal cavities of horses communicate, subclinical, nonseptic peritonitis occurs in many horses following castration (Schumacher *et al.* 1988). Nucleated cell counts in the peritoneal fluid in excess of 10^9 cells/l can frequently be found for at least 5 days following castration, and counts greater than 10^{10} cells/l are common. Greater than normal nucleated cell counts in peritoneal fluid indicate the presence of peritoneal inflammation, and following castration, intra-abdominal haemorrhage may be responsible because free blood in the abdominal cavity can cause peritoneal inflammation (Shearman and Finlayson 1982).

Although peritoneal inflammation occurs commonly following castration, bacterial peritonitis is a rare complication (**Fig 9**). Peritonitis should be considered septic when the horse shows clinical signs of bacterial peritonitis and the peritoneal fluid contains phagocytised bacteria and a high nucleated cell count accompanied by the presence of degenerate neutrophils. Signs of bacterial peritonitis include pyrexia, tachycardia, diarrhoea and signs of colic. Treatment includes administration of antimicrobial and nonsteroidal anti-inflammatory drugs, fluid therapy, peritoneal lavage (**Fig 10**) and removal of the source of infection, such as an infected spermatic cord.

Penile damage

Penile damage is an uncommon complication of castration and usually occurs because the surgeon is unfamilar with genital anatomy and the surgical procedure. **The shaft of the penis can be mistaken by those inexperienced in castration for an inguinally located testis.** Damage to the penile fascia caused by dissection can result in paraphimosis and an incision into the urethra may result in a urethral stricture or fistula.

Penile and preputial damage can result from priapism or penile paralysis, either of which can occur following administration of a phenothiazine-derivative tranquiliser, such as acetylpromazine or propiomazine, prior to castration (Fig 11) (Bolz 1970; Schumacher and Hardin 1987).

Continued stallion-like behaviour

Castration does not always completely eliminate objectionable stallion-like behaviour. Geldings that display stallion-like behaviour are sometimes referred to as **'false rigs'** (Cox 1986). Purported causes of 'false rigs' include incomplete removal of epididymal tissue, heterotopic testicular tissue, production of high concentrations of androgens by the adrenal cortex and psychic causes.

A gelding in which a portion of the epididymis has been retained, which displays stallion-like behaviour, is sometimes referred to as **'proud-cut'** (Cox 1986). Leaving epididymal tissue with the horse is unlikely, however, because the epididymis is usually closely attached to the testis. The epididymis contains no cells capable of producing androgens, and, therefore, a portion left with the horse is probably incapable of causing stallion-like behaviour. Consequently, there is no such thing as a 'proud-cut' horse. The presence of heterotopic testicular tissue in horses has not been demonstrated and the production of androgens by the adrenal cortex has never been shown to be responsible for stallion-like behaviour of geldings. Objectionable sexual behaviour following castration is most probably pyschic in origin and is part of normal social interaction between horses (Cox 1986). Twenty to 30 per cent of geldings display some stallion-like behaviour, regardless of whether or not they were castrated before or after puberty (Line *et al.* 1985).

Amputating the stumps of the spermatic cords has been reported to eliminate objectionable stallion-like behaviour (Smith 1974), but because spermatic cords do not produce androgens, amputating cords to eliminate libido is, doubtlessly, ineffective (Cox 1986). Treatment of innate stallion-like behaviour following castration is limited to establishing stricter discipline and limiting interaction with other horses (Cox 1986). Owners should be forewarned about the incidence of continued stallion-like behaviour following castration so that undeserved blame for performing an improper castration is avoided. The excised testes and epididymides may be shown to the owner to eliminate the possibility of doubt by the owner that the horse was properly castrated in case the horse continues to display stallion-like behaviour .

Continued stallion-like behaviour can occur following improper castration of a **partial abdominal cryptorchid stallion**. A partial abdominal cryptorchid stallion is one whose epididymis, but not testis, has descended from the abdomen into the inguinal canal. The descended epididymis is contained in a well developed tunic and might resemble a small, inguinally located testis to a surgeon unfamiliar with genital anatomy (**Fig 12**). Naturally, if only the epididymis is removed, the horse will still retain the abdominally located testis and continue to display stallion-like behaviour.

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Why Do Some Castrated Horses Still Act Like Stallions, and What Can Be Done About It?

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Objectionable masculine behavior, such as penile erection, mounting, copulation, and aggression toward other horses or humans, is not always eliminated completely by castration.

PROPOSED CAUSES OF MASCULINE BEHAVIOR AFTER CASTRATION

Extragonadal Production of Androgens

A horse that has had both testes removed but still exhibits overt masculine behavior is sometimes called a *false rig*.[1] One cause ascribed to persistence of masculine behavior after castration is extragonadal production of androgens, and one alleged source of extragonadal androgens is the epididymis. Failure to remove all epididymal tissue during castration is sometimes credited for persistence of masculine behavior, and a horse that exhibits such behavior as a result is often referred to as being *proud cut*.[1] The epididymis is close and firmly attached to the normal, descended testis, making it unlikely to be accidentally left during castration. However, the epididymis neither produces nor releases androgens; thus the cause of masculine behavior should not be credited to failure to remove all epididymal tissue.[2] Therefore, there is no such thing as a proud-cut horse.[1]

Persistence of masculine behavior has also been attributed to testosterone production by the adrenal cortex stimulated by an increase in plasma concentration of luteinizing hormone that occurs in response to the decrease in plasma testosterone concentration after castration.[2] However, the serum concentration of testosterone or dihydrotestosterone in false rigs is no greater than that of geldings that display no masculine behavior[1]; therefore, persistence of masculine behavior after castration should not be attributed to testosterone production by the adrenal cortex.

Innate Behavior

Masculine behavior of a false rig should be attributed to innate behavior that occurs during normal social interaction between horses rather than to extragonadal production of androgens.[1,3] After castration, approximately 20% to 30% of geldings display masculine behavior toward mares and aggression toward other horses, and approximately 5% display aggression toward humans.[3] The prevalence of masculine behavior of horses castrated before puberty is comparable with that of horses castrated after puberty. Therefore, at the time of castration, owners should be advised that the procedure may fail to completely eliminate or prevent development of masculine behavior so that, if the horse continues to display some form of objectionable masculine behavior, a mis-

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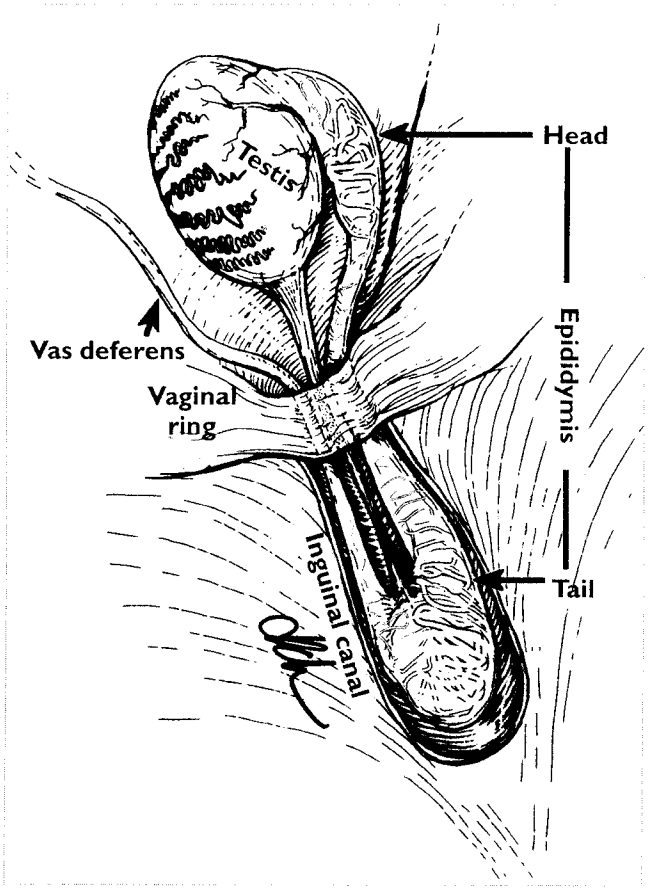


Figure 1. The appearance of a testis and epididymis of a partial abdominal cryptorchid stallion. A portion of the epididymis lies within the inguinal canal, enclosed in the vaginal process. An inexperienced surgeon could mistake the tail of the epididymis for a small inguinal testis and excise it. (©2005 University of Tennessee College of Veterinary Medicine)

conception about the surgeon's ability to perform castration properly can be avoided.

Incomplete Castration

Masculine behavior sometimes persists after castration because the surgeon has, by mistake, incompletely castrated the horse.[4] This occurs when the tail and a portion of the body of the epididymis of an abdominal testis has descended through the vaginal ring into the vaginal process and lies within the inguinal canal (i.e., the horse is a partial abdominal cryptorchid; Figure 1). The tail of the epididymis is improperly identified as a hypoplastic, inguinal testis and is excised. In my experience, the overlooked testis is usually the right one because the epididymis of a right abdominally retained testis is more likely to descend through the vaginal ring than is the epididymis of a left abdominally retained testis. The horse naturally continues to exhibit stallion-like behavior because it is still a stallion, but the owner and surgeon are convinced that both testes have been removed. Before excision of what appears to be an inguinal testis, the vaginal process should be incised and its contents examined to ensure that a testis, and not just a portion of the epididymis, is contained within.

DIAGNOSIS

The first step in determining the cause of persistent masculine behavior after castration is to establish whether the behavior is hormonally induced. Examination of each vaginal ring and surrounding area by per rectum palpation may be useful in determining whether failure to remove an abdominal testis is the cause of continued testosterone production and the persistence of masculine behavior. Per rectum palpation of an abdominal testis is irrefutable evidence that a horse has been incompletely castrated. However, palpating an abdominal testis is difficult because the testis is small and flaccid and an abdominal testis usually has a wide range of movement allowed by the elongated proper lig-

ament of the testis. An abdominal testis can often be palpated per rectum by an experienced examiner, but failure to palpate the testis should not be considered conclusive evidence that one does not exist. Likewise, per rectum palpation of a vaginal ring should not be taken as evidence that the testis has descended through the ring because the vaginal ring can also be palpated when only the epididymis has descended through it.

Ultrasonographic examination of the abdomen, performed per rectum or transabdominally, may be useful in identifying a cryptorchid testis.[5] To image an abdominal testis with the transducer inserted rectally, the abdomen should be examined in a "to-and-fro" pattern in the region of the vaginal rings as the transducer is advanced cranially.[5] To transabdominally image an abdominal testis, the transducer should be applied longitudinally to the inguinal region and advanced cranially in a to-and-fro pattern between the midline and the flank. Via ultrasonography, the testicular parenchyma appears as a spherical homogenous structure surrounded by a more echogenic testicular capsule.[5]

Hormonal assays may be necessary to determine whether masculine behavior after castration is hormonally induced. Horses with testicular tissue have significantly higher concentrations of androgens and estrogens than do geldings, and the concentration of these hormones in the plasma or serum can be used to distinguish between false rigs and horses with an inguinal or abdominal testis.[2,6–10]

In different studies,[1,2,6] the basal serum testosterone concentration in geldings was generally less than 40 pg/ml, and that of horses with testicular tissue was greater than 100 pg/ml and often greater than 1,000 to 2,000 pg/ml. The serum testosterone concentration in horses with testicular tissue depended on age and season, with the concentration being lowest in horses younger than 3 years of age and during the winter. In some studies,[2,6,7,9,10] the wide variation in basal serum testosterone concentrations caused the testosterone concentrations in geldings and in horses with testicular tissue to overlap, leading to error in interpretation. One investigator reported[7] a 14% error using basal serum testosterone concentrations to differentiate geldings from horses with testicular tissue.

Elevation in the serum testosterone concentration in response to administration of human chorionic gonadotropin (hCG) increases the accuracy of predicting the presence of testicular tissue to over 94%.[2,6,7] Serum is obtained for hormonal analysis before and at any time between 30 minutes and 2 to 3 days after intravenous administration of 6,000 or 12,000 U of hCG.[1,6,9] Horses are classified as having testicular tissue if the testosterone concentration increases (and exceeds 100 pg/ml) in response to hCG administration or as geldings if the concentration fails to increase (and is less than 40 pg/ml). The response to hCG administration is poorest during the winter and in horses younger than 18 months of age.[6]

The presence of testicular tissue is highly correlated with circulating concentrations of conjugated estrogen (i.e., estrone sulfate) in horses older than 3 years of age.[6,7] When horses younger than 3 years of age are excluded, the estrone sulfate concentration is about 96% accurate in predicting the presence of testicular tissue in horses of unknown castration status. An estrone sulfate concentration less than 50 pg/ml in plasma or serum indicates that the horse is a gelding, whereas a concentration exceeding 400 pg/ml indicates that the horse has testicular tissue.[7]

The most common causes of continued stallion-like behavior after castration are innate psychic behavior and failure to remove an abdominal testis during castration.

The standard values for normal hormonal concentrations in geldings and horses with testicular tissue may vary between laboratories, so knowing the laboratory's standards is important when assessing the results of a hormonal assay. Comparing test results with those of a known gelding may be necessary if the laboratory cannot provide standards.

TREATMENT

Approximately 30 years ago, shortening the stumps of the spermatic cords was reported to abolish objectionable masculine behavior in three-quarters of 18 false rigs, but the author of that report[11] offered no valid explanation to account for the apparent success of this procedure. The spermatic cords contain no tissue capable of producing androgens; thus shortening cords to eliminate masculine behavior of false rigs seems unlikely to be effective. Because objectionable masculine behav-

ior of false rigs is innate rather than hormonally induced, it is best eliminated or reduced by eliminating or limiting social interactions with other horses or by imposing firmer discipline.[1]

To permanently eliminate hormonally induced masculine behavior after incomplete castration, the abdominal testis must be located and removed. Finding a severed epididymis during exploration of the inguinal canal confirms the diagnosis of incomplete castration and identifies the abdomen as the location of the overlooked testis.[4] The retained abdominal testis can be removed either through the vaginal ring or through a parainguinal incision, but because the contralateral testis has been removed, the retained testis is likely to have undergone compensatory hypertrophy, making its removal through the vaginal ring difficult.

The abdominal testis of a partial abdominal cryptorchid can also be identified and removed laparoscopically, either with the horse standing or recumbent. Finding and removing the testis laparoscopically may be advantageous because the side of testicular retention usually cannot be determined definitively before surgery. The testis is located laparoscopically by inspecting the region surrounding the vaginal ring. The testis is more easily located when the horse is standing rather than recumbent because the viscera are less likely to obscure the vaginal ring and surrounding region. When the horse is standing, an abdominal testis is most commonly located cranioventral to the ring and the epididymis of a partial abdominal cryptorchid can be seen entering the vaginal ring. The contralateral vaginal ring and the region surrounding it can be observed by manipulating the laparoscope under the descending colon or by elevating the descending colon either with an instrument placed through an abdominal portal or with a hand inserted in the rectum.[12]

Immunization against either luteinizing hormone-releasing hormone (LHRH) or gonadotropin-releasing hormone (GnRH) can be used to temporarily suppress hormonally induced masculine behavior.[13-15] However, frequent immunization is necessary to maintain a sufficient titer to completely neutralize LHRH or GnRH and to inhibit the reproductive endocrine axis. Because stallions respond differently to immunization, it does not always totally suppress the libido.[14] A vaccine for immunization against LHRH or GnRH is not commercially available.

CONCLUSION

Stallion-like behavior after castration is highly unlikely to be caused by extragonadal production of androgens and is most likely to be caused by innate, psychic behavior or incomplete castration of a partial abdominal cryptorchid. Tests to determine whether the cause of persistent masculine behavior after castration is psychic or incomplete castration include per rectum palpation of the abdomen, ultrasonographic examination of the abdomen, and hormonal assay. A horse that displays objectionable, psychic masculine behavior can be treated by using stricter discipline or isolating the horse from other horses. To ameliorate objectionable masculine behavior in a horse that has been incompletely castrated, the retained testis must be removed via either a conventional surgical approach or laparoscopy. Hormonally induced masculine behavior can be temporarily suppressed by immunizing against either LHRH or GnRH, but these vaccines are not commercially available.

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Thanks a lot!

Ovariectomy in the Mare: Presurgical, Surgical and Postsurgical Considerations

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Ovariectomy in the mare is frequently indicated for removal of a pathological ovary. Presurgical considerations are: (1) nature of ovarian abnormality, (2) size of ovary to be removed, (3) temperament and tractability, (4) economical considerations, and (5) physical condition of the mare. Surgical planning should include a consideration of: (1) experience of surgeon, (2) anesthesia, anesthesia equipment, and surgical facilities available, (3) availability of selected surgical instruments, (4) preparation of mare for surgery, (5) laparotomy approach, and (6) technique of ovariectomy. Postsurgical care may include by necessity the treatment of the following: (1) hemorrhage, (2) shock, (3) abdominal pain (colic), (4) hematoma, abscess, granuloma development with adhesions, (5) peritonitis, (6) dehiscence of laparotomy incision with occasional herniation or evisceration, and (7) behavioral change of operated mare.

Removal of 1 or both ovaries in the mare (to ovariectomize or spay) has been employed for surgical correction of various ovarian conditions. The most frequent indication for removal of a pathological ovary is granulosa cell tumor.[3,4,14,15] Additional ovarian tumors reported in the mare include melanoma, epithelioma, cystadenoma, adenocarcinoma, and teratoma.[15] Less common indications for ovariectomy are: (1) ovarian abscess and hematoma, (2) ovarian cysts, (3) nymphomania, and (4) prevention of estrus.[4,14,15,16,17]

In the majority of tumor cases, ovariectomy is performed unilaterally for removal of the neoplastic mass, and the tumor is usually granulosa cell type.[3,4,8,14,15,17,18,19,21,22] In this paper the discussion centers around the surgical correction of ovarian tumors, except where otherwise indicated.

Granulosa cell tumors are functional androgen producers, and clinical alterations seen are related to changes in estrus, colic, infertility, lameness, abnormal muscular development, changes in external genitalia, and personality.[3,4,8,9,17,18,19,23,24] This tumor predominantly affects 1 ovary, rarely if ever metastasizes in the mare, and occurs primarily in the young mare.[12,15,16,19] A consistent physical examination finding of granulosa cell tumors is a slow but progressive increase in size. Tumorous ovaries, exceeding 20 kg in weight, have been successfully removed in certain cases.[16] Once a presumptive diagnosis of ovarian tumor is made, ovariectomy is the preferred surgical approach.[21]

Ovarian abscesses and hematomas may accompany neoplastic involvement or occur as primary entities. Rough handling of ovaries by rectal palpation and drainage of ovarian cysts by percutaneous or vaginal centesis are probable causes.[15] Hematomas, abscesses, and cysts may fluctuate considerably in size due to seasonal variation and iatrogenic factors. Ovariectomy is an elective procedure for this group of abnormalities in virtually all instances.

Spaying a mare for correction of nymphomania has had variable and inconsistent results.[3,14,17] Based on clinical observations of nymphomaniac tendencies only during estrus and "normal" ovarian size, bilateral ovariectomy has been reported as a satisfactory means of surgical correction. Conversely, the nymphomaniac mare with marked abnormal behavior that is chronic and constant in nature has not generally been responsive to bilateral ovariectomy.[14,17] This type of mare should be considered extremely dangerous and hazardous to the surgeon, handlers, and animals that come in contact with her. Humane destruction is frequently the end result of this case and the advisability of surgical intervention (ovariectomy) should be questioned for economical as well as therapeutic reasons.[17]

Although the physical attitude, personality, and various other clinical manifestations of ovarian tumor may be altered by ovariectomy, sterility may persist for the life of the mare.[2,3]

Presurgical Considerations

Nature of Ovarian Abnormality — A history of infertility, abortion, alterations of estrus, nymphomania, "mounting" of other mares, personality changes, lameness, masculinity, overdevelopment of external genitalia, and ovarian enlargement contains clinical signs and physical findings consistent with ovarian tumors.

Rectal examination of such a mare may reveal an enlarged ovary, with the opposite ovary normal. Palpation of small, firm, and atrophied ovaries is a consistent finding in the classical form of nymphomania. If the history includes a previous attempt to "tap" an ovarian cyst, careful palpation per rectum may reveal adhesions, abscessation, or fluid-filled structures.

Radioimmunoassays can be performed on blood samples for analysis of hormone concentrations. Results of such analyses from mares with tumorous ovaries may correlate with clinical findings and responses to surgical removal. Function of a remaining ovary may be assessed by comparing hormone concentrations in blood samples collected pre- and postsurgical with those that are recognized to be normal. This type of test may aid the clinician in formulating hormonal treatment and predicting the prognosis of fertility for unilateral ovariectomized mares.[13,19,20,21]

Size of Ovary to be Removed — Accurate assessment of ovarian dimensions by rectal palpation is not always possible. If the ovary is very large or the mare difficult to palpate, an estimation may be necessary. Since this parameter is important in selecting the surgical approach, sedative agents or epidural anesthesia should be employed in the intractable mare. Inaccurate assessment of ovarian size contributes to the following surgical errors: (1) inadequate surgical exposure (wrong laparotomy approach), (2) unnecessary tissue trauma promoting abscesses, hematomas, and seromas, (3) misdiagnosis of problem, and (4) inability to correct problem(s).

Temperament and Tractability of Mare — This parameter influences not only the method of anesthesia and surgical approach used, but is a trait that hopefully will be altered by surgery in certain cases.

With ovariectomy performed in the standing mare (flank approach or via colpotomy) under appropriate anesthesia, it is imperative that the mare's disposition be conducive to surgical incision and manipulation. Such an operation performed on a vicious or intractable mare will consistently lead to disastrous results. Therefore, standing ovariectomies should be reserved for surgical cases that are more than adequately manageable under these circumstances.

Economical Considerations — Cost of surgery and anesthesia will not be a deciding factor in all cases. When economic circumstances influence and compromise the surgical, anesthetic, and postsurgical phases of ovariectomy, the anticipated standard results will not be achieved. The most inexpensive way to spay a mare is via colpotomy. Special instrumentation and a cooperative mare are prerequisites for this procedure to be successful. Conversely, the greatest cost of a similar operation would be ovariectomy through a midline laparotomy under general anesthesia of an inhalation type. Cost should be a factor that is always considered but should never assume priority over the appropriate anesthetic agent or surgical approach for a given case.

Physical Condition of the Mare — In a few instances, physical condition of a mare may influence the surgeon's choice of anesthesia or laparotomy approach to be used. Generally, intravenous anesthesia will be discarded in favor of a standing approach for the debilitated or crippled case. The presence of uterine, cervical, or vaginal infection eliminates the vaginal approach from available laparotomy choices. Excessively fat and short-coupled mares may have inadequate room for surgical exposure through a paralumbar incision. Chroni

TABLE 1

Anesthesia Chart for Selecting Type Compatible with Laparotomy Approach Chosen

Laparotomy Approach	Primary Anesthetic	Complementary Anesthetic
Vaginal	Caudal epidural 1-1.5 ml 2% lidocaine [a] per 100 # (45.6 kg) body weight	Topical application of gauze soaked in lidocaine applied to ovarian pedicle
Flank (local anesthesia)	Line infiltration Inverted L block (Local infiltration of lidocaine with syringe and needle)	Topical application as with vaginal approach
Flank (general anesthesia)	1. Glyceryl guaiacolate (GG) [b] with 5% dextrose and barbiturate [c] added. Used both as inducing agent and maintenance solution. 2. Short acting barbiturate (thiamylal sodium) [c] as inducing agent and 1% solution as a slow intravenous drip for maintenance. 3. Combinations of chloral hydrate-magnesium sulfate [d] with or without pentobarbital [e] for induction and maintenance. 4. Inhalation anesthesia (halothane [f] or methoxyflurane [g]) induction and intubation.	
Midline or paramedian	[1 through 4 under general anesthesia]	

Brand Name	Generic Name	Company
[a] Xylocaine	Lidocaine U.S.P.	Astra Pharmaceutical Products, Inc. Worcester, MA 01606 USA
[b] Gecolate Sterile Pwd.	Glyceryl guaiacolate	Summit Hill Laboratories Avalon, NJ 08202 USA
Guaifenesin, N.F. XIV	Glyceryl guaiacolate	Gane's Chemical Works, Inc. Industrial Park Road Pennsville, NJ 08070 USA
[c] Surital	Thiamylal sodium	Parke, Davis & Company Detroit, MI 48232 USA
[d] Mag-Chloral	Chloral hydrate Magnesium sulfate Chlorobutanol	Haver-Lockhart Laboratories Division of Bayvet Corporation Shawnee, KS 66201 USA
[e] Equi-Thesin	Chloral hydrate Pentobarbital Magnesium sulfate	Jensen-Salsbery Laboratories Division of Richardson-Merrell, Inc. Kansas City, MO 64141 USA
[f] Fluothane	Halothane	Ayerst Laboratories, Inc. Veterinary Med. Division New York, NY 10017 USA
[g] Metofane	Methoxyflurane	Pitman-Moore, Inc. Washington Crossing, NJ 08560 USA
[h] Betadine Surgical Scrub	Povidone-iodine	The Purdue Frederick Company Norwalk, CT 06856 USA
[i] Nolvasan Solution	Chlorhexidine	Fort Dodge Laboratories, Inc. Fort Dodge, IA 50501 USA

alveolar emphysema, if present, is a deterrent to general anesthesia if an alternate method will suffice.

If any doubt exists in the mind of the surgeon as to the status of organ function (liver, pancreas, lungs, heart, kidney, etc.), then appropriate laboratory tests should be performed. In all cases scheduled for surgery, a complete physical examination may provide the most rewarding and fruitful information obtainable.

Surgical Considerations

Experience of Surgeon — Previous experience tends to be a major factor in planning an operative procedure. However, a lack of experience or experience in abundance should not prevent one from integrating personal knowledge, knowledge of literature written on the subject, and the expertise of colleagues in formulating a surgical plan. This plan by necessity must vary with individual cases. No single approach will suffice for all ovariectomy procedures.

Anesthetic, Anesthetic Equipment, and Surgical Facilities — Surgical facilities and a recovery area with inhalation anesthesia are optimal. A standing flank or vaginal approach is facilitated by a stock or chute to confine the operative case. This affords some safety to the operator with mares that become unruly for a period of time. Various acceptable methods of anesthesia for selected laparotomy approaches are listed in Table 1. For a better understanding of specific anesthetic agents and anesthesia procedures, the reader is referred to selected writings.[6,7]

Special Instruments — A chain ecraseur (Fig 1) is a valuable surgical instrument in selected approaches to ovariectomy. It is essential for ovariectomy through the vagina and can also be used with other laparotomy approaches. This instrument is not available commercially, and, unless one is presently owned, the instrument can not be procured.

Preparation of Mare for Surgery — In all operative cases, starving the animal 24 hours prior to surgery is recommended. In vaginal ovariectomies this is absolutely essential to avoid penetration of viscera during colpotomy incisions. Fasting will also increase exposure in the ovarian area and enhance manipulative procedures.

Surgical areas of the external abdominal wall are clipped and scrubbed with appropriate antiseptic preparations.[a] Preparation of the mare for vaginal ovariectomy involves removal of feces manually from the rectum, wrapping of tail, and surgical scrub of perineal area. The vagina is douched with an antiseptic solution[b] and the mare's bladder emptied by catheterization. A small area

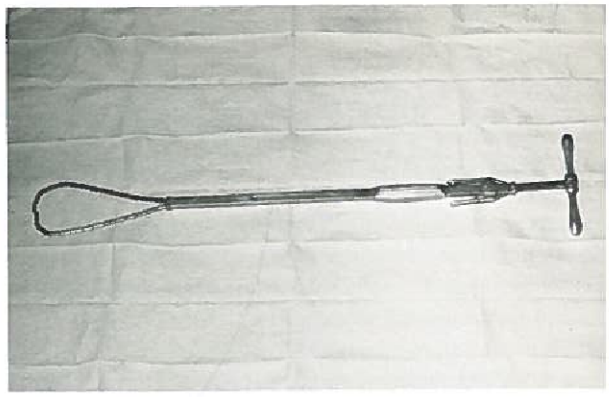


Fig 1— Example of a chain ecraseur that is commonly used fo ovariectomy via colpotomy.

over the tailhead is clipped and scrubbed in preparatio for anesthesia.

Laparotomy Approaches — For concise descriptio of various surgical approaches to the abdominal wal readers are referred to the following writings.[10,22,23] I this discussion, 3 procedures will be discussed: (1) flan approach (paralumbar fossa), (2) midline or paramediar and (3) vaginal (via colpotomy).

Flank (paralumbar fossa) approach — This is a ve satile approach that can be performed standing (loc anesthesia) or in lateral recumbency (general anesthesia) For ovaries that are small (less than 8–10 cm) a gri approach (muscles separated in direction of fiber rathe than incised) may suffice. For greater exposure and re moval of larger ovaries (up to 15–16 cm in diameter) th muscle layers can be transected in a parallel manner t the skin incision. Use of a chain ecraseur is applicable i this procedure, particularly when a bilateral ovariectom is performed. In this case the preferred method would b transfixation ligation of the pathological ovary through laparotomy performed on the same side and chain ec raseur removal of the opposite through the same incisior Removal of both ovaries utilizing the chain ecraseur ca also be performed through a grid incision, if the ovarie are small.

An example of a through and through flank incisio is shown in Fig 2, and the left ovary is indicated by th arrow. Shown in Fig 3 is the correct placement of a chai ecraseur over the hand of the operator. The correc placement of chain loop over the ovary, is shown in Fi 4, and the size of left ovary after removal is illustrated i Fig 5. This ovary could have safely been remove through a grid or vaginal approach also.

Primary advantages of this method are: (1) it can b

a,b See footnotes h and i, Table 1.



Fig 2— Paralumbar fossa laparotomy, left flank approach, with left ovary visualized at the end of the arrow.



Fig 3— Hand of operator placed through chain loop of ecraseur.

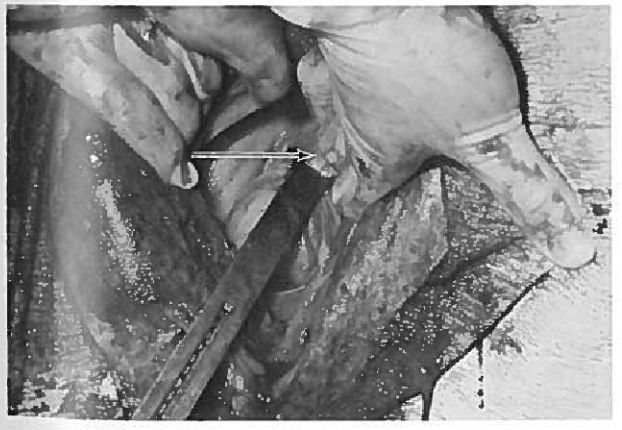


Fig 4— Chain ecraseur has been carried into abdomen (via approach in Fig 2) and properly placed for removal of the left ovary. Mesovarium is at end of pointer.

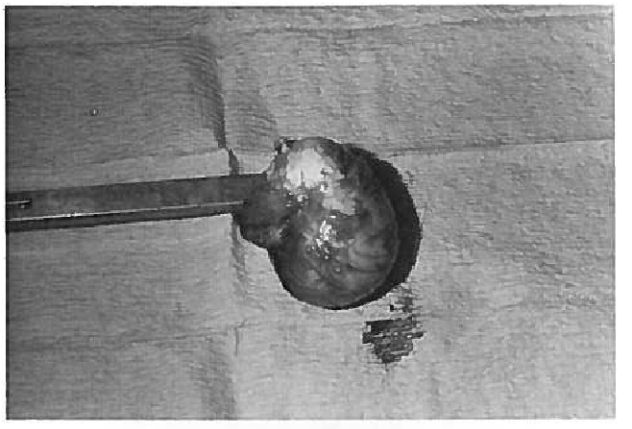


Fig 5— Left ovary after removal, with chain ecraseur also shown.

performed in the standing mare under local infiltration of anesthesia, and (2) it minimizes the problem of postsurgical dehiscence, herniation, and evisceration that are more commonly seen with ventral approaches.

Midline or paramedian — The most versatile approach is one that has inherent allowances for errors committed by the surgeon. This approach is the preferred method of exposure for: (1) large tumorous ovaries, (2) bilateral ovariectomies in vicious mares where an ecraseur is not available, and (3) ovaries for which an accurate assessment of size was not obtained. This laparotomy gives adequate exposure for even unusually large ovaries (Fig 6), and transfixation ligation of the ovarian pedicle with #1-2 medium chromic gut (Fig 7).

Its major disadvantages are: (1) requirement for general anesthesia, (2) surgical time necessary for approach, ovariectomy, and closure, and (3) tendency toward incision problems (dehiscence, etc.). The incision should be located just anterior to the mammary glands and extended forward as necessary.

Vaginal approach via colpotomy[1,23] — A contraindication to this procedure is the presence of vaginal, cervical, or uterine infection. As previously mentioned, the maximal size of ovary (diameter) that can safely be removed via colpotomy is smaller than the other laparotomies discussed and should not exceed 8–9 cm (baseball size). After careful surgical preparation of the perineal and vaginal area, the mare is placed in stocks with her tail wrapped. Epidural anesthesia is used if deemed necessary. A fully gloved hand and arm (obstetric length) is wetted and inserted into the vagina, carrying along a solid scalpel or scissors. Moving the arm back and forth will allow air to enter and distend the vaginal wall. An incision is made in the anterior fornix of the vagina, off the midline and dorsolateral to the exter-

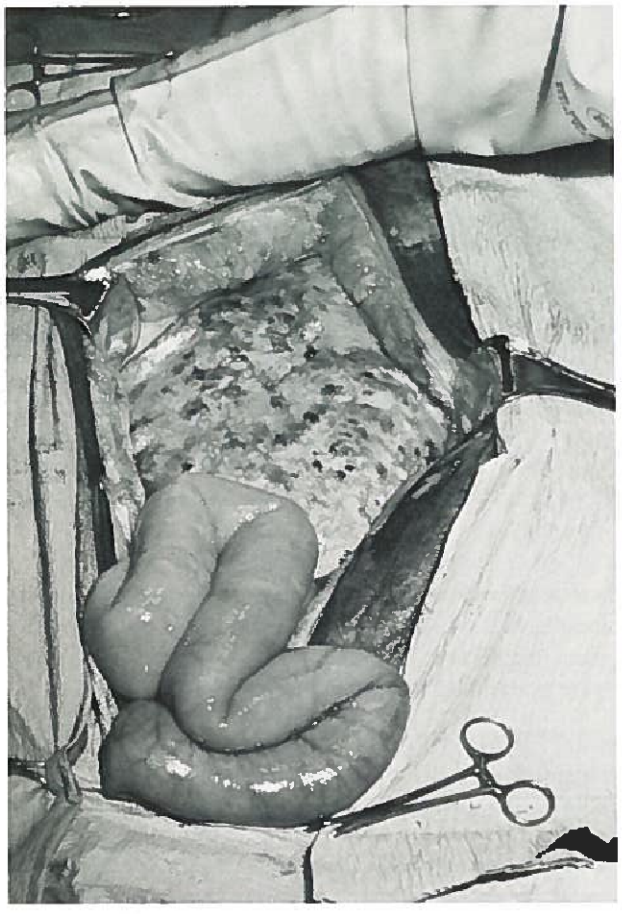


Fig 6— Midline laparotomy with large neoplastic ovary visualized through the incision.



Fig 7— Ovarian pedicle is ligated with transfixation ligature of #1-2 medium chromic gut (arrow). Ovary seen in this figure weighed over 45 pounds (20 kg).

nal os of the cervix. If the right ovary is to be removed, the left hand is gloved and incision of the vaginal wall located at about a 2 o'clock position. Both ovaries can be removed through 1 incision, and the location of the incision in this case is not important. Incising the vaginal wall with a scalpel is effected with a controlled depth incision. The solid scalpel is guarded with the blade properly positioned between the thumb and forefinger with the blade projecting approximately 3 cm. A thrust is made with scalpel in the proper area of vaginal fornix. Directing the knife forward and slightly ventral will prevent accidental laceration of the rectum, bladder, and major vessels in the area. An alternative to sharp incision with a scalpel is perforation of the same area with a pair of scissors. Entry into the peritoneal cavity is through a reflection of pelvic peritoneum, the rectogenital pouch (Fig 8, arrow). If the complete thickness of vaginal wall (mucosa-muscularis-peritoneum) is not incised, the scal-

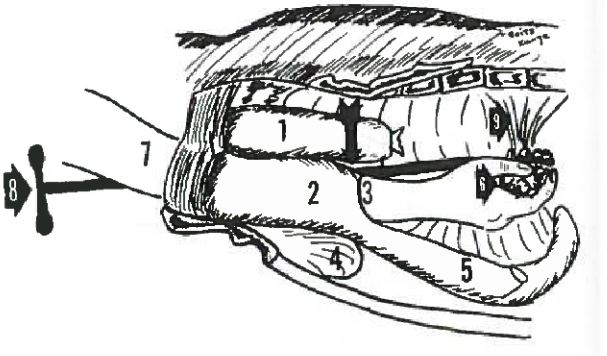


Fig 8— Vaginal approach to ovariectomy via colpotomy—lateral view and important anatomical areas are identified. Rectum (1); vagina (2); colpotomy incision (3); bladder (4); uterine horn (5); ovary (6); operator's arm (7); ecraseur (8); mesovarium (9); rectogenital pouch (arrow).

pel or scissors are withdrawn and the remaining layers perforated with a sharp thrust of a pointed finger. Once the abdomen is entered, enlargement of the incision is accomplished with 2 fingers followed by 3 and then a coned hand. Tracing a uterine horn will aid in locating the proper ovary. If mesovarian anesthesia is desired, a gauze sponge soaked in a local anesthetic preparation is secured to a long piece of suture (umbilical tape) and placed around the ovarian pedicle. This should be held in place for 2–3 minutes and can be retrieved safely if inadvertently dropped.

Passing an ecraseur along the area, the chain is looped over the hand within the abdomen, in a manner similar to that demonstrated in Fig 3. Alternatively, the hand may be withdrawn and reentry made with the ecraseur. The ovary is grasped and the chain loop passed over the ovary which is elevated upward, away from the viscera. The chain loop is closed slowly by an assistant with the surgeon checking the loop repeatedly to insure that no loops of intestine or the tip of a uterine horn have been included (Fig 8). Additional tension is placed on the chain loop until considerable resistance or crushing of mesovarium is palpable. This action should be slowly advanced over a period of 5–8 minutes. A separated ovary may be dropped into the vagina or held until completion of the operation, at which time both the ovary and ecraseur are removed. Once the chain loop has been closed to the point of lying withing the ecraseur handle, the ovarian pedicle is crushed and divided, and the operation virtually completed. Hemorrhage, if it occurs, is palpable by the surgeon. Removal of a secondary ovary may be similarly accomplished if desired.

A Caslick operation completes the surgical procedure and is performed with local or existing epidural anesthesia. This prevents vaginal aerophagia if straining should occur, thus enhancing healing and minimizing herniation or evisceration.

Postsurgical Care

In general, antimicrobial therapy is systemically administered for 3–7 days in ovariectomy cases and longer in selected cases. In uncomplicated cases, a broad spectrum antibiotic should suffice. In cases complicated by sepsis or infection, antimicrobial agents should be chosen on the basis of culture and susceptibility testing results. Tetanus toxoid or antitoxin is administered according to the vaccination status.

Feeding is restored slowly to the ovariectomy case since bowel distention and excessive peristalsis may produce pain. Bulk is slowly added to the diet using bran or pelleted feed. Grain is given in handsful initially and progressively increased over 7–10 days.

Controlled exercise is encouraged to alleviate edema of limbs and surgical site and stimulate bowel movements. This should be controlled as excessive exercise may disrupt incisions and ligatures and produce episodes of severe abdominal pain.

A mare operated through the vagina (unilateral or bilateral ovariectomy) is placed in a stall and cross-tied after surgery. This cross-tied and standing position is continued for 36–48 hours to reduce the possibility of herniation or evisceration.

Hemorrhage — Hemorrhage usually occurs at the time of ovariectomy and should be managed at this point. The mare spayed through the vagina that hemorrhages after ovariectomy with an ecraseur can be ecraseured a second time. A long suture of #2-3 medium chromic gut may be carried into the abdomen through the vaginal incision, passed around the ecraseur positioned snugly on the bleeding pedicle, exteriorized, and knots placed on the outside. This knot is carried in and repeatedly tied until secure. Long ends are cut off with scissors carried into the abdomen while some slight tension is maintained on the suture. Hemorrhage postsurgically occurs when a ligated pedicle is disturbed by movement of ligatures or when an unligated pedicle is disturbed by massage of adhesions. Thrashing during recovery and blood disorders may be contributing causes. Transfixation of ligatures placed in the ovarian stump (Fig 7) and individual ligation of large vessels is the most effective way to control hemorrhage.

Shock — Acute blood loss, hypovolemia, severe abdominal pain, and adverse responses to use of the ecraseur in the standing unanesthetized mare are probable causes of shock. Blood or fluid volume replacement, effective anesthesia (particularly of ovarian pedicle in vaginal approach), and control of postsurgical pain may circumvent this problem. The first few hours after surgery are critical and a surgical case should be carefully watched during this period.

Abdominal Pain — Ovariectomy is attended by various degrees of pain in all cases. Judicious use of analgesics during the first 24–48 hours after surgery will enhance postsurgical recovery. It should be noted that sedatives or tranquilizers are potentially hypotensive and should be used with caution. Severe intractable abdominal pain is suggestive of a more severe state. Rectal palpation, physical examination, and laparotomy results may reveal the cause.

Hematoma, Abscess, Granuloma, and Adhesions – Rectal palpation should be routinely performed 2-3 days after surgery, or immediately in the case that differs from the usual and expected response. Formation of adhesions occurs early in most cases and can be gently broken down by manual massage. Any enlargement of the ovarian pedicle or unmanageable adhesions should be noted. Very large hematomas of the right ovary or ova-

rian pedicle may place extramural pressure on the terminal ileum or proximal jejunum. This interrupts the flow of ingesta and may produce signs of colic. Subsequent rectal examinations will reveal the progress of these conditions.

Unless the hematoma, abscess, or granuloma becomes excessively large or painful, interrupts digestive function, or is complicated by unmanageable adhesions, surgical intervention is elective.

Peritonitis — A degree of localized peritonitis with adhesions is not uncommon as sequelae to ovariectomy. If they are confined to the ovarian pedicle and abdominal wall, minimal problems will be seen. Severe peritonitis is accompanied by more extensive adhesions and, if the animal recovers, will manifest variable degrees of recurrent abdominal pain (colic). Peritonitis in the acute phase is a medicinal problem. Abdominal paracentesis and culture and susceptibility testing are guides to appropriate antimicrobial therapy. A broad spectrum antibiotic should be administered while culture and susceptibility results are forthcoming. If peritonitis is responsive to antimicrobial therapy, surgical intervention for the correction of adhesions is elective.

Dehiscence, Herniation, and Evisceration — Sepsis at the surgical site, unnecessary trauma, hematomas, seromas, and improper laparotomy closure are factors that promote this category of problems. Herniation occurs when the primary confining layers of the abdominal wall are disrupted. If the intact skin and subcutaneous layers prevent evisceration, adhesions may form between a herniated organ and the abdominal wall. Clincial signs of colic, sepsis of incision area, enlargement of incision site, anorexia, depression, and fever are common. Evisceration may occur through any abdominal approach but is more likely to occur with a vaginal approach since the incision is not sutured. If herniated and eviscerated organs are identified before gross injury or contamination has occurred, the organs may be replaced and appropriate reconstruction performed on the laparotomy incision. Herniation may be acceptable, depending on the organ herniated and site of hernia. Herniation following a vaginal approach may be managed by manual reduction per rectum.

Behavioral Changes and Fertility — As mentioned previously, the vicious chronic nymphomaniac mare will probably not be altered by surgical removal of ovaries. An unfavorable prognosis for behavioral changes following surgery is therefore justified. Other mares less severely influenced show varying degrees of response, and some improvement is usually seen. Prognosis for fertility is guarded for the mare that has 1 ovary removed. Radioimmunoassays of blood withdrawn from operated cases before and after surgery may be helpful in suggesting medicinal (hormone) treatment and evaluating progress of mares that continue to be infertile.

Conclusions

This paper is an attempt to relate the various factors involved in the successful approach to ovariectomy in the mare. Of prime importance is the choice of anesthesia used and laparotomy approach employed. Failure to select a combination that is complementary for each case will complicate all aspects of the procedure and promote unfavorable results.

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COMPARISON OF CHEMICAL AND SURGICAL VASECTOMY ON TESTICULAR ACTIVITY IN FREE-ROAMING HORSES (*EQUUS CABALLUS*)

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Abstract: Free-roaming horse (*Equus caballus*) management is a complex issue incorporating social, economic, emotional, political, and environmental factors. Currently, few proven field techniques exist for managing free-roaming horse population growth, which can reach 20–25% annually. Although there are several strategies available for sterilizing mares when managing free-roaming horse populations, surgical vasectomy is the only method used in the field for stallions. Some managers believe that surgically vasectomizing dominant stallions would have significant effects on reducing horse populations. However, sterilizing only dominant harem stallions results in a relatively modest reduction in population growth as substantial reproduction may occur even when 100% of the dominant harem stallions are sterilized if other males perform as little as 10% of the breeding. The overall goal of the current project was to evaluate the efficacy of a novel nonsurgical method for sterilizing free-roaming horses (chemical vasectomy). In September of 2013, stallions that had been previously surgically vasectomized (SURG, $n = 25$), previously chemically vasectomized (CHEM, $n = 16$), or untreated (CONT, $n = 32$) were captured and surgically castrated in preparation for adoption. When comparing both sterilization methods to CONT, serum testosterone and estrone sulfate concentrations did not differ ($P > 0.05$), suggesting that these methods for sterilizing free-roaming stallions would not disrupt herd social hierarchy. However, similar to the CONT, all CHEM stallions had sperm present within the vas deferens seminal fluid samples. CHEM stallions had more morphologically abnormal sperm than did CONT stallions but it is not known if this affected the actual fertility. Additional research is needed using alternative sclerosing agents for chemical vasectomy in free-roaming horse populations.

Key Words: *Equus caballus,* equid, spermatogenesis, stallions, sterilization, testosterone.

INTRODUCTION

Free-roaming horse (*Equus caballus*) management is a complex issue incorporating social, economic, emotional, political, and environmental factors. Under the provisions of the Wild Free-Roaming Horses and Burros Act of 1971,[4] which applies only to lands administered by the Bureau of Land Management and the United States Forest Service, the government is required "... to protect and manage wild and free-roaming horses ..." and "... to achieve and maintain a thriving natural ecological balance on the public lands." However, high population growth rates of these horses have made this mandate a significant management challenge. Free-roaming horses are not normally vulnerable to native predators, and as such, their populations can increase by 20–25% annually.[15] Overpopulation of free-roaming horses causes significant adverse impacts to the ecological integrity of the environment. These impacts have reduced the health and function of native habitats necessary to support native wildlife (e.g. pronghorn, Greater sage grouse).[13]

Reducing the free-roaming horse population will likely result in substantial recovery and restoration of the ecological health and natural conditions. The Sheldon National Wildlife Refuge has identified reducing the free-roaming horse populations as the one of the most important management strategies necessary for achieving the wildlife refuge purposes and goals.[36] As there are no private cattle grazed on this public land, range deterioration can be solely attributed to

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horses. The primary means used to reduce the number of horses has been live removal.[37] Some (typically younger) horses have been adopted, but many have been removed to long-term holding on contract ranches.[37] These removals are stressful for the horses and expensive for the government. The Government Accountability Office noted in 2008 that off-the-range holding costs would continue to absorb the majority of the Wild Horse and Burro Program budget, having increased from $7 million in 2000 to $21 million in 2007, with the expectation that costs would continue to increase rapidly.[13] More recently it has been reported that captive wild horses will cost the United State over $1 billion by 2030 unless management approaches change.[11]

Fertility control has long been recognized as a potentially useful approach in managing free-roaming horse populations because it could reduce or even eliminate the need to remove horses.[21,22] Research on fertility control in mares has been conducted for more than three decades, and several techniques have been successfully employed including hormone implants,[29] intrauterine devices,[17] ovariectomy,[3] and immunocontraception (e.g. porcine zona pellucida vaccine).[18,19] However, there has been significantly less research on sterilization methods for stallions.[9]

Currently, the only method used for sterilizing free-roaming stallions is surgical vasectomy. An advantage of vasectomy over surgical castration is the retention of circulating male sex hormones (testosterone) and consequently maintenance of natural stallion-like behaviors. In addition, the idea of using surgical castration for managing free-roaming horse populations is highly controversial with members of the public, who are concerned about changes in free-roaming horse social organization and behavior (2011 United States District Court case: American Wild Horse Preservation Campaign vs. Department of the Interior). According to the 2013 National Research Council's report, some believe that vasectomizing any one dominant stallion would have a greater effect on reproduction than sterilizing any one fertile female.[21] Indeed, Asa (1999) reported that most surgically vasectomized stallions maintained their social hierarchical status, with harem turnover rates not different than those reported for intact stallions.[2] However, sterilizing only dominant harem stallions results in a relatively modest reduction in population growth as substantial reproduction may occur even when 100% of the dominant harem stallions are sterilized if other males perform as little as 10% of the breeding.[12] In addition, although reduction in foaling occurs when dominant stallions are sterilized, instability of bands or breeding by subordinate males may limit the effectiveness of only sterilizing dominant stallions.[31]

Chemical vasectomy was developed as a method for nonsurgical sterilization in the dog[25] and cat[26] in an effort to reduce overpopulation in these species. Although injection of sclerosing agents directly into the vas has also been reported,[28] this method presented several difficulties because consistently penetrating the lumen of the vas was not easily achieved. Since the epididymal tubule is still coiled at the tail of the epididymis where it merges with the vas deferens, the authors hypothesized that sclerosing agents injected percutaneously into the tail of the epididymis would result in chemical vasectomy. In dogs, 0.6 ml of 1.5% chlorhexidine gluconate in 50% dimethylsulfoxide (DMSO) injected into the tail of the epididymis resulted in azoospermia by 35 days after treatment.[25] In cats, 0.05 ml of 4.5% chlorhexidine digluconate injected into the tail of the epididymis resulted in either intact, nonmotile sperm or sperm with detached heads by 35 days after treatment.[26]

The overall goal of the current project was to determine if chemical vasectomy would be an effective method for sterilizing free-roaming stallions. The first hypothesis was that there was no difference in reproductive hormone concentrations between surgically (SURG) and chemically (CHEM) vasectomized stallions when compared to untreated controls (CONT). The second hypothesis was that both surgical and chemical vasectomy would induce sterilization through posttesticular occlusion.

MATERIALS AND METHODS

Study location

This study took place at the Sheldon National Wildlife Refuge (NWR, refuge) managed by the United States Fish and Wildlife Service (USFWS). Sheldon NWR is located in northwest Nevada along the southern Oregon border to the north and near the California border to the west (41°48′N, 119°14′W). Sheldon NWR covers approximately 232,964 ha of sagebrush-steppe habitat with elevations that range between 1,200–2,100 m. Average precipitation ranges between 18 and 33 cm. Plant communities are dominated by sagebrush (*Artemisia* spp.). Several tree species such as aspen

(*Populus* spp.), western juniper (*Juniperus occidentalis*), and curl-leaf mountain mahogany (*Cerocarpus ledifolius*) are found in scattered stands throughout Sheldon NWR.[35]

Animals

Stallions used in this study were part of a feral horse herd managed in accordance with the USFWS Comprehensive Conservation Plan for Sheldon NWR.[34] Horses were captured primarily by using a helicopter to herd them into a trap corral.

During the capture operations in 2008 and 2009, stallions were surgically vasectomized and then released back onto the refuge. Briefly, stallions were chemically restrained with xylazine (Bayer Animal Health, Pittsburgh, Pennsylvania 15205-9741, USA; 0.5 mg/lb), ketamine (Zoetis, Florham Park, New Jersey 07932, USA; 1 mg/lb), and succinyl choline (Hospira, Lake Forest, Illinois 60045, USA; 0.04 mg/lb) and placed on a hydraulic surgery table with the hind legs secured. The site was surgically prepped and the tail of the epididymis was identified by palpation. An incision was made over the tail of the epididymis exposing the vas deferens and the vas deferens was isolated distal to the epididymis. Two hemoclips were applied 3 cm apart and the vas deferens between the clips was excised. The procedure was repeated on the contralateral side. The skin incisions were left open for drainage and an intramuscular antibiotic (Excede®, Zoetis; 1.5 ml/100 lb) was administered.

During the capture operations in 2010 and 2011, stallions that were not previously surgically vasectomized were chemically vasectomized and then released back onto the refuge. Briefly, stallions were chemically restrained with xylazine (Bayer Animal Health; 0.4 mg/lb) and succinyl choline (Hospira; 0.4 mg/lb). The hind legs were secured and the site was surgically prepped. The tail of the epididymis was identified by palpation and injected with 10 ml of 1% chlorhexidine in 90% DMSO (v/v) using an 18-gauge needle and 20-ml syringe. The procedure was repeated for the contralateral side. Chlorhexidine with DMSO was selected as a sclerosing agent on the basis of previous sterilization successes with intraepididymal injections in dogs[25] and cats.[26] The volume of sclerosing agent was empirically derived from volumes successfully used in dogs and cats. Given that the tail of the epididymis is much larger in mature horses than in dogs and cats, larger volumes were administered. In September of 2013, a subset of the stallions that had previously been surgically (SURG, $n = 25$) or chemically vasectomized (CHEM, $n = 16$) were again captured. In addition, stallions that had been previously untreated (CONT, $n = 32$) were also captured. All of these stallions were to be removed from the refuge, and as such were prepared for placement with adoption agents, which included a routine surgical castration. The surgical castration also permitted assessment of the efficacy of the previous treatments. Briefly, stallions were chemically restrained with xylazine (Bayer Animal Health; 0.4 mg/lb) and succinyl choline (Hospira; 0.04 mg/lb). The hind legs were secured and the site was surgically prepped. Lidocaine was locally infiltrated into the scrotal midline (20 ml) and into each spermatic cord (10 ml). A routine castration was then performed.

Hormone analysis

Prior to castration, a jugular blood sample was collected for equine infectious anemia testing as part of the process for removing horses from the refuge. An aliquot of this sample was used to determine total serum testosterone concentrations. In a randomly selected subset of these samples (SURG, $n = 9$; CHEM, $n = 14$; CONT, $n = 17$), estrone sulfate concentrations were also analyzed. Total serum testosterone was measured using the enzyme immunoassay Immulite 1000 (#LKTW1, Siemens Medical Solutions Diagnostics, Los Angeles, California 90045-6900, USA) according to the manufacturer's instructions. Similar to results published by Illera et al.[14] for stallions, our laboratory found close agreement between radioimmunoassay (RIA) and enzyme immunoassay (EIA) when measuring total serum testosterone concentrations ($EIA = 4.6331 + 0.6633 * RIA$, $R^2 = 0.8306$, $F(1, 38) = 186.3$, $n = 40$, $P < 0.001$). Estrone sulfate was analyzed at the Cornell University Animal Health Diagnostic Center using a semiquantitative direct enzyme immunoassay with antiserum and conjugate purchased from University of California, Davis. The estrone sulfate assay has been previously validated for equine diagnostic applications.[5,32]

Seminal fluid evaluation

A sample of fluid from the vas deferens distal to the tail of the epididymis was collected immediately following castration for microscopic evaluation. For stallions that had been previously surgically vasectomized, the seminal fluid sample was collected from a portion of the remaining vas deferens distal to a hemoclip. A small drop of seminal fluid was placed on a microscope slide

with a coverslip and evaluated using bright field light microscopy at ×200 magnification by a single observer (CMS). The presence or absence of sperm was recorded from almost all of the stallions (SURG, $n = 19$; CHEM, $n = 16$; CONT, $n = 23$). Following direct microscopic examination, a seminal fluid sample was prepared for sperm morphology evaluation. If no sperm were present in the initial direct examination, a sample of fluid for sperm morphology evaluation was collected from the tail of the epididymis (SURG, $n = 10$). The sample was then prepared as a smear, air dried, and stained with Hemacolor® (Harleco, EMD Chemical Inc., Gibbstown, New Jersey 08027, USA) using a 10-min incubation for each stain. Then, the sperm morphology was determined by a single observer (CMS) blinded to the treatments. Briefly, a minimum of 200 sperm were examined using bright field light microscopy at ×1000 magnification under oil immersion to determine the percentage of normal sperm. In addition, each type of abnormal sperm morphology was recorded as well as the number of times the abnormality occurred. Images of representative samples were digitally captured.

Histology

Left and right testicular tissue sections were collected from a subset of the stallions castrated (SURG, $n = 19$; CHEM, $n = 16$; CONT, $n = 10$). Tissues were fixed in 10% formalin and paraffin embedded. Sections were then cut at 4 μm and stained with hematoxylin and eosin. Briefly, randomly selected fields were examined using bright field light microscopy at ×200 magnification. Electronic images of representative samples were digitally captured. A single observer (RL) blinded to the treatments performed histomorphometry to evaluate effects of possible pressure distension following treatment. Two perpendicular diameter measurements across five seminiferous tubules with accompanying tubular basement membrane thickness were recorded for each tissue sample. In addition, a single observer (KMP) blinded to the treatments performed all of the Yoshida scoring (Table 1) to determine the effect on spermatogenesis.

Statistical analyses

All data are reported as mean ± standard deviation (SD). The effect of treatment on the presence of sperm was evaluated using the Fisher exact test and performed using R (version 3.0.2). The effect of treatment on testosterone and estrone sulfate concentration, sperm morphology, histomorphometry, and Yoshida scoring was compared using a one-way ANOVA with a Tukey-Kramer post hoc test. These statistical tests were performed using Graph Pad Prism (GraphPad Software, Inc., La Jolla, California 92037, USA). Significance was defined as $P < 0.05$.

RESULTS

The hormone analysis showed no significant differences in testosterone or estrone sulfate concentrations when comparing either sterilization method (SURG or CHEM) to untreated (CONT) (Table 2). However, SURG stallions had significantly lower testosterone compared to CHEM stallions ($P < 0.05$). This difference was not evident when comparing estrone sulfate concentrations.

SURG stallions had no sperm present in the remaining section of the proximal vas deferens, whereas all of the CONT and CHEM stallions had sperm present within the vas deferens seminal fluid samples. The difference in presence of sperm between the CONT and CHEM compared to the SURG stallions was highly significant ($P < 0.001$).

For SURG stallions that had no sperm present in the remaining section of the proximal vas, a sample of fluid was collected from the tail of the epididymis. There were more morphologically normal sperm in the CONT stallions compared to SURG and CHEM stallions (Table 3, $P < 0.01$). In addition, CHEM stallions had more morphologically normal sperm than did SURG stallions ($P < 0.001$). The majority of the defects recorded in all groups were detached heads, bent tails, and severely coiled tails (Table 3, Fig. 1). There were more detached heads in the SURG stallions compared to CHEM ($P < 0.01$) and CONT stallions ($P < 0.001$). However, there were more severely coiled tails in the CHEM stallions compared to SURG stallions ($P < 0.05$).

The histomorphometry results showed no significant difference in seminiferous tubule diameter ($P = 0.51$) or basement membrane thickness ($P = 0.07$) mediated by changes in intratesticular pressure following treatment (Table 4, Fig. 2). In addition, spermatogenesis as scored using the Yoshida method did not differ between treatment groups (SURG, 11.8 ± 0.3; CHEM, 12.0 ± 0.0; CONT, 12.0 ± 0.0).

DISCUSSION

Measurement of baseline total serum testosterone concentration is often used to assess normal

Table 1. The criteria used for scoring changes within the seminiferous epithelium of stallions that were surgically or chemically vasectomized, or untreated. Adapted from Yoshida et al. 1997.[38]

Score	Yoshida scoring method criteria
12	Many late spermatids or spermatozoa (≥10) present
11	Only a few late spermatids or spermatozoa (<10) present
10	No spermatozoa and no late spermatids, but many round spermatids (≥10) present
9	No spermatozoa and no late spermatids, but only a few round spermatids (<10) present
8	No spermatozoa and no spermatids, but many secondary spermatocytes (≥10) are present
7	No spermatozoa and no spermatids, but only a few secondary spermatocytes (<10) are present
6	No spermatozoa, no spermatids, no secondary spermatocytes, but many primary spermatocytes (≥10) present
5	No spermatozoa, no spermatids, no secondary spermatocytes, but only a few primary spermatocytes (<10)
4	No spermatozoa, no spermatids, no spermatocytes, many spermatogonia (>10) present
3	Only germ cells present are a few spermatogonia (<10) present
2	Absence of germ cells, but Sertoli cells are present
1	Total absence of cells in tubular section

testicular endocrine function in stallions.[1] However, the diagnostic usefulness of a single baseline sample is limited because of the extreme fluctuating nature of testosterone concentrations in circulation. In the horse, serum testosterone levels can range from 0–120 ng/dl over a 12-hour period.[34] Due to this unpredictability, it is often recommended that a more accurate measurement of the testicular function is an hCG-stimulation response test.[7] The field conditions in this study and the wild nature of the subjects made an hCG-stimulation response test impractical. Therefore, to determine with the utmost confidence that the sterilization method under evaluation had no effect on reproductive endocrine status, a baseline estrone sulfate concentration was also measured. Estrone sulfate is the most abundant plasma estrogen in the stallion.[30] The concentration of estrone sulfate in the plasma of mature stallions is nearly 100-fold greater than that of testosterone.[8] In fact, estrone sulfate is believed to be involved in the maintenance of libido in stallions, since it was demonstrated that administration of exogenous estradiol without testosterone to geldings could restore libido.[33] In the current study, serum testosterone concentrations from CHEM stallions did not differ from CONT stallions. However, there was an unexplainable reduction in testosterone in the SURG stallions compared to the CHEM stallions. Because of the unpredictability in baseline testosterone measurements and the importance of estrogens in the maintenance of stallion libido, baseline estrone sulfate concentrations may be a more reliable way to determine whether testicular endocrine tissue is still functioning normally. In the current study, estrone sulfate concentrations did not differ between groups.

Pressure damage can occur within the seminiferous tubule postsurgical vasectomy because sperm production continues without interruption even though there is no outlet for release.[23] However, effects on the seminiferous epithelium following surgical vasectomy appear to differ between species. In rats, changes in testicular histology including loss of spermatogenic cells and vacuole appearance become evident within 1 mo after surgical vasectomy.[6] This is in contrast to rhesus monkeys, where there were no histological changes seen in the testes or the epididymides of

Table 2. Serum estrone sulfate and total testosterone concentrations (mean ± SD) in stallions following sterilization treatment in Nevada, USA, in 2013.

Hormone	Treatment group		
	Surgical vasectomy	Chemical vasectomy	Untreated
Estrone sulfate (ng/ml)	70.95 ± 15.59 (n = 9)	66.94 ± 20.37 (n = 14)	68.05 ± 23.29 (n = 17)
Testosterone (ng/dl)	19.7± 10.70[a] (n = 25)	38.68 ± 30.05[a] (n = 16)	27.17 ± 26.56 (n = 32)

[a] Values in the same row with this superscript differ ($P < 0.05$).

Table 3. Sperm morphology results (mean ± SD) in stallions following sterilization treatment in Nevada, USA, in 2013.

	Surgical vasectomy ($n = 10$)	Chemical vasectomy ($n = 16$)	Untreated ($n = 23$)
Total normal (%)	31.8 ± 13.3[a]	48.0 ± 9.9[ab]	59.7 ± 6.6[ab]
Detached head (%)	48.8 ± 24.7[ab]	27.9 ± 11.1[b]	23.2 ± 6.7[a]
Bent tail (%)	16.0 ± 12.3	16.50 ± 10.2	11.6 ± 5.3
Severely coiled tail (%)	3.6 ± 3.0[c]	7.2 ± 3.4[c]	4.7 ± 3.4
Bent midpiece (%)	0.0 ± 0.0	0.1 ± 0.2	0.2 ± 0.9
Abnormal acrosome (%)	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Microcephalia (%)	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 1.1

[a] Values in the same row with this superscript differ ($P < 0.001$).
[b] Values in the same row with this superscript differ ($P < 0.01$).
[c] Values in the same row with this superscript differ ($P < 0.05$).

vasectomized monkeys when examined 6 mo following surgery.[24] The results appear to be even more confounding in rabbits, where vasectomy results in damage to spermatogenesis within 10 days following surgery, but the effects on spermatogenesis were less severe at 6 and 12 mo following surgery.[20] In the stallions of the present study, the results based on the histologic evaluation performed more than 24 mo following any treatment indicate there were no long-term effects on spermatogenesis ($P = 0.95$), seminiferous tubule diameter ($P = 0.51$), or basement mem-

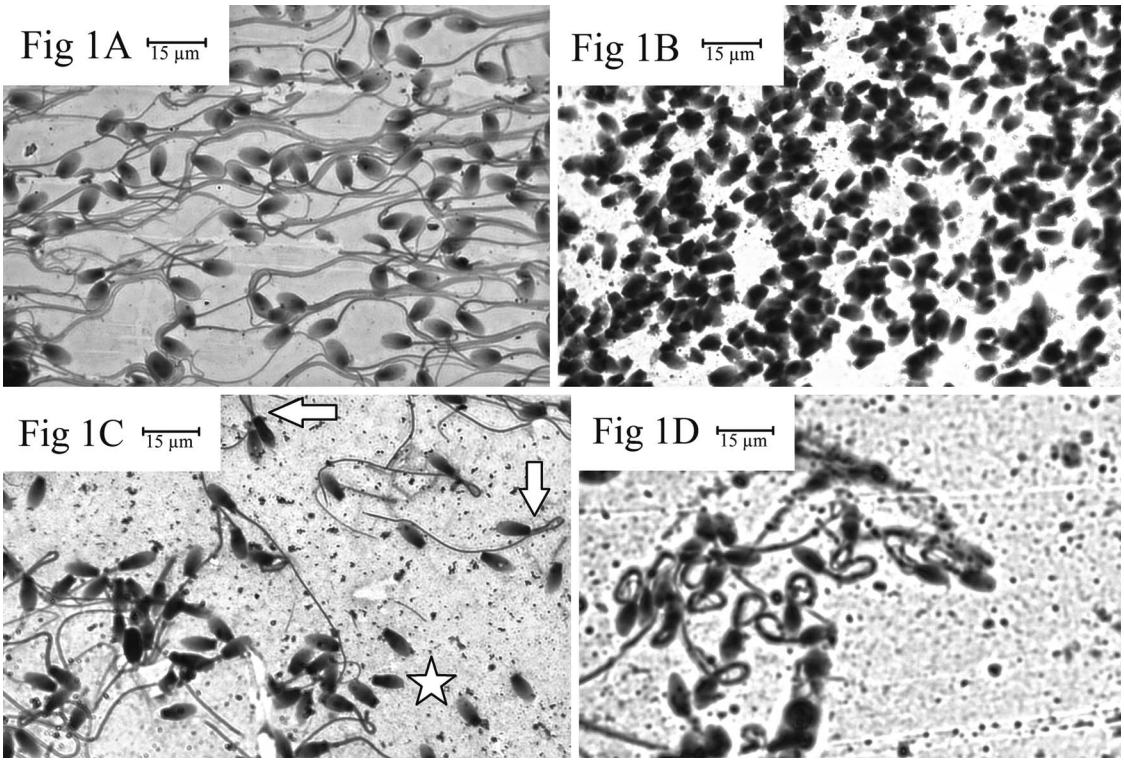


Figure 1. Representative sperm morphologies from an untreated stallion with a majority of normal sperm (**A**), from a surgically vasectomized stallion with almost all sperm having detached heads (**B**), from a chemically vasectomized stallion with several bent tails (arrows) and detached heads (star) (**C**), and from a chemically vasectomized stallion with a high percentage of sperm with severely coiled tails (arrow) (**D**). Sperm were stained with commercial modified Romanowsky stain. Photomicrographs were digitally captured at ×1000 magnification under oil immersion.

Table 4. Seminiferous tubule diameter and basement membrane thickness (mean ± SD) from testicular tissue samples of stallions following sterilization treatment in Nevada, USA, in 2013.

Histomorphometry	Surgical vasectomy ($n = 19$)	Chemical vasectomy ($n = 16$)	Untreated ($n = 10$)
Seminiferous tubule diameter (μm)	192.25 ± 15.37	194.69 ± 18.81	201.535 ± 28.81
Basement membrane thickness (μm)	3.12 ± 0.53	2.83 ± 0.16	3.18 ± 0.53

Values in the same row did not differ significantly ($P > 0.05$).

brane thickness ($P = 0.07$). It is unknown if either the surgical or the chemical vasectomy caused acute pressure damage within the testes (under 1 mo) as the stallions were not evaluated at this time point. However, based on the histology performed several months following either treatment, there was no evidence of irreparable effects on the seminiferous epithelium.

We anticipated that similar to what was reported previously in dogs[25] and cats,[26] percutaneous injection of a sclerosing agent into the tail of the epididymis would cause irritation of the tubular and intertubular tissues, resulting in sclerosis and occlusion, thereby preventing passage of sperm from the epididymides to the vasa deferentia. The finding of significant numbers of intact, motile sperm in the vas following chemical vasectomy may indicate recanalization of a previous obturating tissue reaction. Because the stallions in the current study were chemically vasectomized in 2010 and 2011 and seminal fluid samples were only collected once at the end of the study, it is not

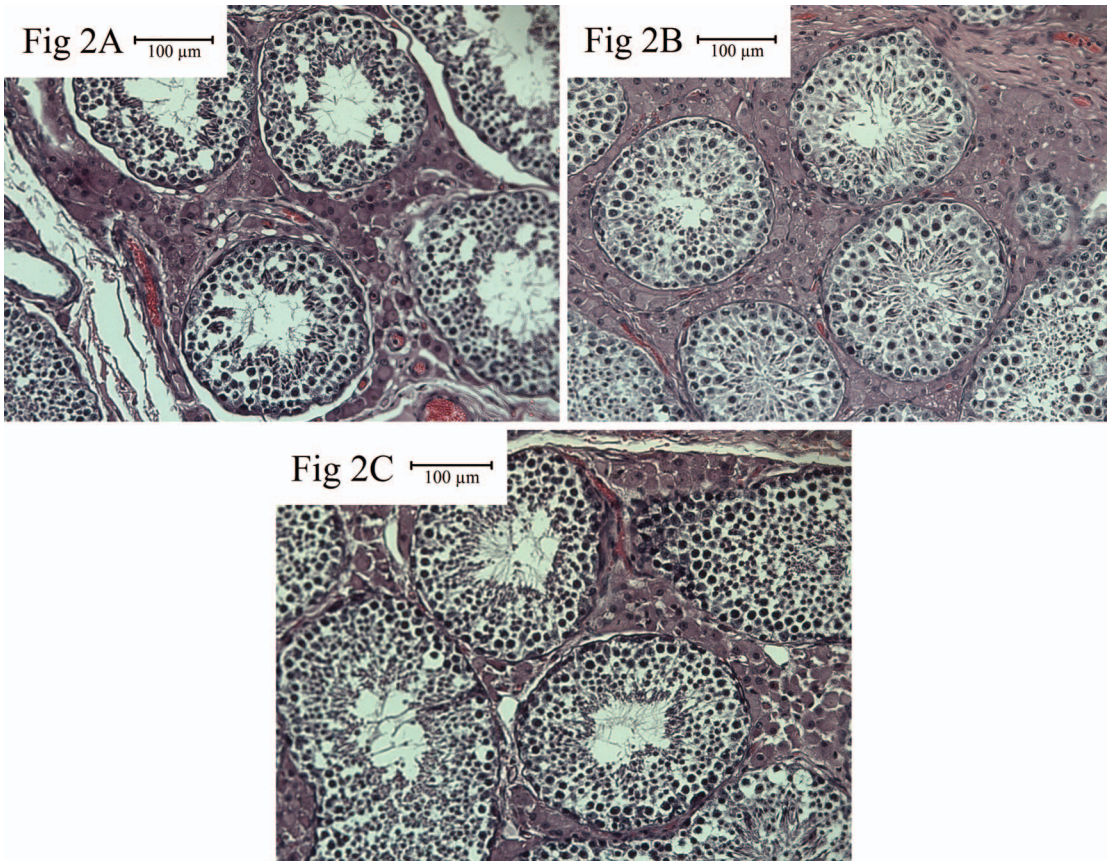


Figure 2. Representative cross sections of the seminiferous epithelium from stallions that were surgically vasectomized (**A**), chemically vasectomized (**B**), or untreated (**C**). Paraffin-embedded testicular biopsy sections (4 μm) were stained with hematoxylin and eosin. Photomicrographs were digitally captured at ×200 magnification.

possible to determine whether or not temporary azoospermia may have resulted from the injection but then recovered over time. A similar study performed in dogs using either 3.0% chlorhexidine digluconate in 50% DMSO or 4.5% chlorhexidine digluconate without DMSO injected into the tail of the epididymis caused azoospermic ejaculates by within 35 or 42 days after treatment.[27] Based upon the histopathology, the authors of this canine study assumed the treatment would be irreversible but they did not do any long-term evaluations so it is possible that the effects were reversible as well. It is of interest to note that in cats, little or no adhesions were present between the scrotum and tail of the epididymides in males that became azoospermic following a similar treatment.[26] In the current study, there was evidence at the time of castration of adhesions and scar tissue surrounding the epididymis and vas deferens in CHEM stallions (Fig. 3). This is suggestive of an increased amount of seepage of chlorhexidine into the vaginal cavity of the scrotum following treatment and may indicate a smaller amount injected (e.g. 5 ml) would have improved efficacy as seen in cats.[26] Other than seepage, another explanation for treatment failure and the formation of the adhesions within the scrotal vaginal cavity would be that DMSO carried the chlorhexidine outside the epididymal luminal walls, calling into question the use of DMSO for chemical vasectomy. Even though CHEM was not effective as a sterilant, it did have long-lasting effects on fertility as illustrated by a significant increase in sperm morphologic abnormalities. However, fertility, in terms of foals produced by each stallion following treatment, was not assessed in this study.

Free-roaming horse populations can be managed with surgical vasectomy but it is time consuming and expensive. Additional research is needed on alternative methods for nonsurgical sterilization in free-roaming horse populations. In dogs, zinc arginine injected into the tail of the epididymis also causes infertility.[10] This application has not yet been tried in horses. A compound that has not yet been tested in the vas deferens of any species is polidocanol. Polidocanol is a powerful sclerosing agent most commonly used in treatment of varicose veins. In baboons, polidocanol administered into the uterine lumen induces oviductal occlusion causing sterility in females.[16] In stallions, polidocanol could be injected into the epididymis, which might result in a permanent occlusion similar to what is seen in the oviducts of baboons.

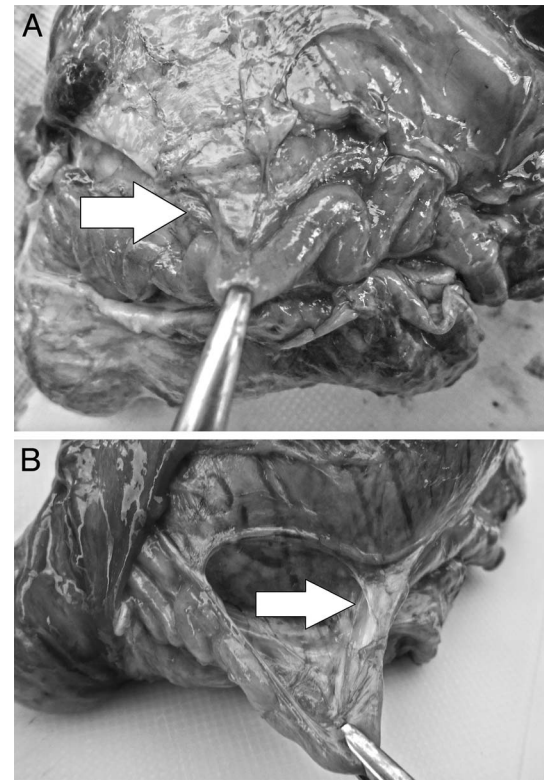


Figure 3. Gross images digitally captured at the time of castration from chemically vasectomized stallions. Note the presence of adhesions and scar tissue near the tail of the epididymis and vas deferens (arrows), presumably resulting from irritation and inflammation induced following injection of the chemical sterilant into this location (**A–B**).

CONCLUSION

In conclusion, only surgical vasectomy induced sterilization of free-roaming horses. Chemical vasectomy with 10 ml of 1% chlorhexidine diluted in 90% DMSO was not an effective method for sterilizing free-roaming stallions. Increasing the concentration of chlorhexidine, eliminating the use of DMSO, and/or decreasing the volume injected may improve the efficacy of this technique. Neither surgical nor chemical vasectomy significantly affected serum estrone sulfate concentrations, which are less unpredictable than baseline testosterone measurements for evaluating testicular endocrine function. Additional research is needed before chemical vasectomy can be a reliable sterilization method in free-roaming horse populations.

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Equine castration: review of anatomy, approaches, techniques and complications in normal, cryptorchid and monorchid horses

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Complications associated with equine castration are the most common cause of malpractice claims against equine practitioners in North America. An understanding of the embryological development and surgical anatomy is essential to differentiate abnormal from normal structures and to minimise complications. Castration of the normal horse can be performed using sedation and regional anaesthesia while the horse is standing, or under general anaesthesia when it is recumbent.Castration of cryptorchid horses is best performed under general anaesthesia at a surgical facility. Techniques for castration include open, closed and half-closed techniques. Failure of left and right testicles to descend occurs with nearly equal frequency, however, the left testicle is found in the abdomen in 75% of cryptorchid horses compared to 42% of right testicles. Bilateral cryptorchid and monorchid horses are uncommon. Surgical approaches described for the castration of cryptorchid horses include an inguinal approach with or without retrieval of the scrotal ligament, a parainguinal approach, or less commonly a suprapubic paramedian or flank approach. Laparoscopic castration of cryptorchid horses has recently been described but the technique has limited application in practice at this time. A definitive diagnosis of monorchidism can only be made after surgical exploration of the abdomen, removal of the normal testis and hormonal testing. Hormonal assays reported to be useful include analysis of basal plasma or serum testosterone or oestrone sulphate concentrations, testosterone concentrations following hCG stimulation, and faecal oestrone sulphate concentrations. Reported complications of castration include postoperative swelling, excessive haemorrhage, eventration, funiculitis, peritonitis, hydrocele, penile damage and continued stallion-like behaviour.

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Castration is one of the most common surgical procedures performed in equine practice.[1,2] Although the procedure is considered routine, surgical complications constitute the most common cause of malpractice claims against equine practitioners in North America.[3] A complete understanding of male reproductive anatomy and physiology and good surgical technique will reduce the rate of surgical complications. Presurgical evaluation and surgical approach to cryptorchid and monorchid horses are a more complex diagnostic and surgical challenge. This article provides practitioners with a review of relevant information on the embryological development and anatomical features of the male equine reproductive tract, the approaches to castration of the normal, cryptorchid, and monorchid horse, and the diagnosis and management of surgical complications.

IM	Intramuscularly
IV	Intravenously

Embryonic development and surgical anatomy of the equine testis

The embryonic gonad differentiates into a testis at approximately 5½ weeks of gestation, at which time it lies on the ventral aspect of the mesonephric kidney.[4,5] A retroperitoneal cord of mesenchyme coursing across the abdominal cavity from the caudal pole of the testis to an extraperitoneal position at the site of the future scrotum forms the gubernaculum.[5] The gubernaculum is divided into two parts by the mesonephric duct which ultimately

differentiates into the epididymis.[4] At day 45 a peritoneal outpouching at the site of the future internal inguinal ring invades the extraperitoneal segment of the gubernaculum forming the vaginal process.[5] Ultimately, when it is completely differentiated, the gubernaculum can be divided into the three different segments identified in the adult horse: the proper ligament of the testis lies between the caudal pole of the mature testis and the tail of the epididymis, the ligament of the tail of the epididymis lies between the tail of the epididymis and the parietal tunic of the vaginal process, and the scrotal ligament lies between the parietal tunic of the vaginal process and the scrotum (Figure 1).[5]

At approximately 6 weeks gestation, the interstitial cells of the testis begin to multiply. By 5 months the embryonic testicle approaches the size of that of a mature stallion and lies in contact with the kidney and the internal inguinal ring.[4,5] Between 7 and 10 months of gestation the testicle atrophies to approximately one tenth of its former size as the gubernaculum shortens.[4,5] The epididymis and the ligament of the tail of the epididymis expand to dilate the vaginal ring and inguinal canal.[5] Ultimately, at 9 and 10 months of gestation dilation of the inguinal ring, contraction of the gubernaculum and increase in intra-abdominal pressure combine to force the small, flaccid testicle into the inguinal ring.[5] The volume of extra-abdominal gubernaculum prevents the testicle from descending completely into the scrotum, so at birth the testicles usually lie within the inguinal rings.[4,5] The mass of gubernaculum is quite large at birth and may be mistaken for a testicle.[5] During the first few weeks of life vaginal rings contract to approximately 1 cm in diameter and gubernacular tissues reduce in size, allowing the testicle to assume a scrotal position.[4,5]

The testicular tissue is encased in the tunica albuginea.[2,5] In the horse the left testicle is usually larger than the right and is suspended more ventrally and situated more caudally.[5] The head of the epididymis is found cranially continuing as the body and tail to become the ductus deferens caudally.[5] The testicles lie within an outpocket of abdominal peritoneum known as the tunica vaginalis.[2,5] The tunica vaginalis consists of visceral and parietal tunics. The visceral tunic is tightly adhered to the testicle, ducts and vessels, and the parietal tunic is continuous with the parietal peritoneum (Figure 1).[5] The cremaster muscle is a caudolateral extension of the internal abdominal oblique muscle and is continuous with the parietal layer of the tunica vaginalis inserting at the caudal pole of the testicle (Figure 2).[5] The parietal tunic is intimately adherent to the scrotal skin through the scrotal fascia and tunica dartos muscle.[2,5] The tunica dartos sends a sagittal septum into the scrotal sac dividing it into left and right pouches.[5] The spermatic cord courses from abdomen to scrotum through the inguinal canal which is bounded by deep and superficial inguinal rings. The deep inguinal ring is a dilatable opening between internal abdominal oblique muscle, rectus abdominus muscle, prepubic tendon and inguinal ligament. The superficial inguinal ring is formed by a small opening in the external abdominal oblique muscle.

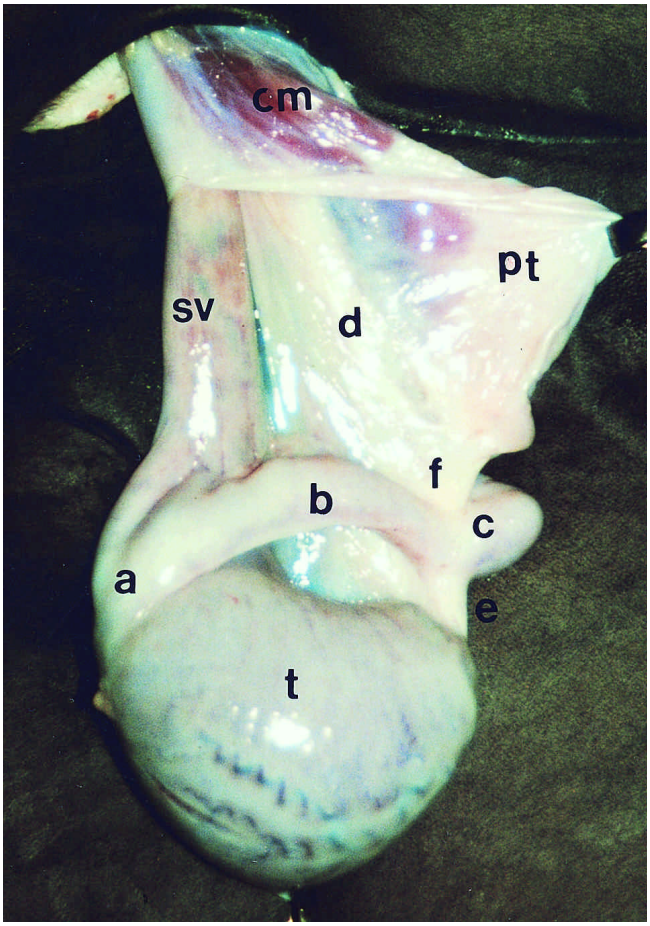


Figure 1.The testicular structures seen during castration of the mature male horse using an open castration technique. The testicle (t) is tightly enclosed in the tunica albuginea.The head of the epididymis (a) continues as the body (b) and the tail (c) the latter continues as the vas deferens (d).The tail of the epididymis is attached to the caudal pole of the testis by the proper ligament of the testis (e).This continues as the ligament of the tail of the epididymis (f) between the tail of the epididymis and the parietal tunic (pt).The visceral tunic covers the visceral surface of the testicle, epididymis, spermatic vessels (sv), and vas deferens. The parietal tunic and cremaster muscle (cm) have been incised and are partially retracted.

Preoperative considerations

Before castration all horses should undergo physical examination including external examination of the reproductive tract.[5] Sedation may be required to palpate the scrotum and the inguinal canals for the presence of two testicles and absence of an inguinal hernia. Tetanus prophylaxis should be current. Some practitioners choose to give procaine penicillin (20 mg/kg IM) prophylactically prior to surgery. The efficacy of this in reducing postoperative complications in castration of normal horses is questionable. The use of antimicrobials for horses undergoing cryptorchid or monorchid castration should be considered on a case-by-case basis but cannot be a substitute for aseptic technique. Preoperative adminis-

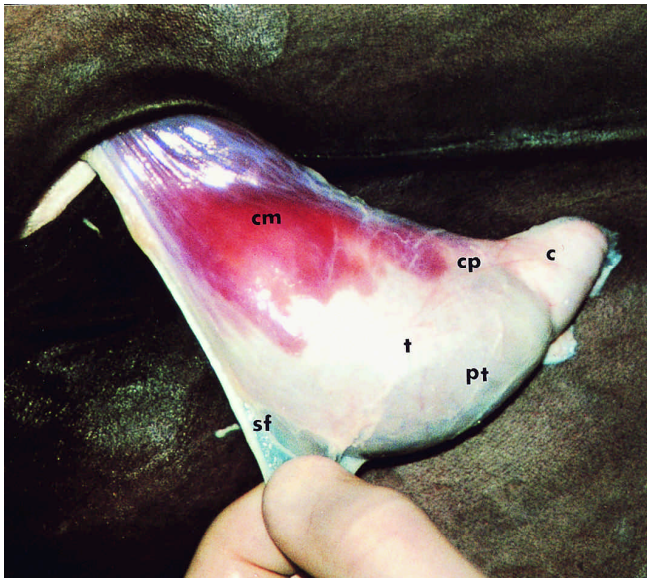


Figure 2.The testicular structures seen during castration of the adult male horse using a closed technique.The testicle (t) and the tail of the epididymis (c) are outlined within the parietal tunic (pt).The cremaster muscle (cm) can be seen fanning out to insert towards the caudal pole of the testis (cp).The scrotal fascia (sf) remaining after the testicle and cord have been stripped can still be seen.

tration of a nonsteroidal anti-inflammatory drug (phenylbutazone 2.2 to 4.4 mg/kg or flunixin meglumine 1 mg/kg), and postoperative administration as required, may help control postoperative pain and swelling.

Castration can be performed in the standing sedated horse using regional anaesthesia or with the horse under general anaesthesia. The choice is largely based on the surgeon's preference and experience. Docile colts whose genitals can be palpated readily without sedation are usually the safest candidates for standing castration.[5]

Sedation and anaesthesia for cas - tration

Standing castration

A combination of physical and chemical restraint and regional anaesthesia is generally recommended.[2] Drugs used commonly, alone or in combination, include acepromazine, xylazine hydrochloride, romifidine, detomidine hydrochloride and butorphanol tartrate. A combination of detomidine hydrochloride (20 to 40 µg/kg) or xylazine hydrochloride (0.3 to 0.5 mg/kg) and butorphanol tartrate (0.01 to 0.05 mg/kg) provides reliable sedation and analgesia.[6]

Various regional anaesthetic techniques have been described for castration of the standing horse.[2,5,6] Local anaes-thetic can be injected into the spermatic cord, testicular parenchyma, or both. The testicular nerves may be anaesthetised by directly injecting 15 to 30 mL of local anaesthetic into the spermatic cord using a 21-gauge 1.5 inch needle. This can be achieved by placing gentle traction on the testicle allowing access to the spermatic cord or by depositing the local anaesthetic at the level of the superficial inguinal ring.[2] Infiltration of the spermatic cord may occasionally cause a haematoma which can interfere with application of the emasculator.[5] When injecting into the testicular parenchyma, the testis is tensed in the fundus of the scrotum and 15 to 25 mL of local anaesthetic is injected into the parenchyma of each testis using a 18-gauge 1.5 inch needle. Given sufficient time the local anaesthetic is proposed to diffuse from the pampiniform plexus to anaesthetise the cord.[2] When combining both techniques, infiltration of the testicle first can facilitate access to the cord in anxious horses. In our hands this combination provides reliable anaesthesia for most horses. Using either technique, 5 to 10 mL of local anaesthetic should be deposited under the scrotal skin either side of the median raphe along the proposed incision line.[2]

Recumbent castration

We prefer to use general anaesthesia because surgical exposure is improved and it carries less risk for surgeon and patient. Various drugs and drug combinations have been recommended for this procedure.[2,5] The placement of a jugular catheter is warranted for drug administration. Thiobarbiturates (thiopentone sodium 4 to 8 mg/kg IV to effect) administered following sedation with xylazine hydrochloride (0.3 to 0.5 mg/kg IV) or acepromazine (0.03 to 0.05 mg/kg IV), or given with or after muscle relaxants such as guaifenesin (100 mg/kg IV to effect) have been recommended for short anaesthetic procedures.[2] While these combinations provide excellent muscle relaxation and analgesia, anaesthetic recovery may be considered less than satisfactory if repeated administration of the barbiturate is required.[2,5]

Xylazine hydrochloride (1.1 mg/kg IV) followed by ketamine hydrochloride (2.2 mg/kg IV) combined with diazepam (0.05 to 0.1mg/kg IV) is an effective combination.[2,5] Diazepam provides muscle relaxation and appears to improve the quality of anaesthetic induction and recovery. The addition of butorphanol tartrate (0.02 to 0.03 mg/kg IV) will provide additional analgesia and may improve anaesthetic recovery. This combination generally allows at least 12 to 15 min of surgical time. Additional doses of xylazine (0.25 mg/kg IV) and ketamine (0.5 mg/kg IV) can be used safely to extend surgical anaesthesia.

Cryptorchid castration

Unless the position of the non-descended testicle can be accurately determined and is easily accessible, this surgery is best performed at a surgical centre under gaseous anaesthesia.[3,5]

Surgical techniques for castration of normal horses

Surgical removal of the descended testis can be performed using an open or closed technique. There is also a variation on the closed technique referred to as half-closed or semi-closed castration.[2,5] There are several modifications of each technique but, regardless of whether the horse is sedated and standing or recumbent and anaesthetised, the surgical principles for castration remain the same.[2,5] Traditionally, castration techniques allow for second intention healing of scrotal wounds, however some have advocated primary closure.[7,8] Primary closure is rarely suited for castrations performed in the field but may be of benefit in select cases where castration is

performed aseptically in a surgical facility. Reported advantages of primary closure include early return to work, minimal postoperative management and a more favourable and early cosmetic outcome with no possibility of eventration.[7]

Open technique

Two incisions are made through the scrotal skin, tunica dartos, scrotal fascia and parietal tunic parallel to the median raphe, approximately 2 cm apart and 8 to 10 cm long. The testis is prolapsed out of the tunic but remains attached to the parietal tunic by the ligament of the epididymis (Figure 1). This ligament can be transected to release the parietal tunic and cremaster muscle and expose the vessels and ductus deferens which can then be emasculated. To reduce the occurrence of postoperative complications such as scirrhous cord and hydrocele, it is desirable to digitally strip the fascia around the parietal tunic as far proximal as possible so it may be emasculated and removed.[2,5] This is easily achieved if the parietal tunic alone (not the parietal tunic and testicle together) is pulled distally with Carmalt forceps while the fascia is stripped proximally using gauze swabs. Alternatively the parietal tunic, the cremaster muscle, the vessels and the ductus deferens can all be emasculated together.

Closed technique

Incisions similar to those for the open technique are made down to but not through the parietal tunic (Figure 2). The testicle, still encapsulated by the tunic, is grasped and the scrotal fascia is stripped from the parietal tunic using a dry swab, until the cremaster muscle and the parietal tunic are clearly exposed. The entire spermatic cord is then emasculated.[2,5]

Half-closed technique

The approach is similar to the closed technique, however after the parietal tunic and the cremaster muscle are exposed a 2 to 3 cm vertical incision is made through the parietal tunic just proximal to the testicle. The inside of the parietal tunic is inspected to ensure there is no evidence of herniated intestine and the entire spermatic cord is emasculated proximally. Alternatively the vessels and ductus can be exteriorised from the parietal tunic and emasculated before emasculating the parietal tunic and cremaster muscle. In another modification the surgeon's thumb can be placed through the incision made in the parietal tunic and the fingers used to prolapse the testicle, vessels and ductus deferens through the incision. This provides the surgeon with a secure grip on the parietal tunic. The vessels and ductus deferens can then be emasculated separately prior to emasculation of the parietal tunic and cremaster muscle or the cord can be emasculated in one.[5]

Cryptorchid castration

The failure of the right and left testicle to descend occurs with nearly equal frequency.[9,10] Of left testicles that fail to descend 75% are found within the abdomen compared to 42% of right testicles.[10] Failure of both testicles to descend is uncommon, affecting 9 to 14% of cryptorchid horses.[5,10]

An accurate castration history is helpful in determining the surgical approach to the cryptorchid horse.[5,11] Palpation of the scrotum and inguinal rings for presence of testicular structures may require sedation in younger or more anxious colts.[5] Transrectal examination generally contributes little to differentiating the type of cryptorchid and carries inherent risks in younger or more fractious animals.[5,11]

Surgical options include inguinal, parainguinal, suprapubic paramedian and flank approaches.[2,5] More recently, standing and recumbent cryptorchidectomy using a laparoscopic technique have been described.[12,13]

Inguinal approach

Two approaches are described for inguinal exploration.[5,11] A 10 cm incision is made through the scrotal skin where the testicle should be and digital dissection is continued towards the superficial inguinal ring.[5] Alternatively, a 8 to 15 cm incision can be made directly over the superficial inguinal ring.[11] A scrotal incision results in less skin haemorrhage and precludes the need to displace inguinal fat to locate the superficial inguinal ring.[11] Recognition of anato-mical features is improved if haemorrhage is controlled. During dissection down to the inguinal ring the smooth, white parietal tunic of the vaginal process is sought. If no structures are identified the inguinal extension of the gubernaculum testis (scrotal ligament) can often be identified exiting from the superficial inguinal ring. It is a thin, flat, fibrous band that courses from the vaginal process to the scrotum. It can be located by carefully inspecting the lateral or medial margins of the cranial third of the superficial inguinal ring.[14] Gentle traction on this ligament using sponge forceps will evert the vaginal process from the superficial inguinal ring and it can be opened longitudinally using scissors. Commonly the tail of the epididymis is the first structure identified and gentle traction on the epididymis will expose the ductus deferens, the proper ligament of the testis, and finally the testis.

If the vaginal ring can accommodate more than the tips of the index and middle fingers then the inguinal canal should be packed with sterile gauze for 24 to 36 h or closed with sutures to reduce the risk of intestinal prolapse through the canal. A sterile gauze bandage can be used as packing to obliterate the inguinal canal and the distal end is left hanging freely from the scrotal wound. The pack is maintained in the inguinal ring by loosely suturing skin edges. Alternatively the superficial ring can be sutured closed using simple interrupted sutures of heavy absorbable suture material.

Surgical approaches for cryptorchid horses

- Inguinal
- Parainguinal
- Suprapubic paramedian
- Flank
- Laparoscopic

Parainguinal approach

Where the testis cannot be exteriorised from the inguinal canal using noninvasive inguinal approaches,[10] several more invasive techniques have been described to explore the inguinal canal.[5,11] These have been used commonly but it has been suggested that they increase the risk of evisceration.[10,11] The parainguinal approach provides a convenient alternative where the noninvasive inguinal approaches

have been unsuccessful.[15] A 4 cm incision is made in the aponeurosis of the external abdominal oblique muscle 1 to 2 cm medial and parallel to the superficial inguinal ring. The incision is centred over the cranial aspect of the ring. The internal abdominal oblique muscle underlying the aponeurosis is bluntly separated along its muscle fibres to expose the peritoneum which is then penetrated to enter the abdominal cavity. The internal inguinal ring can be identified caudolaterally and, with a sweeping action, the fingers should pick up any testicular structures. These can be exteriorised and emasculated. If difficulty is encountered in locating the epididymis or associated structures the incision can be enlarged to allow the surgeon's hand to enter the abdominal cavity for exploration of the urogenital tract. The incision in the aponeurosis is closed using heavy absorbable sutures and the subcutaneous tissue and skin can be closed or left open to heal by second intention.[15]

Suprapubic paramedian approach

An incision of 8 to 15 cm is made cranially from the level of the preputial orifice approximately 5 to 10 cm lateral to the ventral midline.[5,16] The abdominal tunic, which is closely adherent to the ventral sheath of the rectus abdominis muscle, is incised longitudinally and the underlying fibres of the rectus abdominis muscle are bluntly separated in the same direction. Blunt dissection is used to penetrate through the dorsal rectus sheath, retroperitoneal fat and peritoneum. The testicle is usually found near the deep inguinal ring and is easily exteriorised. After emasculation, the abdominal tunic, the subcutis and skin are each closed separately with interrupted or continuous sutures. This procedure has largely been superseded by the parainguinal approach, however it is proposed to allow access to both testicles in the case of a bilateral cryptorchid.[5]

Flank approach

A standard flank approach can be used in standing or recumbent horses.[17] The testicle is located and removed using a similar technique to the paramedian approach. This technique is less acceptable cosmetically than the other approaches and is now generally reserved for removal of large neoplastic testicles.[5]

Laparoscopy

Laparoscopic techniques for castration of cryptorchid horses have recently been described.[12,13] Laparoscopy requires special instruments not readily available in general practice[5] and further refinements are needed in the method.[13] At present this technique has limited application in general practice but it may be more feasible in the future.[5]

Monorchidism

Monorchidism is the complete absence of one testicle and is rare in the horse.[5] A definitive diagnosis can only be made after surgical exploration of the abdomen, removal of the other testis and hormonal testing.[18,19] Useful hormonal assays include basal plasma or serum testosterone and oestrone sulphate concentrations, testosterone concentrations following hCG stimulation, and faecal oestrone sulphate concentration.[5,18,19] The hCG stimulation test in horses older than 18 months, basal oestrone sulphate assay in horses older than 3 years or a combination of both tests appear to be most reliable.[5,11,18,19] The hCG stimulation test measures plasma or serum testosterone concentrations, before and 1 and 24 h after intravenous administration of 6000 to 10,000 IU of hCG.[18,19] There should be no increase above basal testosterone concentrations in horses with no testicular tissue. The response in horses with testicular tissue may be poor during winter or if testicular tissue is abdominal. Both tests should be performed if either test is inconclusive. Samples should be submitted to laboratories that routinely assay equine testosterone and oestrone sulphate concentrations so that reliable reference values are available.[14,19]

Hormonal assays detecting testicular tissue

Hormone detected	*Sample*
Basal testosterone	Serum or plasma
Basal oestrone sulphate	Serum, plasma or faeces
Testosterone following hCG stimulation	Serum or plasma

Complications of castration

Postoperative swelling

Some preputial and scrotal oedema is normal, usually greatest 3 to 6 days after surgery and subsiding by 9 days.[20] Excessive swelling is a common complication and typically occurs with inadequate wound drainage, inadequate postoperative exercise, poor lymphatic drainage, excessive surgical trauma or infection.[1,20]

Postoperative swelling can be reduced by creating a large scrotal incision, avoiding excessive tissue trauma, removing as much tunica vaginalis as possible, manually stretching the surgical incision and removing the median raphe during surgery.[5,20] Following castration, exercise, hosing the wound, and the use of nonsteroidal anti-inflammatory drugs can reduce swelling.[1,5,20] The owner may start riding the horse 24 to 48 h after surgery.[20]

Excessive postoperative swelling is painful and reduces willingness to exercise, causing the wound to seal prematurely and compounding the problem. Treatment involves nonsteroidal anti-inflammatory drugs to decrease pain and inflammation and increase tolerance to exercise.[1,5,20] If scrotal wounds have closed, it may be beneficial to sedate the horse and reopen the wounds manually wearing a sterile glove.[1,5,20] This may need to be repeated on consecutive days until the swelling reduces. Antimicrobial drug treatment may be indicated where the discharge is purulent. Secondary problems associated with severe swelling include phimosis, paraphimosis, cellulitis, wound infection and dysuria.[20]

Excessive haemorrhage

Haemorrhage may occur during, immediately after or several days after surgery.[20] Some bleeding from scrotal wounds after castration is normal but if profuse bleeding continues unabated for more than 15 min haemorrhage should be considered excessive.[1,5,20] Excessive haemorrhage generally occurs from the testicular artery, but may originate from traumatic rupture or laceration of branches of the external pudendal vein.[1,20] Arterial haemorrhage is generally a result of inadequate crushing by the emasculators. The emasculators should be applied perpendicular to, and without tension on, the spermatic cord with the cutting blade closest to the testes.[1,5,20]

Precise guidelines for when to intervene surgically are not established but treatment of postoperative haemorrhage is best performed under general anaes-

Complications of castration

- Postoperative swelling
- Excessive haemorrhage
- Eventration
- Funiculitis
- Hydrocele
- Penile damage
- Continued masculine behaviour

thesia.[1,5,20] Before anaesthesia the patient should be assessed carefully, paying particular attention to cardiovascular status. The site of haemorrhage should be identified. The spermatic cord may be grasped with long forceps passed into the inguinal canal and the testicular artery occluded. The cord can then be emasculated proximally or ligated. If the cord cannot be ligated or emasculated, the forceps can be left in place for 12 to 24 h to allow thrombosis to occur. Where the end of the cord cannot be grasped, haemorrhage can usually be controlled by packing the scrotum with gauze. If the haemorrhage is occurring from a large superficial vein in the scrotal or inguinal area, ligation of the vessel is the treatment of choice.

Eventration

Eventration through the vaginal ring and open scrotal incision is uncommon, generally occurring within 4 h but may occur up to 6 days after surgery.[21] Eventration of the small intestine makes up 67% of cases while omental prolapse comprises the remainder.[22] A survival rate of 85 to 100% can be expected where appropriate treatment is instituted.[22] Suggested predisposing factors include a pre-existing undetected inguinal hernia, presence of visceral structures adjacent to the internal inguinal ring, and increased abdominal pressure following surgery.[22] Palpation of the scrotum and inguinal regions for hernias prior to castration is recommended in foals. Standardbred horses are believed to be predisposed to congenital inguinal herniation.[5]

If evisceration occurs the main objective is to clean and protect the intestine and return it to the abdomen before it is excessively traumatised or contaminated.[21] The intestine should be protected by a moistened towel shaped into a sling and the horse anaesthetised and positioned in dorsal recumbency.[21] In the field the intestine should be lavaged and where possible placed back within the scrotum which is sutured.[21] Broadspectrum antimicrobial therapy should be initiated, an analgesic dose of flunixin meglumine administered and the horse immediately referred to a surgical facility. To replace the intestines into the abdomen, dilation of the vaginal ring and traction on the intestines through ventral midline or parainguinal celiotomy are usually necessary.[5,21] The tunic should be ligated and emasculated as far proximally as possible and the superficial inguinal ring closed with large-diameter, absorbable suture material.[1,5,20] Alternatively, the inguinal canal can be packed with gauze for 24 to 48 h, however intestine may still eventrate around the packing.[1,5,21] Prolapse of the omentum through the inguinal ring can usually be managed using sedation and transecting the prolapsed omentum as far proximally as possible. After recovery from anaesthesia the horse should be kept quiet in a stall for 24 to 48 h.[5,21]

Evisceration of omentum from the scrotal wound is much less serious and is best treated with transection of the omentum which can be performed with the horse standing.[5]

Funiculitis

Funiculitis refers to inflammation of the spermatic cord.[1,5,21] It is usually a septic process developing as an extension of a scrotal infection or from a contaminated emasculator or ligature.[5] Failure to remove the tunica vaginalis and external cremaster muscle during open castrations predisposes horses to septic funiculitis.[23] Signs vary with pyrexia, lameness, inguinal and scrotal swelling and chronic discharge often noted.[1,5] These may not develop for months or years after castration.[5,21]

In the early stages, funiculitis often resolves with antimicrobial therapy and the establishment of drainage, but occasionally surgical removal of the infected stump is required.[5,21] A chronic infection with pyogenic bacteria is commonly referred to as scirrhous cord, however the term 'champignon' and 'botriomycosis' have been used historically to describe infection with *Streptococcus* spp and *Staphylococcus* spp, respectively.[5,21] Extension of the septic process through the inguinal ring and into the peritoneal cavity is possible but uncommon.[5] Treatment of scirrhous cord requires surgical resection and antimicrobial therapy.[1,5,20] The horse should be anaesthetised and positioned in dorsal recumbency. The infected stump is then isolated from healthy tissue using blunt dissection. The cord is emasculated proximal to the infected stump and the wound is left to heal by second intention.[5]

Peritonitis

The vaginal and peritoneal cavities communicate and nonseptic peritonitis is common following castration.[1,5,21] Haemoperitoneum and secondary inflammation can increase peritoneal total nucleated cell counts to more than 10 x 10^9 cells/L for at least 5 days with counts greater than 100 x 10^9 cells/L not uncommon.[23] Septic peritonitis is rare,[1] but should be considered where signs include abdominal pain, pyrexia, tachycardia, diarrhoea, weight loss and reluctance to move.[1,5,21] Toxic or degenerate neutrophils and intracellular bacteria found on abdominocentesis confirm the diagnosis.[1,5,21] Culture of a peritoneal fluid sample is suggested where possible. Treatment consists of appropriate antimicrobial and nonsteroidal anti-inflammatory therapy, and providing analgesia, peritoneal drainage and lavage, and adequate drainage through the scrotum.[5] Until culture results become available, procaine penicillin (20 mg/kg IM twice daily) and gentamicin sulphate (6.6 mg/kg, IV or IM once daily) should be considered.

Hydrocele

A hydrocele is an accumulation of sterile amber-coloured fluid within the vaginal tunic. It can appear months to years following castration.[1,5,21] It is believed to result from failure to resect the vaginal tunic adequately during castration and is reported to occur most commonly in mules.[1,5] It appears as a fluid-filled enlargement of the scrotum that can often be reduced by squeezing the fluid into the abdominal cavity. Treatment is only necessary where the swelling is unsightly or interferes with function. The skin is incised over the sac and the vaginal tunic is isolated proximally to the inguinal ring and emasculated.[1,5]

Penile damage

Complications involving the penis are rare and generally associated with iatrogenic trauma to the penile shaft. Penile prolapse can occur secondary to excessive swelling, and priapism and penile paralysis has been reported following administration of phenothiazine-derivative tranquillizers.[1,5,21]

Continued masculine behaviour

Castration does not always result in elimination of objectionable stallion-like behaviour. This has been attributed to incomplete removal of epididymal tissue, presence of heterotopic testicular tissue, production of high concentrations of androgens from the adrenal gland, incomplete cryptorchid castration and psychological reasons.[1,5] Androgens are not produced by the epididymis and therefore the presence or absence of epididymal tissue cannot influence masculine behaviour. Amputating the spermatic cords was reported to abolish objectionable masculine behaviour in 75% of castrated horses, but no satisfactory explanation was offered for this response.[24] Heterotopic testicular tissue and production of adrenal androgens have never been established in horses and are unlikely to be the cause of continued stallion-like behaviour.[1,5] About 20 to 30% of horses display some masculine behaviour following castration.[3,26] It is likely that this is psychological in origin and represents normal social interaction between horses. Where this behaviour is excessive or the castration history is obscure, hormonal testing (see monorchidism) can be used to establish whether testicular tissue is present.

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Erysipelas in malleefowl

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The malleefowl *(Leipoa ocellata)* is a ground-dwelling Australian native bird originally found in a range of habitats. It belongs to the mound-building family of birds, the Megapodiidae, which are confined almost entirely to the Australian region. Members of this family vary greatly in colour and form. However, they all have in common the fact that they do not build a nest and brood their eggs, but bury them in the ground, or in a mound, or in heaps of fermenting vegetable material built up for this purpose (Figure 1). The incubation process utilises external sources of heat such as decomposing vegetation or incident solar radiation. The adults keep the eggs at a constant temperature by constantly remodeling the mound, adding or removing dirt and leaves. The eggs hatch in the mound and the chicks are left to look after themselves.[1] Numbers of malleefowl have declined appreciably since European settlement.[2] Factors influencing this decline include land clearance for cropping and pastoralism, predation by the European fox *(Vulpes vulpes)*, changes in fire frequency, grazing of malleefowl communities by domestic stock, and the presence of introduced mammalian pests, particularly goats *(Capra hircus)* and rabbits *(Oryctolagus cuniculus)*.[3] No reports of disease as a factor in the decline of the malleefowl are available

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Territory fidelity, space use, and survival rates of wild coyotes following surgical sterilization

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ARTICLE

Territory fidelity, space use, and survival rates of wild coyotes following surgical sterilization

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Abstract Sterilization of wild canids is being used experimentally in many management applications. Few studies have clearly demonstrated vasectomized and tubal-ligated canids will retain pair-bonding and territorial behaviors. We tested whether territory fidelity, space use, and survival rates of surgically sterilized coyote (*Canis latrans*) packs were different from sham-operated coyote packs. We captured and radio-collared 30 coyotes in December 2006. Sixteen of these animals were sterilized via vasectomy or tubal ligation, and 14 were given sham-surgeries (i.e., remained intact). We monitored these animals using telemetry and visual observations through 2 breeding seasons and 1 pup-rearing season from December 2006 to March 2008. Mean pack size was not significantly different between sterile and intact coyote packs. We found no difference in home range size between sterile and intact coyotes. We found differences in home range and core area overlap between sterile and intact coyote packs in some seasons; however, this difference may have existed prior to sterilization. Home range fidelity was not significantly different between sterile and intact coyotes. All coyotes had higher residency rates during the breeding season, with no differences between sterile and intact coyotes. Survival rates were correlated with biological season, but there were no differences in survival rates between sterile and intact coyotes. We concluded that surgical sterilization of coyotes did not affect territory fidelity, survival rates, or home range maintenance.

Keywords Carnivore · Coyotes · Home range · Sterilization · Survival · Territory fidelity

Introduction

Sterilization of canids is being tested for various management purposes including population control of native and non-native species, predation control, and to reduce genetic introgression with other canids (Haight and Mech 1997; Kelly et al. 1999; Spence et al. 1999; Bromley and Gese 2001a; Saunders et al. 2002). Surgical sterilization in particular is a promising management approach for these objectives because hormonal systems remain intact with vasectomy and tubal ligation. Canid reproductive strategies (e.g., monogamy and pseudopregnancy in subordinate pack members) and physiology (e.g., monestrum and prolonged proestrus and estrus) favor pair and pack cohesion (Asa 1997). Without hormonal signals, these characteristics may not be preserved (Asa 1995). For most management purposes, retaining social structure of the pack is critical (Bromley and Gese 2001a, b). If the social structure of a sterilized pack fails, then a vacant territory becomes open to colonization by intact animals (Asa 1995; Mech et al. 1996; DeLiberto et al. 1998; Gese 1998).

Mech et al. (1996) vasectomized 5 wolves (*Canis lupus*) to determine if sterilization was a viable method for controlling population size. They determined the vasectomized wolves' social behaviors were not altered (i.e., the males maintained pair bonds and territories). Due to the success (i.e., pack size remained the same or decreased) of this study, sterilization is one of several proposed methods to

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control wolf populations (Haight and Mech 1997). In Canada, wolves had been implicated in the decline of caribou (*Rangifer tarandus*) and moose (*Alces alces*) calf survival (Spence et al. 1999). Due to public concern of the use of lethal control, fertility control was tested as an alternative to manage wolves (Spence et al. 1999). To determine the applicability of such a tool, territorial behaviors were examined. They found sterilized wolves maintained pair bonds and remained in their territories (Spence et al. 1999).

The sheep industry in the United States has a long history of conflict with coyotes (*Canis latrans*) preying on domestic livestock (Wagner 1988). Ranchers and wildlife management agencies utilize various lethal methods to reduce coyote predation on livestock and wildlife species (Knowlton et al. 1999). The public repeatedly is concerned over the use of lethal management (Arthur 1981; Kellert 1985; Andelt 1987; Messmer et al. 2001). One alternative to reduce predation on sheep is surgical sterilization of coyotes (Knowlton et al. 1999). Bromley and Gese (2001a) sterilized coyotes and found an eight-fold reduction in coyote predation on domestic sheep. This technique is effective because it reduces the energetic needs of the pack during pup rearing (Bromley and Gese 2001a). Bromley and Gese (2001b) further demonstrated the sterile coyotes' territorial and pack affiliative behaviors were not modified.

Coyotes are considered a social canid (Bekoff and Gese 2003; Gese 2004). The basic social unit is the adult, heterosexual pair, referred to as the alpha pair. Coyotes form heterosexual pair bonds that may persist for several years, but not necessarily for life. Courtship behavior begins 2–3 months before copulation. Coyotes may maintain pair bonds and whelp or sire pups up to 10–12 years of age (Gese 1990). Associate animals may remain in the pack and possibly inherit or displace members of the breeding pair and become alphas themselves. Associates participate in territorial maintenance and pup rearing, but not to the extent of the alpha pair (Gese 2004). Other coyotes exist outside the resident packs as transient or nomadic individuals. Transients travel alone over larger areas and do not breed, but will move into territories when vacancies occur. One factor that may affect coyote sociality is prey size or prey biomass. In populations where rodents are the major prey, coyotes tend to be in pairs or trios (Bekoff and Gese 2003). In populations where ungulates are available, large packs of up to 10 individuals may form (Gese et al. 1996a, b, c). Coyotes are territorial with a dominance hierarchy within each resident pack (Gese et al. 1996a, c; Gese 2004). Territoriality mediates the regulation of coyote numbers as packs space themselves across the landscape in relation to available food and habitat. The dominance hierarchy influences access to food resources within the pack (Gese et al. 1996a, b, c). Resident coyotes actively defend territories with direct confrontation, and indirectly with scent marking and howling (Gese 2001, 2004). Only packs maintain and defend territories (Gese 2001, 2004; Bekoff and Gese 2003). Fidelity to the home range area is high and may persist for many years (Kitchen et al. 2000). Shifts in territorial boundaries may occur in response to loss of one or both of the alpha pair (Gese 1998). Dispersal of coyotes from the natal site may be into a vacant or occupied territory in an adjacent area, or they may disperse long distances. Generally, pups, yearlings, and non-breeding adults of lower social rank disperse (Gese et al. 1996a). Dispersal seems to be voluntary as social and nutritional pressures intensify during winter when food becomes limited (Gese et al. 1996a). Dispersal by juveniles usually occurs during autumn and early winter.

Although sterilization has been used in a few canids, only Bromley and Gese's (2001b) study has demonstrated that free-ranging coyotes maintained territorial and breeding-pair behaviors. If coyote sterilization continues to be used as a management tool, it is important to validate that territorial maintenance and pair-bonding behaviors are retained across various circumstances (Asa 1995) and environments. Without this assurance, intact animals could displace sterile packs and threaten the success of the management action (Till and Knowlton 1983; Asa 1995; Mech et al. 1996; DeLiberto et al. 1998). As part of a study to test whether coyote sterilization could increase pronghorn (*Antilocapra americana*) fawn survival (Seidler 2009), we also tested the hypothesis that sterilization would not affect territory fidelity, survival rates, and home range maintenance of coyotes. Using similar methodologies, we examined the same parameters as Bromley and Gese (2001b), including home range size, home range and core area overlap, home range fidelity, pack size, and survival rates of sterile versus intact coyotes. Scientific theory is advanced through repeated studies (Ford 2000; Gauch 2003). Since Bromley and Gese (2001b) was the only study examining the effects of sterilization on coyote behavior and survival rates, additional studies in different environments are needed to increase our understanding of the effects of reproductive control on coyote behavior and broaden our scope of inference. Our study was conducted in a shortgrass prairie and native prey ecosystem, while the study by Bromley and Gese (2001b) was conducted in the sage-brush steppe with a mixture of domestic livestock and native prey species.

Materials and methods

Study area

We conducted this study on the 1,040-km^2 Piñon Canyon Maneuver Site (PCMS) in Las Animas County, Colorado.

The study area within the PCMS was defined by the home range boundaries of the radio-collared coyotes. Mean elevation on the PCMS was 1,520 m, mean temperature ranged from 1 °C in January to 24 °C in July (Shaw and Diersing 1990), and mean annual precipitation was 305 mm (Milchunas et al. 1999). Harvest of coyotes was not permitted during the study. Nearly 60 % of the PCMS was shortgrass prairie dominated by blue grama (*Bouteloua gracilis*), galleta (*Hilaria jamesii*), and western wheatgrass (*Agropyron smithii*) (Shaw et al. 1989). Many shrub communities occurred within the grassland communities along alluvial fans, waterways, and slopes, and included black greasewood (*Sarcobatus vermiculatus*), fourwing saltbush (*Atriplex canescens*), Bigelow sagebrush (*Artemisia bigelovii*), winterfat (*Krascheninnikovia lanata*), small soapweed (*Yucca glauca*), and tree cholla (*Opuntia imbricata*). Woodland communities dominated the canyons and breaks, and were composed of one-seeded juniper (*Juniperus monsperma*) and pinyon pine (*Pinus edulis*).

Capture and monitoring of coyotes

We captured coyotes using aerial net-gunning (Barrett et al. 1982; Gese et al. 1987). Animals captured in the southern portion of the study area were sterilized, while animals captured in the northern portion of the study area were sham-sterilized (i.e., animals were sham-operated but remained intact). The boundaries of the two areas were 4 km apart and both areas were similar in climate, topography, vegetation, and prey availability. We used this clustered experimental design in an effort to swamp a single area with the treatment simulating actual management practices. Due to the uncertainty of capturing the breeding individuals, we sterilized both males and females from each pack.

Captured animals were blind-folded and muzzled, then transported to a licensed veterinarian. Animals were sexed and weighed with a spring scale to the nearest 0.1 kg to determine the initial drug dosage and then sedated with a combination of tiletamine and zolazepam (dosage 10 mg/kg). Continued anesthesia to maintain the anesthesia plane during surgery and processing were with a combination of tiletamine and zolazepam plus xylazine (dosage 2 mg/kg). Temperature, pulse, and respiration were monitored every 10 min. The surgical procedure for the tubal ligation (Howe 2006) involved a 2- to 3-cm incision along the midline of the abdomen, exposing the horns of the uterus, and locating the ovary and oviduct. The oviduct was clamped and then tied off 1 cm either side of the clamp. A 1-cm section of the oviduct was then cut and removed. The ovary and uterus were then returned to the normal positions in the body cavity. The incision was then closed via three separate suturing procedures involving the peritoneum, subcutaneous tissues, and skin.

Surgical vasectomy involved bilateral removal or occlusion of the portion of the ductus deferens (Howe 2006). The vasectomy was performed through a 1- to 2-cm incision located in the inguinal area. Following skin and subcutaneous incision, the spermatic cords were identified, separated, and exteriorized. Manipulation of the testicle identified the spermatic cord and ductus deferens. Following isolation of the ductus deferens, a segment of the ductus was then removed and both of the severed ends of the ductus ligated. The incision was then closed via three separate suturing procedures involving the peritoneum, subcutaneous tissues, and skin.

Following the surgical procedure, each coyote was aged by visual inspection of tooth wear (Gier 1968), ear-tagged, and radio-collared. We reversed the effects of the xylazine with the antagonist yohimbine (dosage 0.15 mg/kg) after the surgery was completed. An analgesic (butorphanol; dosage 0.4 mg/kg) was administered immediately following surgery for post-operative pain management. We applied ophthalmic ointment to prevent corneal desiccation. Animals held overnight were monitored for any post-operative complications. The following morning, animals were inspected and then returned to their respective sites of capture. Control animals (intact coyotes) underwent a sham surgery following the exact same procedures without the final tying of the tubes (thereby remaining reproductively intact), so that all else (including the surgery) was controlled. This method (sterile vs. control) has previously been documented to show no impact to subsequent survival, dispersal, and behaviors of surgically sterilized coyotes (Bromley and Gese 2001b). Close monitoring of all animals released into the wild following surgery showed no complications or deaths due to the surgical procedures. Research protocols were approved by the Institutional Animal Care and Use Committees at the USDA/National Wildlife Research Center (QA-1350) and Utah State University (IACUC #1269).

To determine with greater certainty that treated coyote packs were sterile (i.e., we captured and sterilized one or both of the breeding pair), we conducted howling surveys (Harrington and Mech 1982; Fuller and Sampson 1988) and searched for dens and pups of radio-collared individuals. Howling surveys were conducted during June to mid-August, with personnel going to high points, howling, and recording whether the response included pups. In addition, visual observations of radio-collared individuals allowed us to gain information on pup presence. Any pack found to have pups was considered intact.

Determination of pack size

We compared pack sizes between sterile and intact packs using the observed minimum pack size. We made multiple

visual observations of radio-collared individuals to count associated pack members. Field personnel would home-in on a radio-collared animal, attempting to approach animals from downwind in a stealthy manner to reduce disturbance of additional pack members that may be present. Group size, location, and pup presence were noted. We did not include pups in pack size estimations, but estimated pre-whelping pack size (Gese et al. 1989).

Home range size and overlap

We acquired telemetry locations primarily at dawn and dusk to obtain point locations during the highest activity periods (Andelt and Gipson 1979). We attempted to locate animals every 2 days. We calculated locations using ≥3 compass bearings in Program LOCATE (Pacer, Truro, Nova Scotia, Canada). All home ranges were computed using only locations with an error polygon <0.10 km^2. We calculated home range size using the 95 % fixed kernel (FK) density estimator and core area with the 50 % FK density estimator in ArcGIS 9.2 (Environmental Systems Research Institute, Redlands, CA, USA) with the Hawth's Tools extension (Hawth's Analysis Tools); bandwidth was set to $h = 1{,}000$. We calculated home range estimates (home range size and overlap) for two breeding seasons (breeding season 1: December 2006–March 2007; breeding season 2: October 2007–March 2008), and one pup-rearing season (April–September 2007).

We compared percent overlap among adjacent packs for the 95 and 50 % FK contours using analysis of variance (ANOVA). We used only adjacent packs to calculate percent overlap, including adjacent packs with zero overlap. Packs were considered adjacent if their home range boundaries were <2 km apart; this figure represents the radius of the smallest home range and was the minimum area we used to exclude the potential presence of a home range in which the pack members were not radio-collared. We made comparisons of home range overlap among adjacent sterile–sterile packs, intact–intact packs, and sterile–intact packs. Tests for differences between each pair were performed with a Tukey's Honestly Significant Difference (HSD) test. All statistical tests were performed using SPSS 10.0.5 (SPSS, Chicago, IL, USA).

Home range fidelity

Familiarity of the home range, and therefore territory fidelity, is important in reducing the vulnerability of coyotes to human persecution (Knowlton et al. 1999). We tested home range fidelity of resident coyotes using known fate models in Program MARK (White and Burnham 1999); animals were censored after dispersal. We defined dispersal as the movement of an animal from its point of origin to where it reproduces or would have reproduced if it had survived and found a mate (Howard 1960). We compared models of residency rates between sterile and intact coyotes with Akaike's Information Criterion (AIC; Akaike 1973) corrected for small sample size bias (ΔAICc; Burnham and Anderson 2002). We grouped coyotes by treatment (sterile or intact) and encounter occasions were expressed as 15 1-month intervals. Because treatment was of primary interest, all models included this variable. Hypotheses tested included residency varying by treatment alone, treatment and 4-month season, or treatment and 1-month time interval (Table 1: models 1, 2, 3). For examining home range fidelity, we used 4-month seasons based on biological changes in coyote behavior, including the breeding season (December–March), pup-rearing season (April–July), and dispersal season (August–November; adapted from Gese and Ruff 1998). We also tested a model which examined the interactive effect between treatment and time (the most parameterized model, Table 1: model 4). We censored transient animals from the analysis unless and until they became established as residents later in the study.

Survival rates

We examined survival rates of intact and sterile coyotes because, if sterilization changed coyote behavior and they dispersed, these animals would become more vulnerable to human persecution (Windberg and Knowlton 1990;

Table 1 Model selection for residency rates of sterile ($n = 15$) and intact ($n = 12$) coyotes, Piñon Canyon Maneuver Site, Colorado, December 2006–March 2008

Model no.	Model structure	AICc	ΔAICc	AICc weights	Model likelihood	K[a]	Deviance	Evidence ratio (w_1/w_i)
2	{R(treatment + season)}	62.583	0.000	0.686	1.000	5	14.630	1.00
1	{R(treatment)}	64.151	1.568	0.313	0.457	2	22.344	2.19
3	{R(treatment + time)}	76.242	13.659	0.001	0.001	16	4.761	927.04
4	{global R(treatment × time)}	103.889	41.306	0.000	0.000	30	0.000	NA[b]

[a] Number of parameters

[b] Evidence ratios could not be calculated because model weight was = 0

Table 2 Model selection for survival rates of sterile and intact coyote ($n = 30$), Piñon Canyon Maneuver Site, Colorado, December 2006–March 2008

Model no.	Model	AICc	Delta AICc	AICc weights	Model likelihood	K[a]	Deviance	Evidence ratio
1	{S(treatment)}	47.907	0.000	0.336	1.000	2	43.876	1.00
5	{S(treatment + season)}	48.377	0.471	0.266	0.790	5	38.224	1.27
3	{S(treatment + age)}	49.536	1.629	0.149	0.443	3	43.474	2.26
4	{S(treatment + kg)}	49.871	1.965	0.126	0.374	3	43.810	2.67
2	{S(treatment + sex)}	49.923	2.016	0.123	0.365	3	43.861	2.74
6	{S(treatment + time)}	65.058	17.151	0.000	0.000	15	33.795	5,606.83
7	{global S(treatment × time)}	94.335	46.429	0.000	0.000	30	29.239	NA

[a] Number of parameters

Windberg 1996; Harris and Knowlton 2001). We compared estimates of survival rates between sterile and intact coyotes in Program MARK using known fate analysis (White and Burnham 1999). We compared models of survival rates using ΔAICc (Akaike 1973; Burnham and Anderson 2002). Coyotes were grouped by treatment and models included three covariates: gender, age class, and weight. We analyzed survival over 15 1-month occasions. We created models based on gender, age class, weight, coyote season, or monthly time interval and always included the variable treatment since this was our variable of interest (Table 2: models 1–6). Except a global model (Table 2: model 7), all hypothesized models were restricted to additive models due to limited sample size.

Results

Pack size

We captured and radio-collared 30 coyotes. We sterilized 16 (mean age 3.3 years, range 1–8 years old) animals from the southern portion of the study area and sham-operated 14 (mean age 2.5 years, range 1–8 years old) coyotes from the northern portion; ages were not different between the two areas ($P = 0.12$). Defined home ranges contained 1–2 radio-collared individuals. During the first breeding season, we documented 8 sterile and 10 intact home ranges. During the subsequent pup-rearing season, we defined 8 sterile and 9 intact home ranges. We documented 6 sterile and 8 intact home ranges during the second breeding season. Mean pack size of sterile packs (2.3 ± 0.3; 95 % CI) was not significantly different than intact coyote packs (2.10 ± 0.3; $t_9 = 0.607$, $P = 0.554$).

Home range size and overlap

Home range sizes were not different between sterile and intact coyote packs during any of the three seasons. During the first breeding season, mean home range sizes of intact ($n = 10$) and sterile ($n = 8$) coyote packs were 24.0 ± 3.8 (95 % CI) and 22.3 ± 3.9 km^2, respectively ($t_{16} = 0.601$, $P = 0.556$; Fig. 1a). During the pup-rearing season, home range sizes of intact ($n = 9$) and sterile ($n = 8$) coyote packs were 26.4 ± 6.8 km^2 and 24.7 ± 4.4 km^2, respectively ($t_{15} = 0.405$, $P = 0.692$; Fig. 1b). During the second breeding season, home range sizes of intact ($n = 7$) and sterile ($n = 6$) coyote packs were 20.6 ± 4.9 and 22.0 ± 4.0 km^2, respectively ($t_{11} = -0.421$, $P = 0.682$; Fig. 1c).

Home range overlap was expressed as a proportion of total home range area. During the first breeding season, mean overlap between adjacent sterile home ranges was 0.251 ± 0.081 (95 % CI) and mean overlap between adjacent intact home ranges was 0.139 ± 0.076. Mean overlap between adjacent sterile and intact home ranges was 0.060 ± 0.069. During the first breeding season, core areas of adjacent sterile home ranges had a mean overlap of 0.033 ± 0.032 (SD). Core areas of adjacent intact home ranges overlapped by 0.001 ± 0.001 and core areas of adjacent sterile–intact home ranges had no overlap. We found differences in overlap of adjacent sterile core areas compared to adjacent intact core areas ($P = 0.020$). This relationship appeared to be mainly due to the overlap of core areas between only 2 sets of sterile home range pairs (Fig. 1a). We did not find any other differences in overlap during the first breeding season (Table 3a).

Mean home range overlap during the pup-rearing season among adjacent sterile home ranges (0.266 ± 0.073 95 % CI) was different from the mean overlap among adjacent intact home ranges (0.113 ± 0.061; $P = 0.006$). Differences in overlap among adjacent sterile home ranges and adjacent sterile–intact home ranges were also significant (0.057 ± 0.074, $P = 0.007$). However, there was no evidence of difference in the overlap among adjacent intact home ranges and adjacent sterile–intact home ranges ($P = 0.639$). Core area overlap during the pup-rearing season was also different among adjacent sterile home

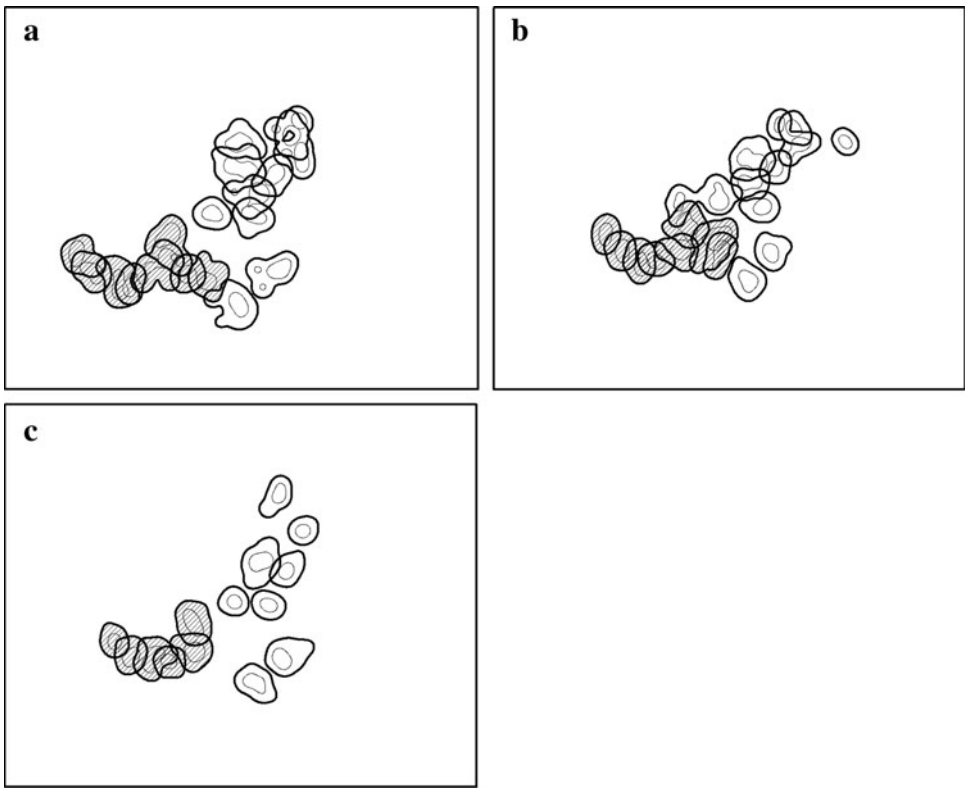


Fig. 1 Plots of 95 and 50 % fixed kernel estimates of individual coyote home ranges during **a** breeding season 2006–2007, **b** pup-rearing season 2007, and **c** breeding season 2007–2008, Piñon Canyon Maneuver Site, Colorado. Sterile home ranges are represented by *cross-hatching*

Table 3 Tukey's Honestly Significant Difference comparison of home range and core area overlap between sterile and intact coyote home ranges during the first breeding season, pup-rearing season, and second breeding season, Piñon Canyon Maneuver Site, Colorado, December 2006–March 2008

Season	Area	Group comparison	*P*
1st breeding	95% home range	Sterile–intact	0.118
		Sterile–sterile	0.181
		Intact–intact	0.734
	50 % core	Sterile–intact	0.020
		Sterile–sterile	0.343
		Intact–intact	0.999
Pup-rearing	95 % home range	Sterile–intact	0.006
		Sterile–sterile	0.007
		Intact–intact	0.639
	50 % core	Sterile–intact	0.043
		Sterile–sterile	0.200
		Intact–intact	0.999
2nd breeding	95 % home range	Sterile–intact	<0.001
		Sterile–sterile	0.011
		Intact–intact	0.982
	50 % core area	Sterile–intact	0.312
		Sterile–sterile	0.733
		Intact–intact	0.999

ranges (0.028 ± 0.030) and adjacent intact home ranges (no overlap, $P = 0.043$). No other differences in core area overlap were found (Table 3b).

Mean overlap of adjacent sterile home ranges (0.208 ± 0.074 95 % CI) during the second breeding season was different from adjacent intact home ranges (0.012 ± 0.017, $P < 0.001$). We also found a difference among mean overlap of adjacent sterile home ranges and adjacent sterile–intact home ranges (no overlap). We found no differences in overlap during the second breeding season (Table 3c).

Because age could affect overlap, we tested for differences in ages between sterile and intact coyotes. We found no difference in mean age between sterile and intact coyotes ($t_{28} = -0.976$, $P = 0.337$). We found no differences between sample sizes used to define home ranges for sterile and intact coyotes (first breeding season: $t_{16} = -0.429$, $P = 0.674$; pup-rearing season: $t_{15} = -0.249$, $P = 0.807$; second breeding season: $t_{13} = -0.147$, $P = 0.885$) which may also influence home range overlap. We also found no correlation between location sample sizes used to determine home range and percent overlap of home ranges (first breeding season: $R^2 = 0.013$, $F_{53} = 0.676$, $P = 0.415$; pup-rearing season: $R^2 = 0.013$, $F_{45} = 0.601$, $P = 0.442$; second breeding season: $R^2 = 0.017$, $F_{29} = 0.480$, $P = 0.494$).

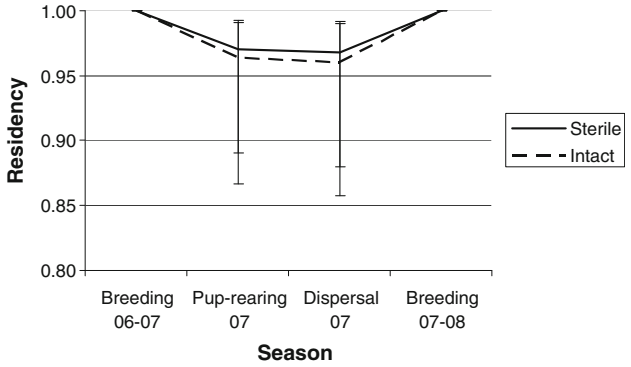


Fig. 2 Coyote residency rates (±95 % CI) from the top model, {R(treatment + season)}, in 4-month seasonal increments for sterile and intact coyotes, Piñon Canyon Maneuver Site, Colorado, December 2006–March 2008

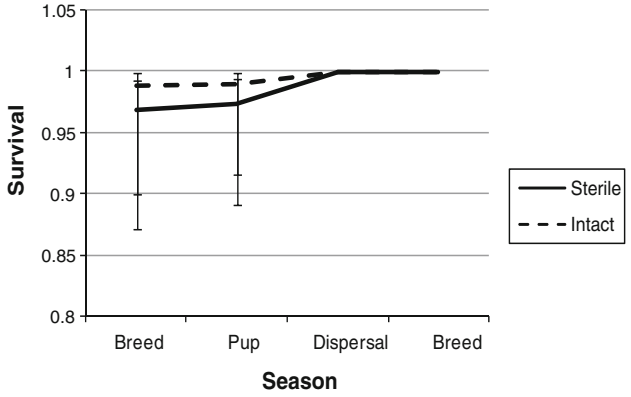


Fig. 3 Coyote survival rates (±95 % CI) from the second-ranked model, {S(treatment + season)}, in 4-month seasonal increments for sterile and intact coyotes, Piñon Canyon Maneuver Site, Colorado, December 2006–March 2008

Home range fidelity

Six radio-collared coyotes (20 %) dispersed during the study. Three of these dispersals occurred during the pup-rearing season and 3 during the dispersal season. No radio-collared coyotes dispersed during the breeding seasons. The best model for coyote residency was {R(treatment + season)} (Table 1: model 2). This model was 2.2 times as plausible as the second-best model {R(treatment)} (Table 1: model 1). Models 3 {R(treatment + time)} and 4 {R(treatment × time)} were not well supported by the data (evidence ratios 927.04 and NA, respectively; Table 1). Residency was highest during the breeding seasons and dropped during the pup-rearing and dispersal season (Fig. 2). Model averaging showed that derived residency rates (the probability of remaining a resident through the duration of the study) were not different between sterile ($\hat{r} = 0.779$, 95 % CI 0.496–0.927) and intact ($\hat{r} = 0.738$, 95 % CI 0.432–0.913) coyotes ($z = 0.239$, $P = 0.406$).

Survival rates

We analyzed survival rates of 17 male and 13 female coyotes; 8 males and 8 females were sterilized. Four coyotes perished during the study: 3 due to gunshot wounds, 1 due to unknown causes. Many of the models used to analyze coyote survival rates were competitive. The first 5 models were within <2.016 ΔAICc values from each other, indicating that all 5 were plausible (Burnham and Anderson 2002). The best-fit model, {S(treatment)} (Table 2: model 1), suggested sterile coyotes had lower survival rates than intact coyotes (sterile: $\hat{s} = 0.805$, 95 % CI 0.540–0.936; intact: $\hat{s} = 0.923$, 95 % CI 0.608–0.989). The second-ranked model, {S(treatment + season)} (Table 2: model 5), showed an increasing trend in survival over the seasons and higher survival in intact coyotes, but the confidence intervals between the groups overlapped (Fig. 3). Model averaged derived estimates of survival rates (i.e., probability of surviving the duration of the study) of sterile and intact coyotes were not different (sterile: $\hat{s} = 0.809$, 95 % CI 0.544–0.938; intact: $\hat{s} = 0.924$, 95 % CI 0.611–0.990). When we calculated statistics for the third-, fourth-, and fifth-ranked models, {S(treatment + age)}, {S(treatment + weight)}, and {S(treatment + sex)}, we found the covariates were not significant ($P > 0.280$). Other models had ΔAICc values >2.016. In a post hoc analysis, {S(·)} (coyote survival rate is not influenced by any variables) was ranked as the top model and {S(season)} was ranked second.

Discussion

As sterilization becomes more widely used in canid research and management practices, we must confirm territorial maintenance and pair-bonding behaviors are being retained. Without these behaviors, the social structure of the pack will dissolve leading to an unguarded, open territory, and management efforts could fail. We found no evidence to suggest territorial behaviors among coyotes were altered by sterilization. Pack size, home range size, home range fidelity, and coyote survival rates were not significantly different between sterilized and sham-operated coyotes. We did find sterile packs exhibited greater home range overlap than intact packs, but it is unknown whether this was due to the effects of sterilization.

Similar to our results, sterile red fox vixens had increased home range overlap compared to non-sterile vixens (Saunders et al. 2002). In contrast, coyotes in Utah did not display differences in home range overlap between sterile and intact packs (Bromley and Gese 2001b). Average overlap between coyote territories in Utah was 21 %, greater than the overall average overlap in our study (14 %). Possibly, sterile coyote

packs on the PCMS were more tolerant of overlap than intact coyote packs. We also tested for age and location sample size differences between the sterile and intact packs to account for the differences in overlap. Younger, low-ranking pack members disperse when resources are not abundant (Gese et al. 1996a). If coyotes in the sterile group were younger than coyotes in the intact group, and location sample sizes were not large enough to accurately detect pre-dispersal forays, then we might mistake these forays for home range overlap. However, we did not find differences in age classes, dispersal rates, or location sample sizes between the groups suggesting that pre-dispersal forays were not occurring differentially between sterile and intact coyotes. Varying location sample sizes were also not correlated to the degree of overlap.

Additionally, 2 dispersers in the second breeding season of previously resident coyotes in the intact area may account for differences observed between home range overlap in this season. One of the dispersers was an adult male coyote located in the center of the intact part of the study area. His initial home range had contributed to overlap in previous seasons. His dispersal coincided with the expansion of adjacent coyote pack home ranges into his previous home range area and may have been the result of displacement (Carbyn 1981). However, the expansion of the neighboring pack's home range was not enough to compensate for the lost overlap. Given consistently high overlap in the sterile home ranges and dispersal events which reduced overlap in the intact home ranges, we believe the disparity in home range overlap was not prompted by sterilization, but most likely had high pre-existing overlap among home ranges in that area.

Home range fidelity of coyotes varied by season and declined during the pup-rearing and dispersal seasons. Pack sizes gradually decline after whelping due to dispersals of non-breeding associated pack members (Bekoff and Gese 2003). We found no evidence that dispersal rates were influenced by sterilization. This corroborates with Bromley and Gese's (2001b) results; they found no difference between residency rates of sterile and sham-operated animals.

Although our results suggested many variables were important to coyote survival rates, sterilization had no significant influence. In addition, a post hoc analysis incorporating the model {S(·)} (coyote survival rate was not influenced by any of the variables), ranked this model at the top when run with the previously described models, further suggesting none of the other variables explained the true effects. Indeed, a Wald's test confirmed them as not significant. Season, as modeled as a coyote biological interval, may have been influential on coyote survival rates. An additional post hoc analysis ranked the model {S(season)} as second only to {S(·)}. However, we must also consider confounding variables such as human persecution. Three of 4 coyote mortalities were caused by gunshot and the fourth mortality suggested human involvement (i.e., the carcass was found <4 m from a gravel road). Although shooting of coyotes was not permitted during the study, 3 of these mortalities were detected during or shortly after military maneuvers involving armed personnel.

Results from this study add to the small body of knowledge we have regarding the effects of sterilization on wild canids. We did not find any results that were in contradiction to other studies on coyotes or wolves. One component lacking in all peer-reviewed studies of coyote sterilization is an assessment of the long-term stability of territorial and social behaviors following sterilization. Mech et al. (1996) monitored vasectomized wolves for 7 years, but the sample size was small and females were not sterilized. Zemlicka (1995) monitored sterilized captive coyotes for 1 year, while Bromley and Gese (2001b) followed the sterile coyotes for 3 years. Despite functioning endocrine systems, after multiple years of no reproductive success sterile coyotes could sever their pair-bond and search for a more successful mate. Hence, we recommend a study of sterile free-ranging coyotes following treated and untreated animals into senescent years. With a long-term study, dispersal by "breeding" individuals (dominant animals which had been sterilized) due to a lack of reproductive success may be detected. Also, by following sterile and intact coyotes to senescence, changes in survival rates may be detected. Because home range overlap of red fox vixens was reported (Saunders et al. 2002) and this study found possible pre-existing home range overlap in sterile coyotes, disruption of territory boundaries may warrant further exploration. Tolerance of trespassers into territories may complicate interpretation of experimental results and could result in failed measures for canid management.

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In situ coagulation and transection of the ovarian pedicle: An alternative to laparoscopic ovariectomy in juvenile horses

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Abstract

The feasibility of leaving the ovaries within the peritoneal cavity after laparoscopic coagulation and transection of the ovarian pedicle was assessed in the juvenile horse. Elective ovariectomy was performed on 10 quarter horses, aged 4 to 5 mo, with the fillies in a Trendelenburg position. The mesovarium was isolated, and multiple coagulation and transection cycles were performed until all ovarian attachments had been severed. The ovaries were dropped within the abdomen, and hemostasis of the transected mesovarium was evaluated before closure. The mean surgical time was 33 min (range, 23 to 48 min). Ten weeks after surgery the fillies were humanely euthanized. At postmortem examination, the ovary location within the abdomen was noted. In 1 horse, there was an abdominal adhesion; viscera had been punctured during insufflation. Of the 20 ovaries, 4 were free-floating within the abdominal cavity; the other 16 were enveloped in the free portion of the greater omentum in the cranioventral abdomen. Histologic examination of the ovaries was performed to assess follicle cell viability. In both the free-floating and the attached ovaries, the deep blood vessels and all examined follicular structures were necrotic and partially mineralized. Laparoscopic electrosurgical transection of the ovarian pedicle without removal of the ovaries should be considered an alternative to other ovariectomy techniques that may be performed in young female horses.

Résumé

La faisabilité de laisser les ovaires à l'intérieur de la cavité péritonéale après coagulation et trans-section du pédicule ovarien par laparoscopie a été évaluée chez des chevaux juvéniles. Une ovariectomie élective a été effectuée chez 10 chevaux Quarter horse, âgés de 4 à 5 mois, avec les juments dans une position Trendelenburg. Le mesovarium a été isolé, et des cycles multiples de coagulation et de trans-section ont été effectués jusqu'à ce que les attaches ovariennes aient été coupées. Les ovaires ont été laissées libres dans l'abdomen, et l'hémostase du mes-ovarium trans-sectionnné a été évaluée avant fermeture. Le temps moyen de chirurgie était de 33 min (écart, 23 à 48 min). Dix semaines après la chirurgie, les juments ont été euthanasiées. Lors de l'examen post-mortem, la localisation intra-abdominale des ovaires a été notée. Chez 1 cheval il y avait une adhésion abdominale; les viscères avaient été perforés au moment de l'insufflation. Parmi les 20 ovaires, 4 étaient libres dans la cavité abdominale alors que les 16 autres étaient enveloppés dans la portion libre du grand omentum dans la partie crânio-ventrale de l'abdomen. L'examen histologique des follicules a été effectué afin d'évaluer la viabilité cellulaire des follicules. Autant chez les ovaires libres que ceux attachés, les vaisseaux sanguins profonds et toutes les structures folliculaires examinées étaient nécrotiques et partiellement minéralisées. La trans-section électrochirurgicale par laparoscopie du pédicule ovarien sans le retrait des ovaires devrait être considéré comme une alternative aux autres techniques d'ovariectomie pouvant être effectuées chez de jeunes juments.

(Traduit par Docteur Serge Messier)

Introduction

Bilateral ovariectomy is an elective procedure commonly used to prevent pregnancy, manipulate the estrus cycle, manage colic associated with estrus, and modify behaviour associated with the estrus cycle (1,2). In an extensive survey of horse owners in 1996, members of the American Association of Equine Practitioners reported that the estrus cycle has a negative impact on the performance of athletic females (3). Horse owners have reportedly discriminated against using intact mares for athletic endeavours because of undesirable behaviour during estrus (1).

Treatment options to minimize undesirable influences of estrus are limited to medical management with exogenous progestins or surgical removal of both ovaries. Progestins are expensive, daily administration is required when they are given orally, and there are health risks to the person administering the daily medication (4). In our opinion, high morbidity rates with traditional ovariectomy techniques have led to reluctance by owners to have the procedure

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performed. Surgical methods of ovariectomy include colpotomy, flank incisions, ventral incisions, and, more recently, laparoscopic techniques (5–12). Serious complications associated with colpotomy for ovary removal include exsanguination, shock, adhesions, abdominal pain, and peritonitis (1,6). Body wall incisions for ovary removal have been reported to require lengthy convalescence and to have incisional complications ranging from seroma formation to dehiscence (13). These complications have led to the development of less invasive laparoscopic techniques (8,11). Laparoscopic ovariectomy (ventral or flank) provides excellent visualization of the ovary and mesovarium and allows tensionless manipulation of the mesovarium during pedicle transection, visual assessment of hemostasis, smaller body wall incisions, and, in some cases, removal of both ovaries through the same operative site (8–12,14).

The numerous methods used for hemostasis of the mesovarium and the associated ovarian vessels have included ligature application (10), laser techniques (11), electrocoagulation (12,15), and use of the harmonic scalpel (16), stapling instruments (17), and vascular clips (18). Common problems are technical difficulty applying the hemostatic devices and the expense associated with consumables. Ligature slippage has also been associated with inadequate hemostasis (19). Compared with other methods, electrocoagulation has resulted in reliable hemostasis, is technically straightforward, and is inexpensive (12).

Laparoscopic surgery can be technically demanding for the operator. Operative time is directly related to the surgeon's experience. After transection of the 1st ovary, the surgeon usually needs to identify an area to store the ovary before removing the 2nd ovary to prevent loss of the pneumoperitoneum (8). If an ovary is inadvertently dropped during removal and cannot be observed with the laparoscope and grasped, the standard of practice is to remove the instrument cannula and extend the incision to allow manual retrieval of the dropped ovary. Extending the incision negates the advantage of minimal invasiveness associated with laparoscopy. The consequence of failing to remove the transected ovary is unknown.

It is common practice to leave amputated ovaries free in the abdomen of cattle undergoing ovariectomy via colpotomy, and no detrimental effects have been reported (20). If ovariectomies in the horse could be performed satisfactorily with laparoscopic coagulation and transection of the ovarian pedicles without removal of the organs, numerous advantages could be realized in addition to rendering the ovaries nonfunctional. Leaving the ovaries within the abdomen would improve on currently described laparoscopic techniques by reducing surgical time and technical demands on the surgeon, improving cosmesis, and minimizing the risk of incisional complications. The purpose of this project was to assess the feasibility of laparoscopic ovariectomy without removal of the ovaries from the abdomen of juvenile horses.

Materials and methods

With approval of the University of Saskatchewan Animal Care and Use Committee, laparoscopic ovariectomy was performed on 10 healthy quarter horse fillies aged 4 to 5 mo and weighing 160 to 180 kg. The animals were housed together in a large paddock and had unlimited access to feed and water. Before surgery, feed was withheld for 24 to 36 h. All the fillies received a tetanus toxoid (Ayerst, Guelph, Ontario) booster intramuscularly before surgery. Procaine penicillin G (Ethacillin; Roger/STB, London, Ontario), 22 000 U/kg, was administered intramuscularly 1 h before surgery and once after surgery. The horses received flunixin meglumine (Banamine; Schering–Plough, Pointe Claire, Quebec), 0.5 mg/kg intravenously every 8 h, beginning 1 h before surgery and continuing for 48 h. During surgery, heart rate, direct arterial blood pressure, and respiratory rate were monitored, and electrocardiographic analysis was performed. Surgical time (defined as the time from initial insertion of the laparoscope to incision closure), intraoperative and postoperative complications, perioperative attitude, and postmortem and histologic findings were recorded.

Anesthesia was induced with a combination of xylazine hydrochloride (Rompun; Bayer, Etobicoke, Ontario), 1.0 mg/kg, ketamine hydrochloride (Vetalar; Vetrepharm, London, Ontario), 2.0 mg/kg, and diazepam (Sabex; Boucherville, Quebec), 0.02 mg/kg, administered intravenously. After endotracheal intubation, the fillies were placed in dorsal recumbency with their hindlegs tied to the surgical table. Anesthesia was maintained with halothane in oxygen, delivered through a rebreathing system. Ventilation was assisted with positive-pressure ventilation. All animals received butorphanol tartrate (Torbugesic; Ayerst), 0.05 mg/kg, administered intravenously, at the start of the surgical procedure. Surgical preparation included clipping the ventral abdomen from the xyphoid to the inguinal region, followed by aseptic surgical preparation.

Surgical procedure

A 2-cm-long skin incision was made 1 to 2 cm caudal to the umbilicus at the midline. A 5-mm-long stab incision was made in the middle of the skin incision through the linea alba, and a teat cannula was passed through the peritoneum into the abdominal cavity. Correct placement was confirmed by the ability to feel abdominal viscera with the end of the teat cannula. The teat cannula was connected to a high-flow electronic insufflator (Karl Storz Veterinary Endoscopy, Goleta, California, USA), and the abdomen was distended with carbon dioxide to a pressure of 15 mm Hg. The teat cannula was then removed, and a guarded sharp pyramidal trocar sheathed in a cannula with a diameter of 11 mm (Dr Fritz Endoscopy and Video Systems, Tutlingen, Germany) was thrust firmly into the abdominal cavity in the same location. Care was taken to ensure that pressure applied to the trocar–cannula unit was perpendicular to the linea alba. The sharp trocar was removed and replaced by a 10 mm × 33 cm, 30° laparoscope (Karl Storz Veterinary Endoscopy) connected to a 300-W xenon light source (Karl Storz Veterinary Endoscopy) and laparoscopic camera (Linvatec Canada, Mississauga, Ontario). Insufflation was maintained at a pressure of 10 to 15 mm Hg during the procedure. The surgery table was inclined to roughly 30°, with the head downward (Trendelenburg's position), to displace the abdominal viscera cranially.

After the caudal abdomen was observed, 2 instrument portals were made, 15 to 20 cm caudal to the laparoscope portal and 5 to 10 cm to the right and left of the midline. Two incisions, 10 to 15 mm long, were made through the skin and the external abdominal oblique fascia, parallel to the linea. Instrument cannulas 10 mm in diameter were placed with blunt trocars under direct observation

with the laparoscope. The instrument cannulas were fitted with adapters (Karl Storz Veterinary Endoscopy) that reduced the diameter of the portals from 10 mm to 5 mm to accommodate the 5-mm bipolar electrosurgical forceps (Kleppinger bipolar forceps; Richard Wolf Medical Instruments, Rosemont, Illinois, USA) and the 10-mm laparoscopic scissors. Laparoscopic atraumatic Babcock grasping forceps were inserted through both working portals, and the ovaries or uterine horns were located in the caudal abdomen abaxial and caudal to the apex of the bladder. Large and small intestine was gently manipulated to locate the reproductive tract, then traction was applied to the ovaries to allow manipulation of the ovarian pedicles. Atraumatic forceps passed through the ipsilateral portal were used to grasp an ovary at the cranial pole. The proper ovarian ligament was extended between the ovary and the tip of the uterine horn. Proper positioning was accomplished by passing the ovary back and forth between 2 atraumatic grasping forceps until the mesovarium was easily accessible.

The ovary was manipulated with a Babcock forceps placed through the ipsilateral portal to ensure that adequate distance was maintained between the mesovarium and the viscera. A 5-mm bipolar electrosurgical forceps was inserted through the contralateral instrument portal and placed across the cranial aspect of the mesovarium. Electrical current (11.5 W) was applied until the grasped tissue bubbled and turned white, indicating adequate coagulation. The tissue was then transected with laparoscopic scissors. Multiple coagulation and transection cycles were required to completely transect the ovarian pedicle. After the ovarian artery was transected, it was specifically targeted for additional electrocoagulation. Once coagulation and transection of the entire mesovarium was complete, the ovary was dropped in the abdomen, and the tip of the uterine horn and mesovarium were elevated and observed for hemorrhage. The 2nd ovary was coagulated, transected, and dropped in a similar manner.

After completion of the surgical procedure, the pneumoperitoneum was relieved by opening the valves of the 3 cannulas and placing manual pressure on the ventral abdominal wall. The incisions in the abdominal wall were apposed with 1 polyglyconate cruciate suture (Maxon; Tyco Health Group Canada, St. Laurent, Quebec). The skin incisions were closed with 3-0 poliglecaprone sutures (Monocryl; J & J Medical Products Ethicon, Markham, Ontario) in a continuous subcuticular pattern. The horses were then returned to a horizontal position, allowed to recover from anesthesia, and gradually introduced to feed over the next 8 h.

After close observation for 48 h after surgery, the fillies were returned to a large outdoor paddock for twice-daily examination for the remainder of the study. They were introduced into an antibiotic pharmacokinetic trial 6 wk after surgery and euthanized with a barbiturate 10 wk after surgery.

Postmortem and histologic examination

Postmortem examination concentrated on location and retrieval of the transected ovaries, the state of the mesovarium, and intra-abdominal adhesion formation. The ovaries were photographed, transected longitudinally and processed for histologic examination. The ovarian remnants were fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. All serial sections were completely assessed.

Results

The surgical procedure was performed by a board-certified surgeon and a surgical resident. The procedure was not considered technically difficult by either surgeon, and no major operative complications were experienced. There was a trend towards shorter surgical times as experience increased. The mean surgical time was 33 min (range, 23 to 48 min).

In 1 filly, the teat cannula used for insufflation was inadvertently placed into an abdominal viscus, as indicated by the presence of greenish liquid in the hub of the cannula. The cannula was removed and replaced with a clean teat cannula. This filly was not elevated into the Trendelenburg position until laparoscopic evaluation of the abdomen was performed. The punctured viscus was not located, and the surgical procedure was completed as planned. The horse showed no signs of discomfort or colic after the ovariectomy. At necropsy, a fibrous adhesion 2 cm in diameter was noted, extending from the tip of the cecum to the omentum.

The bipolar electrosurgical system was easy to use and provided adequate coagulation of the mesovarium. The proper positioning of the ovary (outstretched proper ovarian ligament) with the grasping forceps was vital to efficient application of the electrosurgical forceps across the mesovarium. The sequential coagulation and transection was considered the rate-limiting step of the surgical procedure. Roughly 5 mm of tissue could be coagulated at 1 time; on average, 6 to 10 coagulation and transection cycles were required per ovarian pedicle. Intraoperative hemorrhage was minimal and only occurred if the mesovarium was transected in an area before adequate coagulation. Transection of the right ovarian artery in 1 horse without appropriate coagulation resulted in excess hemorrhage; the hemorrhage was quickly controlled by grasping the cut end of the artery with the electrosurgical forceps and then cauterizing the vessel. Repeat coagulation was required less than 3 times for all ovarian pedicles.

None of the fillies showed any signs of colic or inappetance after laparoscopic ovariectomy. All had mild swelling at the laparoscopic portal sites, but these regions were not painful on palpation. No incisional complications occurred in any of the fillies.

Four of the ovaries were found free-floating within the abdomen. The other 16 ovaries were enveloped in the free portion of the greater omentum, adjacent to the spleen, in the cranioventral abdomen. All the ovaries had brownish discolouration throughout the parenchyma of the cut sections and appeared to have multiple old follicles. The areas of transected mesovarium and associated uterine horn showed no adhesions or signs of inflammation on gross examination. There were no adhesions directly related to the surgical procedure except in the horse with visceral puncture.

Histologic examination of the attached ovaries consistently showed a band of fibrous tissue, interpreted to be omentum, surrounded the ovary. In all ovaries, the superficial parenchyma contained many mononuclear cells laden with golden pigment. Areas of the tunica albuginea, and some superficial stromal cells, appeared viable. The deep stromal cells were partially mineralized, and all

deep blood vessels and all examined follicular structures were necrotic and partially mineralized. The attached and free-floating ovaries had similar histologic changes in the deep vasculature and follicular structures.

Discussion

Ventral abdominal laparoscopy was performed in these fillies because of their smaller size (standing procedures would be more difficult) and the relatively unhandled nature of the group before the study. There should be no reason the same procedure could not be undertaken with the horse standing, as previously described (9–12). The standing horse, if cooperative, could minimize the added challenge of proper orientation of the ovary before coagulation and transection: suspension of the ovary from its dorsal attachments would allow excellent access to the mesovarium for coagulation. Standing surgery would also decrease the risks and expense of general anesthesia.

The mesovarium, once the ovarian bursa is removed, is divided into a thick medial component and a thin lateral component (12). During our operations, both components were cauterized and transected. Because the medial segment contains the vascular supply of the ovary, other authors have performed coagulation and transection of the medial segment and simply transected the lateral component, without any complications (12). Operative time could be reduced if this technique were used.

Electrosurgical coagulation and transection of the ovarian pedicle was chosen for several reasons. First, electrocoagulation is a reliable means of mesovarium hemostasis in the horse (12). It was also an economical alternative: other techniques necessitate the purchase of costly consumable materials. Confidence in the degree of coagulation. Standing surgery before transection increased greatly with the number of surgical procedures. Other instruments that we considered for coagulation included the recently described bipolar vessel-sealing device (15) and the tripolar cutting forceps (Kendall; Tyco Healthcare Group LP, Mansfield, Massachusetts, USA). The feedback-controlled bipolar vessel-sealing device provides excellent hemostasis while precise energy is applied to seal the vessels; however, it was considered economically unfeasible at the time. The tripolar instrument was not available.

The use of only 2 instrument portals was feasible because of the ease with which the electrosurgical forceps could be applied to the mesovarium and exchanged with the laparoscopic scissors via 1 portal. Limiting the number of portals decreased the time required to suture the abdomen closed and theoretically decreased patient morbidity. The described techniques for multiple working portals may be more appropriate for the removal of unilateral diseased ovaries, such as those with granulosa cell tumours (8).

An unexpected result of the study was the noticeable lack of abdominal discomfort and inappetance among the fillies after surgery. The use of flunixin meglumine, 0.5 mg/kg, could be expected to give some degree of analgesia (21). Multiple studies have found that mares undergoing laparoscopic ovariectomy sporadically exhibit signs of colic in the face of a higher dose of flunixin (1.1 mg/kg) or phenylbutazone (4.4 mg/kg, given orally every 12 h) (8–10,12). In a large proportion of these previously described horses, a local anesthetic was injected into the ovarian pedicle before transection. In our experience, postlaparoscopic colic seems to be limited to ovariectomy patients, whereas horses undergoing cryptorchidectomy and exploratory laparoscopy rarely exhibit postoperative pain. The addition of butorphanol tartrate (0.05 mg/kg, given intravenously at the start of the procedure) should also aid in short-term analgesia (22), but long-term medication for pain control was not necessary. The nerves associated with the ovaries are derived from the sympathetic system through the renal and abdominal aortic plexuses (23). To our knowledge, no study has examined the development of the ovarian nervous supply in the maturing horse. Younger animals may have incomplete or less developed innervation of the ovarian pedicle. Although we studied only 10 juvenile horses, our findings suggest that patient morbidity might be decreased if ovariectomy were performed in younger animals. In addition, the juvenile vascular structures are smaller, and hemostasis may be more readily achieved.

The adhesion between the tip of the cecum and the omentum in 1 filly was not surprising. The location of the adhesion and the fact that intestinal contents were seen in the hub of the insufflation needle at the time of surgery suggest that the teat cannula was placed in the cecum. Before the filly's hindquarters were elevated for surgery, the entry point of the teat cannula was not observed with the laparoscope. Sufficient insufflation and preoperative fasting should decrease the risk of inadvertent puncture of a viscus. Careful insertion of the teat cannula through the peritoneum will also decrease the chance of visceral damage.

Similarly, the attachment of a large proportion of the ovaries to the omentum can be rationalized. Gravity would tend to displace the unattached ovaries to the cranioventral aspect of the abdominal cavity. The omentum is particularly prone to adhesion formation because it can move freely throughout the peritoneal cavity (24). The omentum and its ability to cover serosal defects have been extensively described (25,26). It has been experimentally demonstrated that the omentum will adhere to ischemic bowel within 48 h of contact (25). Contributing to adhesion formation is the fact that the entire surface of the ovary is not covered with a mesothelial layer: the dorsal or convex surfaces, where the vessels and nerves enter, are devoid of a peritoneal lining (27). Coagulation and transection exposes the cut edge of the mesovarium and the portion of the ovary without a peritoneal lining while causing local inflammation and ischemia. Adherence of the ischemic ovaries to the free portion of the greater omentum in the ventral abdomen is likely if the exposed connective tissue is unable to repair itself with mesothelial cells. Ischemic bowel adhering to omentum undergoes neovascularization 3 to 4 d after attachment (25). Since, as we observed histologically, the deep vascular supply and all follicular structures of the ovaries were nonviable, attachment of the omentum to the ovaries and neovascularization must have occurred at a rate incompatible with cell survival. In contrast, in feline species in which ischemic ovaries were surgically transected and implanted into the small intestinal mesentery in the abdomen, the ovarian tissue attached and revascularized, producing measurable hormone levels, before complete cell death (28). The difference in this study, other than species, was

that the ovaries were tethered to the mesentery with suture and sectioned before implantation. This eliminated the time required for the ovaries to come into contact with the omentum and, therefore, decreased the time required for revascularization of the ovarian tissue. Similarly, complete transection of the mesovarium and not the ovarian vessels alone was deemed necessary to promote avascular necrosis of the ovary. In situ coagulation and transection of the testicular cord of cryptorchid testicles without removal from the inguinal ring resulted in revascularization and subsequent production of testosterone (29).

Unlike the ovaries of other domestic mammals, equine ovaries have a peripheral zone of connective and vascular tissue around a central parenchymatous zone containing developing and atretic ovarian follicles, corpora lutea, and corpora albicantia (30). Transection of the ovarian pedicle removes the blood supply to the ovary. Revascularization by the omentum, if rapid enough, could provide the ovarian cells with a new blood source before avascular necrosis. The fact that the structures responsible for hormone production and follicle development are deep in the ovaries of the horse may account for the difference observed between the cat and the horse. It is plausible that the deeper structures of the transected ovaries underwent cellular death and necrosis before revascularization.

Hormonal assays were not performed in this group of horses because of their age (4 to 5 mo). Puberty in the horse is generally accepted to occur between 12 and 24 mo (31), and for that reason serum hormonal analysis in these horses could not be relied upon. The procedure needs to be repeated in mature, actively cycling mares and the behavioural and hormonal effects analyzed.

The envelopment of the majority of the transected ovaries in the free portion of the greater omentum brought about the question of potential complications. The possibility of the attached ovaries acting like a mesenteric lipoma and causing strangulation of a portion of bowel was considered. The fact that the ovaries were enveloped in the omentum and did not have a stalk decreased the likelihood of strangulation. The gravitational force associated with a suspended abdominal mass is generally regarded as a major factor in the development of a stalk. In our horses, the enveloped ovaries lay in the cranioventralmost aspect of the abdomen, such that stalk development was unlikely.

Our results were encouraging, and use of this surgical procedure to control undesirable estrus behaviour in the mare should be considered. In the young, acyclic horse, laparoscopic ovariectomy involving coagulation and transection of the ovarian pedicle without removal of the ovaries from the abdominal cavity appears to be an alternative to other ovariectomy techniques. There was no postoperative morbidity, no peritoneal adhesions were associated with the dropped ovaries, and avascular necrosis was achieved in all the deep blood vessels and all follicular structures of the coagulated and transected ovaries.

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Routine castration in 568 draught colts: incidence of evisceration and omental herniation

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Keywords: horse; castration; eventration; evisceration; complications; herniation

Summary

Reasons for performing study: **Castration is one of the most common routine surgical procedures performed in the horse, from which a number of potential complications can arise. We undertook a prospective evaluation of short-term complications associated with castration of draught colts over a 3-year period (1998–2000).**

Objectives: **To compare castration complications in a large number of draught foals with previously published literature.**

Methods: **Five hundred and sixty-eight draught colts, age 4 or 5 months, were castrated in field conditions. Foals were observed for complications for 24 h post operatively.**

Results: **There was no significant difference in complication rates between open and closed surgical techniques. Inguinal/scrotal hernia rate was 4.6% (26/568) prior to surgery, and evisceration of the small intestine occurred in 4.8% (27/568). Foals observed to eviscerate underwent immediate surgical correction with an overall survival rate of 72.2% (13/18). Omental herniation was seen in 2.8% (16/568) of colts.**

Conclusions: **This study showed no difference between the closed and open techniques of castration and the rate of omental herniation or evisceration. The evisceration rate in combination with the omental and presurgical herniation rates approached 12.2%, which is high enough to warrant further examination.**

Potential relevance: **Future investigation should help to assess predisposing factors for evisceration. Regardless of the technique employed, herniation appears to pose a significant risk to draught foals undergoing castration.**

Introduction

Castration has been reported as one of the most common routine surgical procedures performed in the horse (Hutchins and Rawlings 1972; Thomas *et al*. 1998; Schumacher and Trotter 1999). The reason most often cited for castration in the horse is to reduce masculine behaviour in animals unsuitable for the genetic pool and, less commonly, due to testicular neoplasia, trauma, inguinal hernias or torsion of the spermatic cord.

Castration can be performed in either the standing or recumbent animal. In the standing horse, common practices include use of a combination of sedation and analgesia with local anaesthesia (Schumacher and Trotter 1999). In the recumbent animal, surgery is performed typically after the administration of general anaesthesia with short-term i.v. agents (Searle *et al*. 1999). Techniques used to castrate horses include open, half-closed or modified open, and closed, which have been described previously (Schumacher 1996; Searle *et al*. 1999; Schumacher and Trotter 1999). Most scrotal incisions are stretched for adequate drainage and allowed to heal by second intention, although primary closure has been advocated (Cox 1984; Barber 1985).

Complications that can arise following surgical castration include scrotal swelling or oedema, excessive haemorrhage, schirrous cord formation, peritonitis, omental herniation, incisional infections, hydrocele, penile trauma and evisceration (eventration) (Hunt 1991; Moll *et al*. 1995; Railton 1999). With the exception of peritonitis and evisceration, the majority of castration complications are not considered life-threatening. Evisceration or prolapse of a portion of intestine through the inguinal canal and out the scrotal incision is considered an emergency. Evisceration typically occurs within the first 4 h of castration (Hunt and Boles 1989), although intestinal prolapse has been reported up to 6 days after surgery (Boussauw and Wilderjans 1996). The majority of eviscerations in juvenile horses occur as an indirect herniation (through the vaginal tunic). Recent publications have identified the following clinical features that have a significant negative influence on survival following evisceration: an inguinal surgical approach for correction; an increased length of prolapsed bowel; and bowel resection and anastamosis (Thomas *et al*. 1998). One aspect still lacking in published literature is an acceptable explanation regarding eventration in the horse.

This paper presents a large study population of castration complications in which the surgical setting and the nature of the surgical patient allowed for accurate identification and reporting of only a few complications commonly associated with castration in the horse.

Materials and methods

Horses and facilities

From 1998–2000, the Western College of Veterinary Medicine accumulated data from the castration of 568 draught (Belgian and

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Percheron) colt foals. Large numbers of foals are assembled in the autumn at a local stockyard for processing and castration. The colts were run through a hydraulic chute system and processed, including an i.m. injection of procaine penicillin G (Ethacillin)[1] (22,000 u/kg bwt) and a tetanus toxoid vaccine[2]. The foals were administered a short-term injectable anaesthetic i.v. and released from the chute complex. Castrations were performed on the floor of a large pen (14 x 14 m) covered with 10–15 cm sand and ample amounts of clean wheat straw. All castrations were performed in either left lateral or dorsal recumbency with the legs restrained using heavy cotton ropes. Once recovered, the foals were given access to feed and water, observed by a veterinarian for 24 h and then transported to surrounding ranches.

Anaesthesia

Foals were estimated to weigh 325–350 kg. Anaesthesia was induced with 2.2 mg/kg bwt i.v. ketamine hydrochloride (Vetalar)[3] combined in the same syringe with 1.0 mg/kg bwt xylazine (Rompun)[4]. In the majority of cases, the initial dose of injectable anaesthesia was adequate to perform a routine castration. If the anaesthetic plane was deemed inadequate for the procedure, a second dose of the combined injectable anaesthetic was titrated to effect. Towels were placed over the eyes during the procedure and foals recovered unaided. If a prolonged anaesthetic period was anticipated, anaesthesia was maintained with 0.3% thiopental[5] and 5% guaifenesin[6] dissolved in 5% dextrose solution delivered by gravity feed to the patient via an i.v. catheter. In these cases, anaesthetic depth was monitored with ocular reflexes, heart rate, patient movement, respiration rate and strength of palpable arterial pulses.

Surgical procedure

Castrations were performed using either the open or closed technique. The open technique was performed by making 2 parallel scrotal incisions 1–2 cm apart and 8–10 cm long. The incisions were carried through the *tunica dartos*, scrotal fascia and parietal tunic. The ligament of the tail of the epididymis was transected bluntly and the testis, epididymis and distal aspect of the spermatic cord removed with emasculators. Ultimately, this left the entire vaginal tunic *in situ*. The closed technique differed in that the parietal tunic was not incised so that it and all its contents (testis, epididymis and distal aspect of the spermatic cord) were removed. Haemostasis and transection of the spermatic cord and/or parietal tunic was achieved by application of Serra emasculators. Emasculators were applied to the spermatic cords for a minimum of 90 secs. If, at the time of castration, a foal had an inguinal or scrotal hernia, the spermatic cord was transfixed and ligated proximal to the emasculators using 2 polydioxanone (PDS)[7] or 1 polyglyconate (Maxon)[8] suture in a transfixation pattern. Once standing, colts were herded into a common recovery pen where they were observed periodically for signs of excessive haemorrhage, omental herniation or evisceration.

If excessive haemorrhage was observed, the foals were re-anaesthetised and the surgical field explored. Once the offending vessel was located it was ligated with 2-0 polydioxanone (PDS). Omental herniation was corrected surgically once the horse was physically or chemically restrained. The exposed omentum was abducted from the external inguinal ring until clean tissue was encountered and the exteriorised portion was trimmed with Metzenbaum scissors.

Foals that eviscerated shortly after castration were re-anaesthetised, placed in dorsal recumbency and prepared for aseptic surgery. Anatomical identification of the prolapsed viscera was undertaken and the intestines examined for trauma, vascular compromise and motility. The exposed intestines were washed repeatedly with copious amounts of sterile isotonic saline. In all cases where surgical repair was attempted, herniated bowel was replaced into the abdominal cavity via the open vaginal tunic using manual reduction. Care was taken to avoid excessive handling or force being placed on the intestines. If manual manipulation of the intestines failed to achieve reduction, the cranial aspect of the external inguinal ring and/or vaginal ring was enlarged using Metzenbaum scissors. Once intestines had been replaced, the superficial inguinal rings were closed bilaterally using 2 polydioxanone (PDS)[7] in a simple continuous pattern or 1 polyglyconate (Maxon)[8] in a cruciate pattern.

Data recorded during the castrations included technique (open vs. closed), abnormalities found on inguinal palpation prior to and during surgery, omental herniation and eventration. Data were analysed using the Chi-square test and considered significant at $P<0.05$.

Results

A total of 568 foals were studied. Castrations spanned 3 years and only those in which records were complete were included.

Inguinal/scrotal hernias were detected by palpation prior to surgery in 26 horses (4.6%) and, in these, the vaginal tunic was sutured closed without immediate complication. One of these horses eviscerated post castration. Bilateral inguinal herniation was palpated in one colt prior to surgery.

There were a total of 233 castrations performed utilising the open technique (41.0%) and 335 using the closed technique (59.0%). Omental herniation had an overall incidence of 2.8% (16/568). Six of these (37.5%) occurred following open castration and 10 following closed castration (62.5%). Evisceration occurred following castration in 27 horses (4.8%). Open and closed castration techniques resulted in 8 (29.6%) and 19 (70.4%) eviscerations, respectively. One foal showed marked abdominal pain (violent rolling) just prior to eventration. Small intestine was reported as the eviscerated viscera in all cases. There were no significant differences between techniques (open vs. closed) with regard to omental herniation or evisceration rates ($P = 0.973$ and 0.294, respectively).

The time from castration to evisceration, method of castration and outcome of surgery if performed were noted for all foals (Table 1). Records showed that 20 foals eviscerated within 3 h of castration (3.5%) compared to 7 (1.2%) after 3 h for an overall rate of 4.8%. Foals without surgical correction died or were subjected to euthanasia. Survival rate for foals undergoing surgery for evisceration was 72.2% (13/18).

Discussion

These foals were under the direct supervision/care of a veterinarian for the first 24 h after castration. The fact that the veterinarian was informed only when complications were considered life-threatening (evisceration) and that foals were moved away within 24 h made it unrealistic to evaluate complications such as swelling and infection rates. Evisceration and omental herniation were considered complications that

TABLE 1: Surgical outcome associated with evisceration in 27 cases

Case	Method	Time of evisceration	Outcome
1	Closed	Recovery	Surgery, died
2	Closed	Recovery	Surgery, survived
3	Closed	<3 h	No surgery, euthanasia
4	Closed	<3 h	Surgery, died
5	Closed	<3 h	Surgery, survived
6	Open	Recovery	Surgery, survived
7	Closed	Recovery	Surgery, survived
8	Open	<3 h	Surgery, survived
9	Closed	<3 h	Surgery, survived
10	Open	<3 h	Surgery, survived
11	Closed	<24 h	No surgery, dead
12	Open	<3 h	Surgery, survived
13	Open	<3 h	Surgery, survived
14	Closed	<3 h	Surgery, dead
15	Open	<6 days	No surgery, dead
16	Closed	<3 days	No surgery, dead
17	Closed	<6 days	No surgery, dead
18	Closed	<6 days	No surgery, dead
19	Closed	<6 days	No surgery, dead
20	Open	<3 h	Surgery, survived
21	Closed	<24 h	No surgery, euthanasia
22	Open	<3 h	Surgery, dead
23	Closed	<3 h	Surgery, survived
24	Closed	<3 h	Surgery, euthanasia
25	Closed	<3 h	Surgery, survived
26	Closed	<3 h	Surgery, survived
27	Closed	<6 days	No surgery, dead

allowed for accurate clinical evaluation. The incidence of inguinal hernias detectable by palpation prior to surgery of these draught foals is difficult to compare to other breeds because of the lack of reported incidence in the literature. It is also probable that a number of incipient or small hernias were not detected at the presurgical examination. Ligation of the common vaginal tunic of all foals found to have an inguinal hernia was a precautionary procedure. It was understood, however, that ligation of the tunic proximal to the site of emasculation might not prevent inguinal herniation but would limit evisceration (Boussauw and Wilderjans 1996). The ligature essentially seals off the common vaginal tunic to the exterior but not the inguinal canal from the abdomen. Therefore, it is possible for bowel to herniate into the inguinal canal, as reported by Boussauw and Wilderjans (1996). The fact that one foal eviscerated following bilateral ligation of the tunic suggests that the ligature was improperly placed or broke, or that the tunic ruptured, allowing the intestines to pass through the inguinal canal and out through the scrotal incision.

Omental herniation has not been reported clearly or consistently over the years. Most reference to omental herniation is in the context of overall herniation, including omental and intestinal prolapse. van der Velden and Rutgers (1990) stated that eventration of the small intestine makes up 67% of cases while omental prolapse comprises the remainder. The incidence of 2.8% we observed does not vary substantially from this value. If combined, the omental herniation and evisceration of small intestine in our study showed an overall number of 43 cases. The percentage of these that were omental herniations approached 37% (16/43). Of the 16 horses with omental herniation, no colt proceeded to eviscerate or show any negative signs associated with the surgical procedure of trimming the omentum. This result supports the suggestion by others that omental herniation is not considered a surgical emergency (Railton 1999; Searle *et al.* 1999). The lack of statistical significance between the open and closed techniques for both omental herniation and evisceration was not surprising. If the surgeon does not place a ligature around the parietal tunic during a closed castration, there is the same communication between the peritoneal cavity and the exterior of the scrotum no matter which technique is employed. The application of the emasculators provides only a tenuous seal after the crushing action of the device on the severed end of the tunic. This seal would provide little or no mechanical resistance to the passage of intestines from the inguinal canal to the exterior of the scrotal incisions. There still seems to be an unjustified opinion amongst practicing veterinarians that one method or the other is associated with more complications (Railton 1999).

The incidence of evisceration reported in this study is much higher than previous reports of 2.96% (Hutchins and Rawlings 1972), 0.2% (Moll *et al.* 1995) and 0.4% (van der Velden and Rutgers 1990). It has been reported that draught and Standardbred horses have a higher rate of evisceration compared to other breeds (Hutchins and Rawlings 1972; Moll *et al.* 1995; Schumacher 1996). The breed predilection for evisceration may require an anatomical variation of the inguinal structures within breeds (Cox 1987; Hutchins and Rawlings 1972) but this study could not address this because the parentages of all the foals were not known.

The pathogenesis of evisceration following castration has been the subject of hypotheses (Hunt and Boles 1989; van der Velden and Rutgers 1990) related to the size of the inguinal ring (internal and external), leg position on recovery, increased intra-abdominal pressure and excessive exercise. The significance of inguinal ring size has been disputed (Green 2001) and the vaginal ring is considered the more appropriate structure responsible for the passage of viscera into the inguinal canal. This extension of the peritoneal lining forms a slit at the dorsal aspect of the entrance of the deep inguinal ring (Schumacher and Trotter 1999). Green (2001) stated that it is impossible for a testis to pass normally through the vaginal ring into the inguinal canal after the first few weeks *post partum*. Clinically, the difficulty in delivering an abdominally retained testis through the vaginal ring would support this contention. In contrast, some believe the vaginal rings can be enlarged by flexing the hind legs of a horse in dorsal recumbency (van der Velden and Rutgers 1990). Once again, this is hard to explain anatomically. The problem with current theories is that they fail to explain the difference between the castrated individual and the intact horse. If it were just a matter of vaginal ring size, leg position or increased intra-abdominal pressure, why is there a lack of evidence for a higher rate of inguinal and scrotal herniation in intact males? The surgical procedure of castration must itself alter some biomechanical aspect of the equine abdomen and inguinal canal.

The abdominal cavity has been described as a pressure chamber from which several openings lead (Hahn-Pedersen *et al.* 1994). At each opening the mechanical problem arises of allowing normal content to exit while avoiding herniation. The vaginal ring is just one example of a normal opening in the abdominal cavity. Research in male human subjects has shown the importance of the pampiniform plexus in the prevention of inguinal hernias (Hahn-Pedersen *et al.* 1994). In normal inguinal canals, a rise in intra-abdominal pressure caused a parallel increase in the cross-sectional area of part of the pampiniform plexus located in the medial end of the canal, thereby filling the empty space and preventing herniation. Castration using emasculators would alter the haemodynamics by removal of the pampiniform plexus. The alteration in the venous aspect of the testis could make the vaginal

ring more accessible to intestinal contents. The rarity of evisceration in geldings after the perioperative period may be explained by inflammation creating a mechanical seal to the inguinal canal.

In another study, tension (dilation) across the internal inguinal ring of pigs was found to increase under various conditions (Peiper *et al.* 2001), such as coughing and pneumoperitoneum. It appears important to have increased intra-abdominal pressure to exert a significant force on the inguinal rings. Once the emasculator is applied to the parietal tunic during castration, the new communication created between the abdomen and exterior of the peritoneal cavity should have immediate effects. We know abdominal surgery causes increased intra-abdominal pressure in dogs (Conzemius *et al.* 1995). A clinically important cause of the increased pressure in the horse may be continuous straining after castration. In the absence of increased abdominal pressure, the vaginal ring should physically retain the intestines within the peritoneal cavity. The abdomen or pressure chamber now has a leak and this hole, if patent, should not allow large pressure increases to occur. If the increase in intra-abdominal pressure is important in the events leading up to evisceration, something must inhibit the free flow of gas in and out of the abdomen. It is possible that the vaginal ring, now tensionless following transection of the spermatic cord, acts as a release valve for the abdomen. Under low pressures the vaginal ring is sealed and it is not until elevated intra-abdominal pressures are achieved that air is allowed to escape past the valve. The intestines and abdominal gas act similarly to any material under pressure. If compressed gas or fluid is allowed sudden access to a path of decreased resistance, the material follows that path. This could account for the lodging of small intestine into the inguinal canal and possibly through the scrotal incisions.

The importance of the size of the vaginal ring should not be underestimated. If the ring is too small it should resist the passage of intestine unless extremely elevated intra-abdominal pressures are experienced. If the ring is too large it will not form an effective seal post castration and therefore will not allow elevations in intra-abdominal pressures necessary to herniate intestines. It seems plausible that the combination of altered venous distension of the spermatic cord, vaginal ring size, creation of a patent lumen to the exterior of the abdomen and pressure changes within the peritoneal cavity are all factors in evisceration in the horse.

Survival rate of colts following surgical repair of evisceration in our study was 72.2% (13/18); previously reported survival rates are 36.4% (Hutchins and Rawlings 1972), 72.0% (Thomas *et al.* 1998), 87.5% (Hunt and Boles 1989) and 85–100% (van der Velden and Rutgers 1990). Although our survival rates were considerably lower than some reported values, surgical correction of evisceration in our cases took place in the field under less than ideal surgical conditions and post operative care consisted of close monitoring at the local ranches in the following days. The main feature of the foals that eviscerated was the rapid surgical correction of the prolapsed intestines. This supports the findings that prognosis for survival decreases with increasing time, contamination of and damage to eviscerated intestines and deteriorating condition of the horse (van der Velden 1988; Thomas *et al.* 1998). Historical data suggest that an inguinal approach to correcting eviscerations is 5 times less successful than a midline approach (Thomas *et al.* 1998). No midline approach was attempted in these foals; however, the high success rate using the inguinal approach would challenge this assertion. The placement of the foals in dorsal recumbency with their hind legs flexed provided rapid adequate access to the inguinal canals (van der Velden 1988) in a less than ideal surgical environment.

In conclusion, this study found no difference between the closed and open techniques of castration and the rate of omental herniation or evisceration; and indicated that either method of castration is equally safe/risky in the short term. The overall palpable inguinal herniation rate (4.6%) in the presurgical patient was substantial and shows the importance of a thorough presurgical physical examination. The combined herniation rate in this population of horses, when considering presurgical hernias, omental hernias and evisceration, approached 12.2%. The authors believe that this alarming rate warrants further examination. Future investigation should help to assess genetic predisposition. In the interim, modification of standard castration techniques aimed at limiting post operative evisceration needs to be critically assessed.

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Manufacturers' addresses

[1]Rogar/STB Inc., London, Ontario, Canada.
[2]Ayerest, Guelph, Ontario, Canada.
[3]Vetrepharm, London, Ontario, Canada.
[4]Bayer Inc., Etobicoke, Ontario, Canada.
[5]Abbott Laboratories, Ltd., St Laurent, Quebec, Canada.
[6]BDH Inc., Toronto, Ontario, Canada.
[7]Ethicon, c/o Johnson & Johnson, Peterborough, Ontario, Canada.
[8]Tyco Health Group Canada, St Laurent, Quebec, Canada.

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ORIGINAL ARTICLE

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The long-term effects of ovariectomy on bone metabolism in sheep

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Abstract Osteoporosis and associated fractures are major public health concerns, and as such require appropriate large animal models to further our understanding of this disease. Although sheep appear to be an ideal model with which to study bone loss caused by estrogen depletion, limited data are available concerning the long-term effect of ovariectomy on bone in sheep. The goal of the present study was to observe the ovariectomy-induced changes in bone mass, structure, and metabolism in sheep over a period of 18 months. Six ewes were ovariectomized (OVX) and compared to an age-matched control group by analyzing bone mineral density, trabecular structure, biochemical markers of bone formation and resorption, and plasma estrogen levels. Bone loss (13%, $P < 0.01$) occurred during the first 4 months after surgery, then stabilized and returned to pre-OVX levels for the remainder of the study. Trabecular architecture was also altered and tended toward osteopenia with recovery to baseline values. Markers of bone formation and resorption were elevated up to 6 months post-ovariectomy, after which time levels returned to baseline values. Although estradiol measurements demonstrated a clear decline following surgical ovariectomy, levels returned to normal after 6 months. Therefore, the detrimental effect of ovariectomy on sheep bone metabolism seems to be reversible, with normal bone parameters being reestablished within 6 months after surgery. These data seem to indicate that the sheep is not an appropriate model for human postmenopausal osteoporosis.

Key words osteoporosis · experimental model · sheep · ovariectomy · estradiol

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Introduction

Because of the dramatic increase in the elderly population and the rise in the incidence of fractures, osteoporosis has become a major public health concern [1–3]. Because new treatment strategies and better fixation methods in such fragile bones cannot be developed in vitro, there is a great need for suitable animal models that closely simulate osteoporosis in humans [4].

Various experimental models of human osteoporosis have been described in several different species [4]. The ovariectomized rat is probably the most widely studied rodent osteoporotic model. This model mimics postmenopausal cancellous bone loss over a relatively short time period [5,6]. However, extended studies over 12 months have demonstrated increased bone mineral content, bone area, and body weight, which is not comparable to the human situation [7,8]. Furthermore, the lack of the Haversian system in cortical bone, the absence of impaired osteoblast function during late stages of estrogen deficiency, and the absence of multicellular unit-based remodeling in young rats also limits this model [9]. An additional requirement of experimental osteoporosis models is the ability to assess the performance of surgical implants. Such procedures are clearly impractical in the experimental rat model due to limitations in size. The "FDA Guidelines for Preclinical and Clinical Evaluation of Agents Used in the Treatment of Prevention of Postmenopausal Osteoporosis" recommend agent evaluation in two different species including ovariectomized rats and one nonrodent large animal model [10]. Although these guidelines primarily pertain to pharmaceutical drugs, preclinical evaluation is also required for new surgical treatment options. Therefore, there is an obvious need for large as well as small animal models of osteoporosis.

Large animal osteoporosis models include nonhuman primates, dogs, and sheep. Monkeys such as baboons and macaques have an advantage over other osteoporosis models because of their close physiological resemblance to humans, including gastrointestinal tract, endocrine system,

and bone metabolism [11–13]. Female monkeys have hormonal patterns and a monthly cycle very similar to those of humans. Aged macaques and baboons experience a similar decline in ovarian function and irregular menstrual cycles as in peri- and postmenopausal women. However, for ethical reasons and reasons of handling costs, the experimental use of these species is rather limited.

Dogs possess cortical bone with a Haversian system and manifest internal cortical and cancellous bone remodeling processes similar to those in humans. Studies have evaluated the effects of ovariectomy on histomorphometric variables in cancellous and cortical bone in several breeds of dog [14–19]. Although significant changes in cancellous bone volume were observed in these studies, the data are conflicting and suggest that canine experimental osteoporosis may not be the most suitable animal model for studying bone loss following ovariectomy. More importantly, fracture rates in aged, sedentary dogs without ovaries and uteri are not comparable to those of postmenopausal women [20]. Finally, emotional attachment to dogs, along with the stringent legal housing requirements, contribute to the difficulties in using this experimental model of osteoporosis.

Sheep have been extensively studied as an experimental model of bone disease [4]. Bone loss in sheep is associated with estrogen deficiency, and their hormone profiles are similar to those of women [21–24]. Their size permits the implantation of prosthetics, and isolation of large quantities of fluid samples as well as iliac crest biopsies [25]. Although reports have not described consistent decreases in bone mineral density or bone mass at different anatomical locations, significant changes in these parameters have been demonstrated in the distal radius and spine [26]. Biochemical markers of bone formation such as bone-specific alkaline phosphatase were reported to increase in sheep after ovariectomy [24], indicating an increase in bone turnover similar to the human postmenopausal situation. However, only a 10% reduction in sheep bone mass has been attained through old age or estradiol privation following ovariectomy, whereas in osteoporotic humans this value is at least fourfold higher [27]. Reports describing the long-term effects of ovariectomy in sheep are limited, and studies have predominantly focused on animals post mortem or in large time intervals [22]. Furthermore, the reported effects of ovariectomy on estrogen levels in ewes are highly controversial [28,29]. In the present study, we examined the long-term effects of ovariectomy on bone density, structure, and metabolism in sheep. Multiple parameters of bone turnover including mineral density [using peripheral quantitative computed tomography (pQCT)], 3-D trabecular architecture, and biochemical markers are investigated and their association with estradiol hormone levels determined.

Materials and methods

Study design

The European and Swiss laws on animal experimentation were strictly observed during the entire study, and the animal research protocol was approved by a competent ethical committee. Fourteen female white alpine sheep with a mean age of 3.1 ± 0.7 years at the beginning of the study were included in this study. They were divided into two groups based on bone mineral density (BMD) and body weight so that there would be no statistical differences between the means. Six sheep were ovariectomized (OVX) while the others (n = 8) were used as a control group (control).

The sheep were held in group boxes under standardized conditions and natural light, and their body weight was measured weekly. During the entire observation period, the animals were fed twice daily with silage (1.5 kg/animal·day; 10.3 g/kg calcium, 4.1 g/kg phosphorus), hay (0.25 kg/animal·day; 6.3 g/kg calcium, 2.8 g/kg phosphorus), and straw as well as water ad libidum. Urine and venous blood samples were collected on days 28, 14, and 1 before surgery and every fourth week after surgery for quantification of bone markers and hormones. The BMD was measured at the distal radius by pQCT 2 weeks before surgery and at 3-month intervals after surgery. For micro-CT measurements, bicortical iliac crest biopsies were taken at the time of the ovariectomy and at 3, 6, and 9 months after surgery. The study ended 18 months after surgery.

Surgical procedures

In April 2003, six ewes (treatment group) were subjected in supine position to midventral laparatomy under general anesthesia with isofluran (Halocarbon Laboratories, River Edge, NJ, USA) and intravenous Temgesic (buprenorphine HCl 0.3 mg/ml; Reckitt & Colman Pharmaceutics, Hull, UK). Bilateral ovariectomy was performed, with care being taken to ligate doubly the ovarian ligament, artery, and vein. Postoperative pain medication was given as necessary.

Bicortical transiliacal biopsies were taken under general anesthesia in all 14 animals bedded in prone position. To cut these out, a trephine drill (TL 05 trephine 2.0; external diameter 7.3 mm, internal diameter 6.4 mm) under constant irrigation was used. The length of the biopsies was given by anatomical conditions, as the transfixion was made 1 cm below the iliac crest, alternating medial and lateral from the spina iliaca, either on the right or on the left pelvic bone.

BMD measurement

Bone mineral density (BMD in grams per cubic centimeter) was measured by pQCT using a Densiscan 1000 (SCANCO Medical, Bassersdorf, Switzerland). The distal radius of both sides was examined. To avoid artefacts caused by movement, the measurements were performed under general anesthesia. After fixation of the limb in the CT scanner, a projectional scout view was accomplished to determine the axial position. The joint surface was defined as the starting point using a reference line. A highly filtered low-energy X-ray source was used to enhance the bone contrast. The effective energy of the X-ray beam was always set to 40 keV and 0.5 mil modus. The slice thickness was 1 mm, and a

matrix of 512 × 512 pixels was used, resulting in a spatial resolution of 290 × 290 µm. Ten consecutive slices were obtained, and a contour surrounding the radius on each cross section was detected. The µ50-CT value was calculated using the interior 50% of a section to ensure that only cancellous bone was measured.

Structural parameters in micro-CT

After harvesting transiliacal biopsies and immediate fixation in 70% ethanol, micro-CT scans using MicroCT 40 (SCANCO Medical) were performed. This system uses a fan-beam in a multislice mode. An X-ray tube with microfocus is used as source and a charge-coupled device (CCD) array as detector. The spatial resolution was 22 µm in all directions. The volume of interest included a cube with a side length of 4 mm and was displayed in 14 × 14 × 14 cubic micron voxels. Bone volume/total volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and the degree of anisotropy (DA) were measured.

Bone markers

Venous blood samples were allowed to clot for 30 min at room temperature. The serum then was collected by centrifugation (30 min at 4000 rpm, room temperature). Serum samples were stored at –20°C until assay.

Bone-specific alkaline phosphatase (bALP)

Serum bALP levels were determined by enzyme-linked immunosorbent assay (ELISA), using the Metra BAP EIA kit (TECOmedical; Suhr CH). Principle of the procedure: Metra BAP is an immunoassay in a microtiter strip format utilizing a monoclonal anti-BAP antibody coated on the strip to capture BAP in the sample. The enzyme activity of the captured BAP is detected with a pNPP substrate. Control samples were within the given range [14.9 and 15.4 U/l for low control (range, 9.8–21.1 U/l) and 64.3 and 86.6 U/l for high control (range, 62.0–97.2 U/l)].

Deoxypyridinoline (DPD) cross-links

As no reliable method to measure DPD in sheep urine was found except for the HPLC method, which is very time consuming and laborious for such a large amount of samples, the METRA DPD ELISA was used. HPLC and DPD ELISA have been compared in a parallel measurement with a few samples showing a significant relationship. The METRA DPD ELISA is a competitive enzyme immunoassay (TECOmedical). DPD in the sample competes with conjugated DPD-alkaline phosphatase for the antibody, and the reaction is detected with a pNPP substrate. Control samples were measured as 16.8 and 15.1 nmol/l for low control (range, 13.1–21.4 nmol/l) and 86.2 and 92.2 nmol/l for high control (range, 71.0–115.9 nmol/l). The analysis using the ELISA method was verified by HPLC. The DPD results were corrected for urinary concentration by creatinine, which was quantified by the METRA Creatinine assay from TECOmedical based on a quantitative, colorimetric assay (modified Jaffé method).

Pyridinoline (PYD)

For a quantitative measure of the excretion of pyridinoline (PYD) cross-links in serum, the METRA Serum PYD EIA kit (TECOmedical), a competitive enzyme immunoassay in a microtiter plate, was used. PYD in the samples or standards competes with PYD immobilized on the plate for polyclonal rabbit anti-PYD antibody. Bound antibody is detected by goat antirabbit antibody conjugated to ALP, and the reaction is detected with the pNPP substrate. Control samples were measured as 2.0 and 2.3 nmol/l for low control (range, 1.6–2.6 nmol/l) and 6.5 and 8.1 nmol/l for high control (range, 6.3–10.4 nmol/l).

Hormone estradiol (E_2)

Serum estradiol levels were determined with the 17β-Estradiol ELISA from IBL Hamburg (Germany). Principle of the procedure: an unknown amount of estradiol present in the sample and a fixed amount of estradiol conjugated with horse-radish peroxidase compete for the binding sites of a polyclonal estradiol antiserum coated onto the wells. The manufacturer's protocol was followed as described, and samples were assayed in duplicates. The intra- and interassay coefficients of variation and the limits of detection were 4.9%–5.9%, 6.2%–10.1%, and 3 pg/ml, respectively. All values below the detectable limit were recorded as 0 pg/ml. The controls were within the given ranges (low control, 115 and 101 pg/ml vs. 50–130 pg/ml; high control, 384 and 218 pg/m vs. 168–432 pg/ml).

In addition to the method mentioned above, the serum estradiol levels were determined at an external, independent laboratory (Diavet, Bäch CH) with extensive experience in biochemical veterinary diagnostics. 17β-Estradiol was measured using an electrochemiluminescent-sandwich ELISA.

Statistical analysis

Statistical analysis was performed using SPSS software package (SPSS, Chicago, IL, USA). Results are presented as mean ± standard deviation. For comparison between the groups, analysis of variance (ANOVA) was used for bone markers, and nonparametric testing (Mann–Whitney test) was used for others. The level of significance was set at 0.05.

Results

All animals tolerated the surgical procedures well without any side effects. One ovariectomized sheep died at 16 months of observation of an esophageal infection that was

not related to the treatment methods of this study. During the entire observation period, the mean body weight was 67.6 ± 4.5 kg for control and 67.5 ± 5.3 kg for OVX. The body weight fluctuated over time but the individual changes were below 10% in all sheep. No differences at any time point were found between the groups.

Bone mineral density

The cancellous bone mineral density at distal radius in control sheep remained constant with time at ~0.71 g/cm^3 to 17 months. The course of cancellous BMD in the control group showed small changes with a slight decrease at the beginning of the study followed by minimal increase at 15 months. All changes were not significant compared to baseline. OVX sheep exhibited an rapid, initial phase of cancellous bone loss of –12.7% ($P = 0.008$) between 0 and 4 months postovariectomy. Then, a period of relative stabilization of cancellous bone volume at this osteopenic level occurred between 4 and 12 months, followed by a phase of minimal increases in bone mass until 17 months postssurgery (Fig. 1).

Static bone morphometry and trabecular architecture by micro-CT

Changes of the structural parameter in cancellous bone of the iliac crest are shown in Fig. 2. Following ovariectomy, the parameters bone volume to total volume (BV/TV) (3 months, $P = 0.007$; 6 months, $P = 0.010$) and trabecular thickness (3 months, $P = 0.010$) decreased up to 6 months

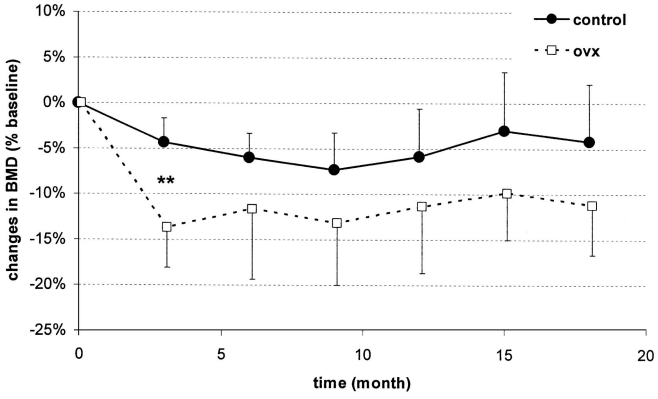


Fig. 1. Cancellous bone mineral density (*BMD*) at distal radius in control and ovariectomized (OVX) sheep. Values are expressed as changes relative to baseline. $^{**}P < 0.01$

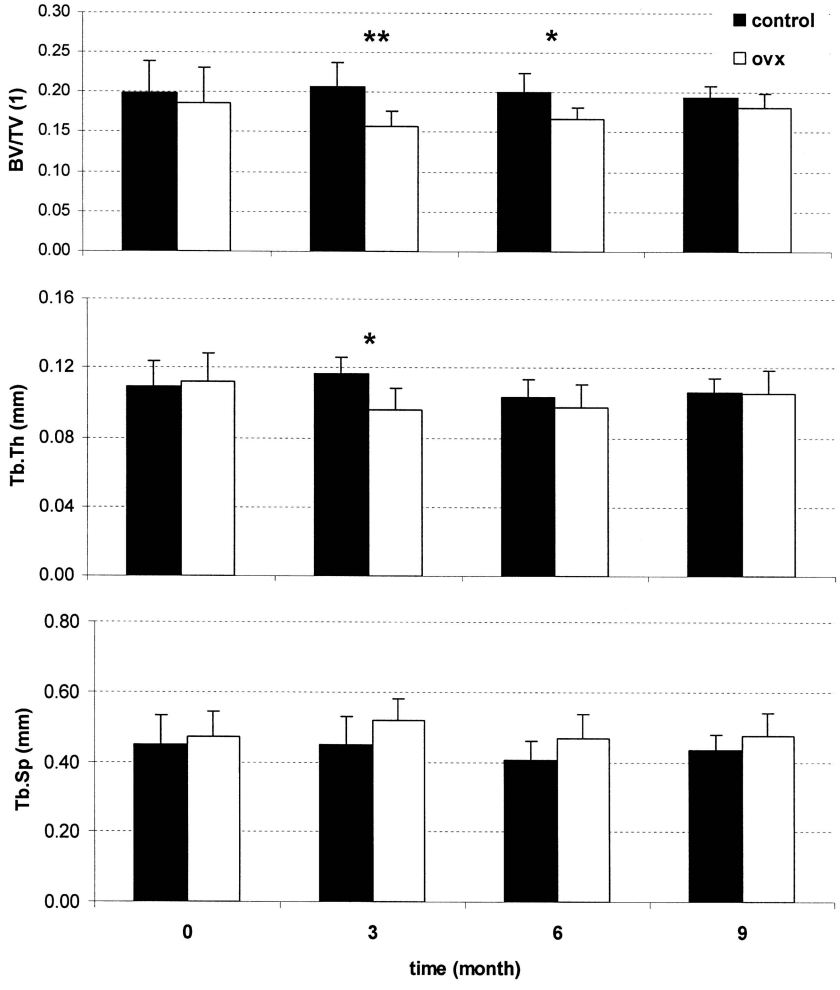


Fig. 2. Structural parameters of cancellous bone at iliac crest biopsies in ovariectomized (OVX) and control sheep. *BV/TV*, bone volume/total volume; *Tb.Th*, trabecular thickness; *Tb.Sp*, trabecular separation. $^{*}P < 0.05$; $^{**}P < 0.01$

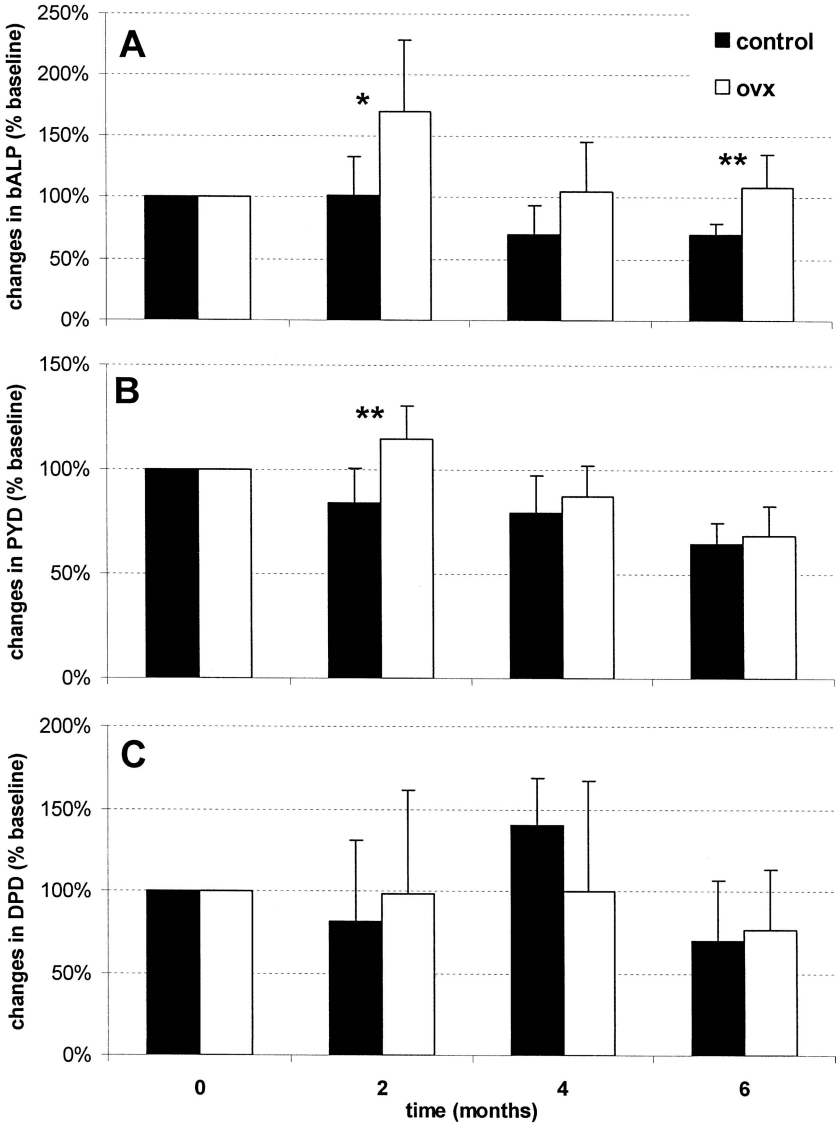


Fig. 3. Markers of bone formation [**A**, serum bone alkaline phosphatase (*bALP*)] and resorption [**B**, serum pyridinoline (*PYD*); **C**, urine deoxypyridinoline (*DPD*)]. Values are expressed as changes relative to baseline (%). $*P < 0.05$; $**P < 0.01$

with a subsequent increase to baseline values. Trabecular number and separation did not change during the observation period.

Bone markers

Figure 3 depicts the serum bALP patterns over the first phase (6 months) of observation. The control group shows seasonal variations that must be considered, whereas in the OVX group there is a 50% elevation of bALP at 2 months after ovariectomy ($P = 0.015$). In this group, bALP decreased until a fall toward baseline values, but was still elevated at 6 months compared to the control group ($P = 0.006$). No differences were found at any later time point. Increased levels of serum pyridinoline were found at 2 months after ovariectomy only ($P = 0.005$), and no differences between the groups were determined at any later time point up to 18 months. The urine DPD values did not show significant differences between the controls and the ovariectomised sheep.

Estradiol

17β-Estradiol decreased 3 months after ovariectomy ($P = 0.021$) in the treated group to values below the detection limit (3 pg/ml) and then increased massively in both groups. The developing of the control values showed a period of anestrus in May and June, followed by the breeding season in fall and winter with higher serum estradiol levels (mean, 20.8 pg/ml) (Fig. 4A). With the exception of significant decrease of estrogen levels following ovariectomy, no differences were found between the two groups (Fig. 4B). The data were validated using a different method at an independent laboratory.

Discussion

This study investigated the long-term effect of ovariectomy on ewes, demonstrating an early bone loss (–13%) followed

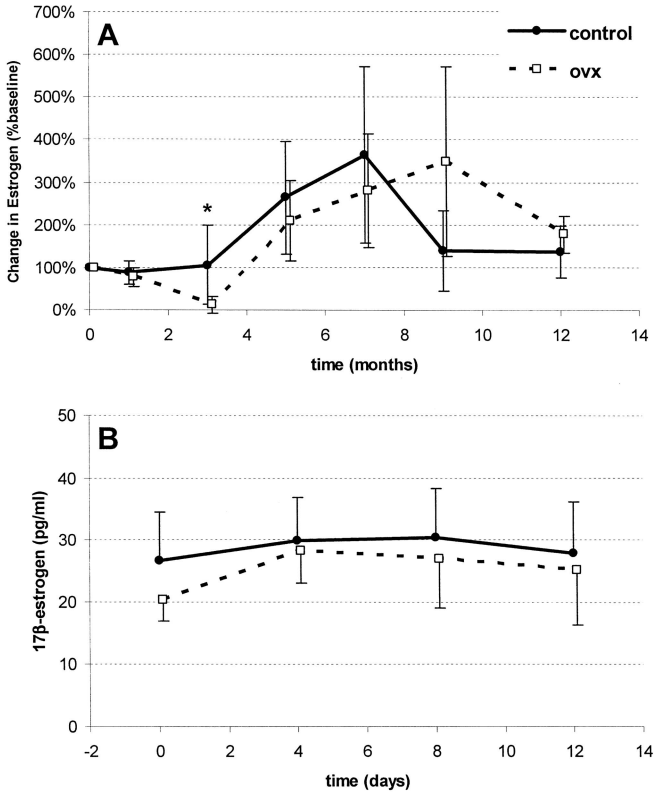


Fig. 4. **A** Serum levels of 17β-estrogen over the course of 12 months presented as changes relative to baseline values. **B** At the end of the study, estrogen was determined at short-term intervals. $*P < 0.05$

by a rebound effect in the later phase of the observation period.

To investigate postmenopausal bone loss and its consequences, there is a great need to develop an osteoporotic animal model for the testing of orthopedic implants as well as for pharmacological research on prevention and treatment. Sheep show several advantages as a model for osteoporosis: easy handling and keeping, availability in large numbers so that large-scale studies are possible, implant dimensions comparable to human implants, similar metabolic rate and bone remodeling process as humans [4,25], and osteopenic response to menopause-simulating manipulations [24,28,29].

The present study evaluated the effect of ovariectomy on bone metabolism in sheep by determining bone mass, bone microarchitecture, and biochemical markers of bone formation and resorption as well as estradiol. Dietary effects were excluded by standardized feeding, and a bias due to physical activity was constrained by single group husbandry. The surgery was performed in spring when the lowest bone mass is usually observed to minimize seasonal influences on bone loss following ovariectomy. As ovariectomy is a standard procedure in our institution [30], the authors omitted a sham group to avoid the use of more animals in the study, as requested by the ethical committee, although all sheep had bilateral transiliacal biopsies. Therefore, a limitation of this study is the lack of a true sham-operated control group because the effect of ovariectomy cannot be distinguished from any influences of wound healing. Prior studies have demonstrated an increased bone turnover following ovariectomy in sheep leading to a loss of bone mass of approximately 10%. Geusens et al. observed a nonsignificant bone loss of –3% to –9% at the femur [31], Lill et al. measured a decrease of 5.5% of BMD at the distal radius in ovariectomized sheep with a calcium-wasting diet [30], and several publications by Turner et al. report a decline of BMD from 3% to 8% [20,24,25]. However, it has not been determined whether these small ruminants experience a short-term effect of ovariectomy or if they develop osteoporosis over a longer period of time as it is the case in humans.

In the present study, most of the bone loss (–13%) occurred during the first 4 months postovariectomy. This initial, rapid phase was followed by a period between 7 and 9 months during which cancellous bone mass appeared to stabilize at the mentioned osteopenic level. Afterward, the bone mass seemed to increase toward baseline values. The changes of bone mass in control group are the result of seasonal changes [20]. During the period between 7 and 14 months, both bone loss and bone turnover subsided. The rapid, initial phase of bone loss is coincident with the maximal increase in bone turnover, which confirms previously reported studies [32]. These data clearly suggest short-term effects on bone mass in ovariectomized ewes.

Several other studies have been determining BMD using dual-energy X-ray absorptiometry (DXA), either on spine, femur, calcaneus, or distal radius, with different results [23,24]. BMD measurements by DXA (a two-dimensional technique) are affected by skeletal size, and changes in density may be obscured by the mass of wool, soiling of the skin, and subcutaneous fat layer [33]. Measurement of volumetric BMD by pQCT, however, is advantageous as it is independent of skeletal size; it allows for selective measurement of cortical and trabecular density and bone area [34].

As many recent studies have shown that BMD alone is not sufficient to determine the strength of cancellous bone, we also looked at static bone morphometry and trabecular architecture using microcomputed tomography [35,36]. Those parameters are important factors in determining bone strength and the risk of fractures. Microcomputed tomography (micro-CT) enables the complete three-dimensional digitization of small specimens at resolutions between 10 and 75 μm. It has the ability to provide quantifiable measures of bone architecture and morphology in three dimensions, giving the exact structure of a bone biopsy. The decrease in BV/TV accompanies a decrease of trabecular thickness. Although the loss of bone mass in postmenopausal women is driven by decrease of trabecular number [37], the results are similar to previous reports [26] and indicate increased bone resorption and thinning of the trabecular structures.

Bone-specific alkaline phosphatase (bALP) has been analyzed to measure bone formation. While the control group shows seasonal fluctuations, an elevation of serum bALP levels until 6 months postovariectomy can be observed indicating an increased bone turnover. No differences between the groups were observed at any time point later than 6 months. Turner et al. [24] as well as

Chavassieux et al. [21] also describe a high bone turnover after ovariectomy. Similar to our results, a trend toward decreasing levels until the end of the study (6 months) can be found. However in both publications, the seasonal timepoint of the ovariectomy is unknown.

To show bone resorption, the pyridinoline cross-links deoxypyridinoline (DPD) and pyridinoline (PYD) have been quantified. Those cross-links are formed during the process of collagen maturation between the chains, and as bone collagen undergoes a higher turnover rate than other tissue sources of collagen, these are specific markers for bone resorption. We found increased levels of bone resorption marker at 2 months after ovariectomy only. Chavassieux et al. [21] observed the highest increase of type 1 collagen C-telopeptide at 3 months after ovariectomy.

By removing the ovaries, the major source of estrogens is eliminated to simulate as much as possible the situation after menopause in women. Very few authors of studies about ovariectomy have tested the estradiol levels of the animals after operation. Johnson et al. writes that ovariectomy does not totally eliminate 17-β-estradiol synthesis in sheep (values between 4 and 6 pg/ml) [28], whereas Karsch et al. postulates that serum estradiol concentrations in intact anestrous ewes average 1 pg/ml and that estradiol levels in ovariectomized animals are well below the limit of detection of any biochemical assay [29]. As the opinions over this topic are divergent and not much literature can be found, estradiol has been quantified in all animals of this study. The results show a significant drop in systemic estrogen levels as a result of ovariectomy followed by increased values without differences in the control group. These data highly suggest the presence of non-ovarian estradiol generation in ovariectomized animals.

Although the ovaries are the principal source of systemic estrogen in the premenopausal nonpregnant woman, estrogen biosynthesis beyond menopause is mainly peripheral, through conversion of androstendione or C19 steroids from the adrenal cortex. Other major sites of estrogen production such as adipose tissue and skin, muscle, and others have the capacity to convert C19 steroids to C18 steroids, but as they lack the ability to synthesize C19 precursors, they are dependent on circulating precursor C19 steroids for estrogen biosynthesis [38,39]. Sheep somehow seem to compensate particularly well for estrogen deficiency, which probably is one of the reasons why ovariectomy only causes slight effects on ovine bone [30]. Further investigations are needed to determine the source of extragonadal extrogen. The ovariectomized sheep still might become a large animal model of postmenopausal osteoporosis, but reduction of nonovarian estrogen production using aromatase inhibitors is indicated. Contrary to glucocorticoid excess, this inhibitor would simulate perfectly the mechanism of bone loss in postmenopausal women. In the study by Chavassieux et al., the administration of lentaron, a specific inhibitor of peripheral aromatase, during 6 months tended to amplify slightly the effects of ovariectomy on bone markers [21]. Because of seasonal variations and calcium intake, experiments in sheep should include all four seasons to minimize the seasonal fluctuations and also a calcium-wasting diet [25].

In summary, this study used a large variety of assessment methods to evaluate the long-term effect of ovariectomy in sheep to establish a large animal model of osteoporosis. Ovariectomy induced bone loss, leading to decreased bone mineral density (–13%) and deterioration of microarchitecture. However, the destructive effect was limited to a few months, and a rebound was observed in all treated animals. Our results suggest a short-term bone loss after ovariectomy in sheep is probably caused by extragonadal estrogen production. These data seem to indicate that ovariectomy in sheep does not simulate postmenopausal osteoporosis and that sheep are therefore not suitable for long-term investigations.

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SOCIAL RELATIONSHIPS IN A GROUP OF HORSES WITHOUT A MATURE STALLION

by

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Summary

1. The social relationships in a group of Icelandic horses without a mature stallion were studied. The horses were all familiar to each other. Mutual grooming and play relationships, spatial associations, dominance-subordinate relations and the effect of kinship on these relationships were analysed.

2. The social structure was clearly dominated by the behaviour of the adult mares. The horses preferred to form bonds within their social class (sex/age) and they kept close proximity with their friends. The group was effectively divided into two social subgroups, adult mares as one group and adult geldings and sub-adults as another group. The sub-adults and adult geldings formed associations, which were based on mutual grooming and play, while the adult mares did not play. Differences between the sexes were evident. Males played more than the females, had more playing partners and were more popular as playmates.

3. Aggression rates were low. The dominance hierarchy was linear. Adult mares ranked higher than adult geldings, sub-adults and the foals. Rank was significantly correlated with age. The closer the adult mares were in rank, the more they groomed with each other. Such relationships were not found amongst the other social group.

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4. Kinship was calculated between all pairs of animals for up to 4 or 5 generations. Allogrooming and play frequencies and proximity were all positively correlated with kinship. Adult mares, which were close in the dominance hierarchy, were on average more related than those further apart.
5. The social relationships in the Icelandic herd were, to some extent, different from relationships reported from unmanaged and feral horse-herds with mature stallions and bachelors. Our results suggest that adult mares groom more in groups without a stallion. Furthermore, they have more preferred partners than in natural harems and their partners are other adult mares, not their weaned offspring as seems to be the case in feral herds. The sub-adults also seem to be more socially active in the absence of stallions. Interestingly, in the Icelandic group, the adult mares showed stallion like behaviours, like mounting and protecting foals. Only by studying the behaviour and the nature of the relationships of horses in groups of different compositions, can we expect to gain a comprehensive understanding about individual social strategies and cognitive capabilities of the species. Such knowledge is valuable for management and welfare of the horse.

Keywords: social relationships, Icelandic horses, kinship, dominance hierarchies, suppression.

Introduction

For a variety of reasons the domestic horse is an ideal species to study bonding in social groups. Unlike in most mammals, where the sexes only maintain bonds during the breeding season (Clutton-Brock, 1989), dominant males stay with their group all year, thereby forming strong bonds with the adult females that make up their harem (Rees, 1993; Waring, 2002). The domestic horse is non-territorial like the plains zebra, the mountain zebra and the Przewalski horse, and this is thought to have contributed to a more complex social organization than that seen in territorial equids, like the Grevy's zebra and African wild asses (Klingel, 1975 in Carson & Wood-Gush, 1983). Under feral and semi-feral conditions, both sexes disperse from their natal group and dispersal coincides with age at first reproduction (Rutberg & Keiper, 1993; Linklater, 2000). The organisation of horse groups into harems or family bands, (dominant male(s) and (un)related mares with their immature offspring) and bachelor groups, is generally considered to be remarkably similar across different breeds and populations of feral horses (McCort, 1984; Linklater, 2000; Boyd & Keiper, 2002; Feh, 2002). The same group types have developed in a small population of Przewalski horses under natural conditions (Feh, 2002).

Many researchers have studied relationships between individuals in horse groups, especially dominance-subordinate relations (see Rutberg & Greenberg, 1990; van Dierendonck *et al.*, 1995), but in order to get a deeper understanding of the social structure, affinitive relations have to be known as well since they may form the basis for bonding among individuals of a group (Hinde, 1976). Bonding is a very strong feature of the social life of horses (Rees, 1993; Waring, 2002). The long term bonds between stallions and their harem mares may have evolved due to predator pressure from cooperative hunting predators (Feh, 2002) or be a manifest of a male strategy protecting the mares against harassment from other stallions as well as reducing inter-mare aggression (Linklater *et al.*, 1999). Social attachment or bonding is indicated in various ways, e.g. by individuals taking part in interactive behaviours like mutual grooming and social play and tolerating the close presence of each other.

Mutual grooming or allogrooming is a very common behaviour in primates (Cheney & Seyfarth, 1990; Dugatkin, 1997) and in some ungulates (cattle: Clutton-Brock *et al.*, 1976; the impala: Hart & Hart, 1992 in Dugatkin, 1997). Apart from removing parasites, dead skin and excessive hairs, allogrooming is thought to cement the relationship between individuals (Feist & McCullough, 1976; Dugatkin, 1997). In horses it is known that allogrooming lowers the hart rate and calms the animals (Feh & Mazieres, 1993). Social play is generally considered to play an important role in the social development of young animals (Feist & McCullough, 1976; Bekoff & Allen, 1998; Waring, 2002). Data on play-relationships in mixed herds of feral horses is limited. Play is probably most common within peer groups and foals often play with foals of their dam's most preferred partner (Wells & van Goldschmidt-Rothschild, 1979; Araba & Crowell-Davis, 1994; Weeks *et al.*, 2000; Waring, 2002).

Inevitably, bonding and affinitive behaviour between individuals are often strongly correlated with their relatedness, *e.g.* in family groups (parents and offspring, siblings). Nepotism is a widespread phenomenon amongst animals which seem to have various means to recognize related individuals, even without social learning (Sherman *et al.*, 1997; Heth *et al.*, 1998; Petrie *et al.*, 1999). A preference for females to bond more with near kin than other individuals is seen in many social mammals (Cheney & Seyfarth, 1990; Emlen, 1997) including horses (see Wells & van Goldschmidt-Rothschild, 1979; Berger, 1986; Duncan, 1992; Monard *et al.*, 1996; Weeks *et al.*, 2000).

In a more general sense, however, the effect of relatedness on the behaviour of horses is not well documented.

Among horses the stallions play a central role in the structuring of bands and herds by controlling the movements of harem members (Feist & McCullough, 1976; Miller, 1981; Stevens, 1989; Monard *et al.*, 1996; Waring, 2002). In this way the stallions minimize the risk of extra-group fertilizations, future male coalitions and straying of the harem mares. Because they vigorously strive to keep the mares and their juvenile associates in an ordered and thereby defendable group, their behaviour (actively) and presence (passively) is significantly influencing all members of the group. This suggests that interactions among individuals and social structure in groups without a stallion could be significantly altered from the natural state. In 1976, Feist & McCullough suggested that the presence of stallions in feral groups might cause the mares to form less stable bonds and have a more poorly developed social hierarchy than in groups without stallions. Thus the mares might be more socially active when the dominant stallion is not around and even show stallion like behaviours (Tyler, 1972; Waring, 2002). In terms of behavioural strategies this phenomenon can be seen as a conflict between stallions and other group members where the stallions have the upper hand.

The compositions and husbandry of managed horse herds are quite varied. In Iceland the most common composition of herds is several adult mares, foals, yearlings, a few adult geldings and unbroken sub-adults. Colts are most often castrated at one year of age. The horses are kept outside all the year and many herds roam free in natural habitats during the summertime but are kept close to the farm during winter, and fed silage to compliment winter grazing. They are only rounded up for necessary treatments, when the mares are taken to a stallion in the summer or for training. In this paper, we present a detailed study of social interactions and social structure in such a semi-wild Icelandic herd, without a stallion present. Our study involves observations of affinitive and aggressive interactions and measurements of proximity. We analysed the effects of dominance rank, age, sex, and relatedness on the relationships. By comparing our results to results on social relationships in herds with stallion(s) present, we can shed a light on how and to what extent the presence of a stallion can modify or change the behaviour of other individuals in a herd. Specifically, we address the suggestion that stallions subdue affinitive interactions among other group members and that the absence of stallions may reveal some underlying and interesting aspects of female and sub-adult behaviour.

Study group

The research took place at Skáney Farm, W-Iceland in 1997. The composition of the study group was typical for Icelandic horse herds (see above). Most of the mares had been trained, but after being judged when they were five-six years old they were kept as breeding mares only. The herd, from which the study group was taken, was kept outside all the year and the horses mostly roamed free in a 200 ha enclosure in a hilly area. At the time of the study 34 horses; 12 pre-partum mares, five barren mares, two adult geldings and 15 yearlings and sub-adults were in the group and 8 foals were born (of which one died soon after birth). The yearlings were still suckling their mothers. (See Table 1.)

Methods

Fieldwork

During the observations, the horses were kept in an eight ha unmanaged field and were given supplementary silage on a daily basis. The observation period lasted five weeks, starting the 6th of May. After the birth of the first foal the groups were observed throughout the 24 hours. At this time of year, light conditions in Iceland allow observation throughout the night. A handheld computer pre-programmed by the Observer© software was used to collect data on social interactions between the horses, categorized in some pre-defined behaviours (see below). The behaviour of the adult mares was also recorded by focal sampling for 15 minutes each in random order, to collect data on frequencies of some of the behaviours and to calculate time-budgets (manuscript in preparation). The horses were monitored from an observation hut or outside and care taken not to interfere with them. In this study we focused on two types of friendly behaviour; allogrooming (*i.e.* two horses stand head to tail and scratch each other's skin) and play (*i.e.* two or more horses run after each other in a friendly fashion or play fight) eight types of aggressive behaviour; bite threat, bite, chase, attack, ears laid back, kick, threat to kick, and three types of submissive behaviour; move slowly away from threatening horse, flee and teeth clapping. (For descriptions of the behaviours, see McDonnell, 2003.)

To assess which horses were preferred associates, judged from spatial positions, a map was drawn every half hour on which the positions of all the individuals with respect to each other were plotted. For each animal a circle was drawn including all the horses, whose head or tail were within two horse lengths. That distance represents the personal space and the individual flight zone of an individual (Cameron *et al.*, 1999; Mills & Nankervis, 1999).

Data treatment and analysis

In total, behavioural data from 488 hours were analysed and 534 of the maps were analyzed for spatial positions (maps, which were drawn while the horses were eating silage, were not included). The foals were only included in the analysis of data on play frequencies.

Matrices were made which showed how often each pair of horses (a dyad) had an interaction (allogrooming, playing, agonistic interactions). For the agonistic interactions it was recorded which horse was the initiator of an act and which was the receiver. A friendly relations matrix based on allogrooming and play was made for the sub-adults and the adult gelding group (= Gsa) by adding the two together. Because three of the sub-adults were introduced to the groups few days after observations started, the raw frequency data had to be

corrected for each pair of animals. A proximity matrix was generated from the map data, showing for all dyads, how often a horse was within two horse lengths to another (data was transfered to percentages).

For most of the horses the pedigrees for five generations were known, so it was possible to calculate the genetic relation between all animals. The method used was to calculate the inbreeding coefficient, F, between all the dyads (Hartl & Clark, 1997). The horses were all related to varying degree with two exceptions. One mare (code: J) had no genetic relation with 11 horses and her offspring (code: w) had no genetic relation with two horses. All the horses were familiar with each other prior to the study and the adult mares had been together for most of their lives.

The Observer©, Systat, Sigmastat and MatMan© softwares were used for data analyses. For the statistics the following statistics were used: Chi squares (for preferred associates), Mann-Whitney U tests, t-tests (when conditions for normality were fulfilled), Kruskall Wallis test for group comparisons, Spearman's rho correlations coefficients: permutation τ_{RW} Kr and partial $\tau_{RW,XY.Z}$ Kr correlations (for matrix comparisons); and Landau's H for dominance (Sokal & Rohlf, 1981; Lee & Lee, 1982; Siegel & Castellan, 1988). Preferred associates or partners were those individuals with whom a horse interacted (allogroomed or played) significantly more often than expected if they had interacted equally with all horses (expected frequencies for each dyad were standardized with respect to time spent together in the herd). To calculate which horses were prefered as allogrooming partners the whole group was used, but only the geldings and the sub-adult group when play partners were identified since play amongst the adult mares was almost non-existant. Correlations were run between the matrices showing frequencies of allogrooming, play and proximities or values for relatedness between all dyads and between these matrices and test matrices. One test matrix showed rank distance between all dyads. Another one showed if the two horses in a dyad were of the same sex and in the same age-class (5 classes) (code = 1) or not (code = 0). The τ_{RW} Kr permutation test (Hemelrijk, 1990) was employed to test for correlations between matrices. In some cases partial correlations ($\tau_{RW,XY.Z}$) were run to control for the effect of being in the same age-group.

Rank-order of individuals was found by using the MatMan software, which works with both aggression and submission matrices and adds them together (see van Dierendonck *et al.*, 1995). The aggression and submission matrices were based on the total frequencies of aggressive acts and submissive responses caused by these aggressive acts. The horses were ranked according to the number of dominated individuals and if the order did not agree with the relationship between two adjacent horses the two individuals were reversed (de Vries, 1993). The improved method of de Vries (1995) was used to find Landau's linearity of each dominance hierarchy that was calculated (the whole group, the adult mares as a group and the Gsa as another group).

Results

Allogrooming

All the horses, except the youngest foals (less than seven days old), were frequently seen allogrooming. On average an individual (foals excluded) took

part in allogrooming 0.24 times per hour (range: 0.05-0.39). Overall, the horses preferred to allogroom with others of their same sex and age group ($\tau_{RW} = 0.41$, $N = 34$, $p < 0.001$). Nevertheless, some allogrooming occurred between horses of different sub-groups so allogrooming preferences were calculated for the whole group. All individuals had one or more preferred partners (see Table 1). No significant differences were between the five subgroups (adult mares, adult geldings, sub-adult geldings, colts, sub-adult mares), with respect to number of partners (Kruskall Wallis: $H = 6.897$, $df = 4$, $p = 0.141$). The adult mares had other adult mares as preferred partners — the exceptions were one adult gelding, which was a partner of one adult mares, and three of the suckling yearlings which were partners of their mothers. Within the sub-adults and the adult geldings group (= Gsa), only two yearlings and one adult gelding had an adult mare as a preferred partner. The yearlings preferred other yearlings ($\chi^2 = 18.49$, $df = 1$, $p < 0.001$), the sub-adult geldings allogroomed significantly less with the colts than expected ($\chi^2 = 5.76$, $df = 1$, $p = 0.016$), while the sub-adult mares and the adult geldings chose more randomly ($\chi^2 = 1.50$, $df = 3$, $p = 0.68$ and $\chi^2 = 4.72$, $df = 3$, $p = 0.193$, respectively).

Some individuals were much more popular to have as a friend than others (see Table 1). Popularity was not related to rank amongst the mares (Spearman's rho for mares: -0.154, $N = 17$, $p = 0.546$), nor amongst the geldings and the sub-adults (Spearman's rho $= 0.239$, $N = 17$, $p = 0.346$). There was no correlation of popularity with age in the two social groups (the adult mares: Spearman's rho $= 0.125$, $N = 17$, $p = 0.625$; Gsa: rho $= -0,031$, $N = 17$, $p = 0.898$). The sexes were equally popular in the Gsa group ($t = 0.484$, $df = 15$, $p = 0.635$).

Play

During the study period, 728 playing sessions were recorded. The adult mares were only involved in 11 sessions and are not included in the analyses reported below. On average the sub-adults (males and females), adult geldings and the five foals that reached at least the age of one week during the observation period, played 0.121 times per hour. The frequencies for the four sub-groups differed (Kruskal Wallis: $H = 16.6$, $df = 3$, $p < 0.001$). The sub-adult males played most or on average 0.215 times per hour. The sub-adult females played on average 0.031 times per hour which was significantly less than the value for males (Mann-Whitney U, $T = 21$, $p = 0.001$).

TABLE 1. *Names, code, age (years), classification, which horses are half siblings through a mother, which are offspring of a mare, dominance rank of individual horses in the study and identities of preferred partners (play partners in italics and bold)*

Horse	Code	Age	Group	Sibling to:	Offspring of:	Rank order	Preferred grooming and ***play*** partners of subject	Individuals preferring the subject as grooming- or ***playing*** partner
Bylgja*	Y	19	pm			7	B,J,o,F	B,J,F,o
Blída	B	18	pm			6	Y,T,x,l	Y,T,R,x,l
Stjarna	S	18	pm			17	K,H,J,P,G	J,H,K,P,G
Rönd*	R	18	pm			3	H,T,G,B,i	G,H
Gerdur*	G	16	pm			9	J,D,l,R,S	R,D,J,l,S,F
Drífa*	D	16	pm			11	K,J,P,T,G,a,q	G,J,T,K,P,a,F
Sjöfn*	J	15	pm			12	Y,G,D,S	Y,S,G,D
Hvida*	H	15	pm			10	S,T,R	S,R,T,i
Tilraun*	T	12	pm			4	B,H,D,x,l	B,D,H,R,x,l
Freyja*	F	11	pm			13	Y,K,G,D	Y
Blika**	K	11	pm			15	P,D,S,i,a	S,D, P,i,a,F
Perla*	P	9	pm			19	K,D,S	S,K
Rispa	i	20	bm			5	K,l,I	Kx,l,I
Stroka	o	17	bm			8	Y,x,l,O	Y,l,O,x
Faxa	x	15	bm			2	B,T,i,o,l	B,T, o,l
Glæda	l	13	bm			1	B,T,G,i,x,o	B,G,T,i,o,x
Pipa	a	10	bm			14	K,D,A	D,K,A
Geysir	q	10	g		R	18	D,z,m,n,c,C,N-***I,X,O,n,m,w***	D, N,C,z,u,n,m-***n,I,O***
Tumi	u	9	g			16	v,q,m,M-***m,N,v***	w,m,c,M,v-***N,M***
Vari	v	4	g	e		25	u,N,z-***X,z,w,m***	z-***w,z,m,X***
Losti	w	4	g	n	J	22	z,u,n,m,C,M,c-***v,m***	z,u,n,C-***v, m,c,q***
Bliki	z	4	g	M	K	24	L,v,w,q,m,c,M,w,n-***X,v,n,m***	L,v,w,q,n,m,c,M-***v,n,u,m,X***
Birna	N	3	sm		T	23	n,m,q-***u,m***	v,q,m-***u***
Mjöll	M	3	sm	z	K	20	z,C,c,N,u,n-***u***	w,z,u,n,C,c-***A***
Týr	n	2	g	w	J	32	w,q,c,e,m,C,M,z-***L,z,q,m***	q,z,w,M,m,C,e, c,N-***z,m,L,q***
Safír	m	2	g		P	33	I,u,q,c,N,L,n,C,z-***L,w,n,v***	I,L,u,n,c-***q,u,v,w,z,N,n,X,L***
Albína	C	2	sm	A	a	27	I,L,m,c,M,q,n,e,A-***L,e***	X,L,q,n,m,c,e,I, M,w
Þrista	e	2	sm	v		21	X,c,L,n,A-***X,L,c***	L,n,C,c-***C,c***
Kría	c	2	sm	X	x	34	L,z,w,q,n,C,e,M,m-***e,w***	z,w,n,m,C,e,M-***e***
Mosi	I	1	c		i	29	m,A,L,C,i-***X,O,L,q***	L,A,O,m,C,i-***q,O***

TABLE 1. *(Continued)*

Horse	Code	Age	Group	Sib-ling to:	Off-spring of:	Rank order	Preferred grooming and ***play*** partners of subject	Individuals pre-ferring the subject as grooming- or ***playing*** partner
Blakkur	X	1	c	c	x	30	I,L,C,A,O-***O,L,z, v,m***	OL,A,e-***q,v,z,e,I, O,L***
Blesi	O	1	c		o	31	A,I,X,L,O-***I,X,q***	X,L,A,o-***q,I,X***
Tígull	L	1	c		l	28	X,z,m,C,e,I,z-***X, n,m***	X,m,e,I,O,C,c-***n,m,C,e,I,X***
Blesa	A	1	sm	C	a	26	I,X,a,O-***M***	I,X,O,a

pm = pregnant, bm = barren mare, sm = sub-adult mare, g = gelding, c = colt.

* These mares gave birth.

** The foal died 20 minutes after birth.

The five foals played with others on average 0.106 times per hour (means for two colts is 0.16 and for three fillies is 0.07). Overall the horses preferred to play with others of the same sex and in the same age group ($\tau_{RW} = 0.331$, $N = 17$, $p < 0.001$). More detailed analyses showed that the sub-adult geldings and the yearlings showed preference for their own class ($\chi^2 = 22.8$, df = 1, $p < 0.001$ and $\chi^2 = 9.53$, df = 1, $p = 0.022$, respectively). The sub-adult mares and the adult geldings chose playing partners more randomly ($\chi^2 = 1.51$, df = 3, $p = 0.680$ and $\chi^2 = 3.44$, df = 3, $p = 0.328$, respectively). Both the sub-adult geldings and the colts played significantly less with the sub-adult mares ($\chi^2 = 5.62$, df = 1, $p = 0.017$ and $\chi^2 = 4.69$, df = 1, $p = 0.030$, respectively) than the other groups.

There was a significant difference in number of preferred playmates between the sub-groups (Kruskal Wallis: $H = 8.961$, df = 3, $p = 0.03$). The main difference was between the sexes; the males had significant more play partners than the sub-adult mares ($t = 3.99$, df = 15, $p = 0.001$, means: 1.83 and 3.91, respectively). Some individuals were also more popular than others as playmates (see Table 1). Males were significantly more popular than females ($t = 3.92$, df = 15, $p = 0.001$). There was no correlation between popularity and rank position nor between popularity and age (Spearman's rho = 0.261, $N = 17$, $p = 0.307$ and: rho = −0.019, $N = 17$, $p = 0.936$).

The allogrooming matrix for the Gsa group was significantly correlated with the play frequency matrix ($\tau_{RW} = 0.207$, $N = 17$, $p < 0.001$). Conse-

quently a combined positive relations matrix was constructed by adding the two together (see Table 3).

Proximity

The horses stayed close to others of similar age and same sex ($\tau_{RW} = 0.25$, $N = 34$, $p < 0.001$). Furthermore, they tended to allogroom with the same individuals as they were spatially associated with (whole group: $\tau_{RW} = 0.39$, $N = 34$, $p < 0.001$). This relationship is not confounded by being in the same age-group as results from partial correlations show (whole group: $\tau_{RW,XY.Z} = 0.33$, $N = 34$, $p < 0.001$; the adult mares as a group: $\tau_{RW,XY.Z} = 0.35$, $N = 17$, $p < 0.001$; Gsa as a group: $\tau_{RW,XY.Z} = 0.34$, $N = 17$, $p < 0.001$).

The adult geldings and the sub-adults were also spatially associated with their playmates ($\tau_{RW} = 0.29$, $N = 17$, $p < 0.0005$).

Agonistic behaviour

The calculated dominance rank order for the whole group was based on 1372 threatening and 1497 submissive acts. The frequencies of the aggressive acts (0.08 per horse per hour) are minimum values for the group as a whole because some of the more subtle acts have probably been overlooked. The higher number of submissive acts is also an indication of an underestimation of recorded aggressive acts. The value for the adult mares is higher or 0.135 per hour, and is more accurate because only the adult mares were observed by focal sampling.

The rank orders were significantly linear in the whole group and also within the two main social groups (see Table 2). The rank order was significantly related to age in the study group (Spearman's rho = 0.882, $N = 34$, $p < 0.001$). The two year-old geldings and one two year-old mare had the lowest ranks. This suggests that the yearlings, which were still suckling, had some benefit of their mother's social status.

The adult mares groomed more with those which were closer in rank (Spearman's rho = 0.22, $N = 136$, $p = 0.018$), while no such correlation was found amongst the Gsa group (Spearman's rho = 0.089, $N = 136$, $p = 0.300$).

TABLE 2. *Linearity of the dominance hierarchy as measured by Landau's improved H*

Group	Landau's *H*	Chi	df	*p*	% blank	% one way
Whole group	0.717	309	39.9	0.001	16	83
Mares only	0.638	82	24.1	0.001	10	87
Geldings and sub-adults	0.532	64	24.1	0.001	30	68

The blank relationships are the percentage of dyads where there were no interactions recorded and one-way relationships are those in which the submission and the aggression results point in the same direction.

Other behaviours

On 47 occasions, adult mares, five pre-partum and two barren, were seen mounting barren mares when they were on heat and in 26 of these cases this was done by the same pregnant mare. Interestingly, being very close to having a foal, did not seem to preclude this behaviour. Higher ranking mares were not more likely to show this behaviour than lower ranking ones.

Interventions, where horses prevented others in taking part in allogrooming or play, or actively prevented others from approaching a mare with a young foal occurred quite often. Such behaviour was recorded on average every other hour in the study group. Six adult mares and all the sub-adults and geldings intervened in allogrooming. Two mares and 14 of the 17 sub-adults and geldings intervened in play. Protecting a mare and/or a foal characterized certain individuals. Two adult mares, one pregnant and one barren, and one two-year-old mare, exhibited this behaviour quite frequently or in 55% of all cases. (MS in prep.)

Kinship

Correlation between affinitive behavioural matrices (allogrooming, playing, friendship) and the genetic relation matrix showed that relatedness had a significant positive effect on frequency of affinitive interactions, both within the adult mares group and the adult geldings and the sub-adults group (Table 3). Although rank distance and relatedness was not correlated in the whole group, splitting the group showed that amongst the adult mares the correlation was negative, while that was not true for the Gsa group. Also, the horses tend to associate spatially more with the more related individuals. (Table 3.)

TABLE 3. *Results from correlations (A) between (x) kinship (based on five generations) and several social matrices (y) and (B) partial correlations between the same matrices, controlling for (z) age-groups (5 age-classes)*

Matrix *y*	(sub) group	τ_{RW} (A) or $\tau_{RW,XY.Z}$ (B)	*N*	*p*
Allogrooming				
A	All	0.081	34	0.001
B		0.076	34	0.002
A	Mares	0.142	17	0.003
B		0.138	17	0.001
A	Gsa	0.142	17	0.005
B		0.090	17	0.032
Play				
A	Gsa	0.206	17	0.002
B		0.191	17	0.001
Play + allogrooming (friendship)				
A	All	0.070	34	0.001
B	All	0.070	34	0.005
A	Gsa	0.200	17	0.0005
B	Gsa	0.170	17	0.0005
Rank distance				
A	All	0.016	34	0.674
B		0.005	34	0.550
A	Mares	0.090	17	0.049
B		0.100	17	0.041
A	Gsa	0.022	17	0.375
B		0.030	17	0.301
Spatial association				
A	All	0.12	34	0.0005
B		0.12	34	0.0005
A	Mares	0.19	17	0.0005
B		0.18	17	0.0005
A	Gsa	0.23	17	0.0005
B		0.18	17	0.0005

Gsa: Adult geldings and sub-adults.

Discussion

Allogrooming and play

Horses took part in mutual grooming on average 0.24 times per hour in this Icelandic herd. In two other Icelandic groups without stallions, which were composed of: (1) 30 adult geldings and adult mares and (2): a mixed group

of 28 sub-adults and adults, allogrooming frequencies were 0.28, and 0.16 times per horse per hour, respectively, in December-April (H.B. Ingolfsdottir & H. Sigurjonsdottir, in prep.). Scottish Highland ponies (14 adult mares and three 1-2 year old sub-adults) groomed more often or on average 0.65 times per hour in July-September (Clutton-Brock *et al.*, 1976). When compared with groups with a stallion these values are relatively high. Thus, in a group of Camargue horses (one stallion, eight mares, five foals and 12 1-2year old sub-adults) the horses only allogroomed 0.06 (mares and yearlings) and 0.13 (males) times on average per hour in March till June and in November (Wells & van Goldschmidt-Rothschild, 1979). And in one family of eight in a herd of Przewalski horses in France, allogrooming in September occurred infrequently or on average only 0.07 times per horse per hour when the two foals are excluded (unpubl. results — the group was observed for 40 hours by the first author in 2000). In that study, the stallion and one of the adult mares were never seen to allogroom while the other adult mare allogroomed with her three offspring. The intensity of allogrooming is influenced by many factors such as season (winter coat shedding), weather, parasites and flies (Wells & van Goldschmidt-Rothschild, 1979) as well as social factors, such as the existence of newcomers in a group and importance of establishing bonds. Therefore, it is necessary to know the number and the identity of partners to understand the nature of grooming relationships.

In most published studies, where relationships have been studied in details, the number of preferred partners is 0-3 (Tyler, 1972; Clutton-Brock *et al.*, 1976; Monard *et al.*, 1996; Kimura, 1998). In our study, the horses had more partners. When looking at the frequency of allogrooming and the identity of the preferred partners it seems that the presence or absence of a stallion could make a difference. Thus, mutual grooming seems to be more common in groups without stallions. Also, in the groups that did not have stallions the adult mares preferred to groom with other mares of similar age (Tyler, 1972; Clutton-Brock *et al.*, 1976; this study), while they tended to groom with their 0-3 year old offspring when in a harem (Feist & McCullough, 1976; Wells & van Goldschmidt-Rothschild, 1979, Przewalski horses, pers. obs.). It is more difficult to see the effect of the stallion on the preferences of the sub-adults. In Wells & van Goldschmidt-Rothschild's study (1979), most mutual grooming occurred within the family but the yearlings and the foals also preferred to groom with their peers. After immigration, sub-adult mares quickly established bonds with mares of similar age (Monard *et al.*, 1996). It is interesting

that in the no-stallion Icelandic herd, the sub-adults (2-4 year olds) did not associate much with their mothers; instead they bonded with their peers and the adult geldings. In Clutton-Brock *et al.* study (1976), the sub-adult mares preferred to bond within their age group.

On the whole there was a good correlation between allogrooming and play preferences within the group of sub-adults and geldings, which supports the common notion that the horses play with the ones they associate mostly with. Data on play frequencies in natural herds is, however, very limited. Young dispersing mares in the Camargue herd played 0.25 times per hour prior to emigration (Monard *et al.*, 1996), which is similar to what we found for the sub-adult geldings in our study. Since dominant stallions are less protective of the dispersing daughters than other members in their band (Waring, 2002), the young mares may in effect be more socially active than the other sub-adults. Feist & McCullough (1976) reported that the stallions in their study allowed the foals to play with foals from other groups but prevented other members in the harem to join other groups. In a herd of Przewalski horses in France the stallion acted in the same way. The only intergroup social interaction that the stallion tolerated was play amongst the foals, which was rare (four foals and sub-adults played on average 0.04 times per hour) (H. Sigurjonsdottir, pers. obs.).

Not only did the males play more in our study, they also had more preferred playmates than the females. The young males preferred their own sex-age class, while the sub-adult mares played with both sexes. Monard *et al.* (1996), found that prior to emigration dispersing sub-adult Camargue mares preferred to play with immatures of both sexes and the stallion in their natal groups. As Wells & van Goldschmidt-Rothschild (1979) argue, all the sub-adults like to mingle with their peers. Differences in popularity amongst group members were striking (see Table 1). Popularity as a playmate was not related to rank position, instead individual temperament may be more important.

Dominance relationships

Aggression rates were lower in the Icelandic group than reported from other studies — only 0.17 per hour on average amongst the adult mares. Monards *et al.* (1996), Stevens (1989) and Rutberg & Greenberg (1990), who were all studying females in natural harems, reported aggressive rates of 0.3-0.5, 0.24

and 0.9-3.6, respectively. In Clutton-Brocks *et al.* study (1976), mean aggression rate was 1.9 per hour. The differences between the groups may be due to different levels of competition for resources and age of the mares, since younger mares tend to be more aggressive (Rutberg & Greenberg, 1990). Newly dispersed females also receive more aggressive acts than other mares and aggression is more frequent between females during male competition for mates (Linklater *et al.*, 1999). Clearly, more factors need to be controlled for before the effect of stallions on female aggressiveness can be revealed. A clear and linear dominance hierarchy was found to characterize the group, where adult mares dominated the sub-adults. This finding is in an agreement with some studies (Houpt & Keiper, 1982; Jeriezski & Gebler, 1984; Keiper & Sambraus, 1986; Rutberg & Greenberg, 1990; van Dierendonck *et al.*, 1995) while in others the linearity was much less clear (Feist & McCullough, 1976; Schoen *et al.*, 1976). For the group as a whole there was a significant correlation between rank and age. The adult geldings were relatively young compared to the adult mares so it is not possible to reveal wether sex was more important than age. The top ranking mares were, however, not the oldest in the group (see Table 1). The same pattern was found to characterize the dominance hierarchy amongst the Exmoor ponies (Wood-Gush & Galbraith, 1987). Positive correlation between age and rank has been found in a study on the Przewalski horse (Feh, 1988; Keiper & Receveur, 1992) and in the domestic horse, both feral and domestic groups (Clutton-Brock *et al.*, 1976; Keiper & Sambraus, 1986; Rutberg & Greenberg, 1990; van Dierendonck *et al.*, 1995; Kimura, 1998). Other studies on the domestic horse have not shown a significant correlation between age and rank, (Houpt *et al.*, 1978; Houpt & Keiper, 1982) although adults were dominant over juveniles. In some studies, offspring of dominant mares achieve a higher rank (Araba & Crowell-Davis, 1994; Weeks *et al.*, 2000) but in others they do not (Keiper & Sambrus, 1986). In our study, the five yearlings which were still suckling their mothers, ranked higher than some of the two and three year old subadults, so it seems that they benefited from their mother's presence in the group.

As Houpt *et al.* (1978), Houpt (1982), Berger (1977) and Weeks *et al.* (2000) point out, many factors influence dominance relations. It could be aggressiveness, weight, residence time or some other attribute. The longer a horse stays in a group, the more time he has to bond and gain social experience, which is likely to be the basis for strategic planning. Residency has

been shown to influence rank among horses (Clutton-Brock *et al.*, 1976; van Dierendonck *et al.*, 1995; Monard *et al.*, 1996). The social structure of horse groups is, without any doubt, much influenced by dominant/subordinate relationships between the horses since the dominant horses can choose which they want to associate with. Earlier findings, (Clutton-Brock *et al.*, 1976; Wells & van Goldschmidt-Rothschild, 1979; Ellard & Crowell-Davies, 1989; Monard *et al.*, 1996; Kimura, 1998) where individuals of similar rank formed affinitive relationships, are supported in this study. Interestingly, although found amongst the adult mares, such a relationship was not apparent amongst the immatures. That is not surprising since they are probably still forming bonds and their preferences are less clear than the mare's.

Although the dominance status of the stallions in natural herds is clear when they herd their harems it is not necessary so in other contexts, like access to drinking or in aggressive encounters within the harem (see Waring, 2002). For this reason the nature of affinitive relationships within a harem could be dependent on the dominance status of the stallion. The social structure of groups without a stallion could therefore be more like that of harems with a low ranking single-stallion. More research is clearly needed in this area.

Kinship

The horses preferred to have positive social relations (allogroomed, played and stayed close to) within their social groups with the more related individuals. This is an important finding, that has not been described before for horses except in the context of close kin preferences within harems (in the Camargue — see Wells & van Goldschmidt-Rothschild, 1979). Also, the more the adult mares are related, the closer they are in rank. A possible explanation for this is that relatives share genes, which influence the outcome of agonistic interactions and thus determine their rank positions in the hierarchy.

Kin recognition is a widespread phenomenon (Tang-Martinez, 2001; Parr & de Waal, 1999) and in some cases animals are able to discriminate between unfamiliar kin and non-kin (Sun & Müller-Schwartze, 1997; Heth *et al.*, 1998; Petrie *et al.*, 1999). Little is known about kin discrimination in horses since familiarity is generally a confounding factor. Horses recognise herd members by sight, sound and smell (Feist & McCullough, 1976; Waring, 2002) and learning is definitely important. In tight family bands grooming

occurs mostly within the family (Tyler, 1972; Feist & McCullough 1976; Well & von Goldschmidt-Rothschild, 1979; Houpt, 1982). Monard *et al.* (1996) showed that before dispersal, young mares reject the sexual advances of familiar males in their natal groups. That their mothers often interfered when close kin males were involved could be evidence of kin recognition. Preference for related individuals outside the family group as reported for the New Forest ponies herd, especially amongst the sub-adults (Tyler, 1972) is probably another example. Although kinship relations might not be as important for the individuals in female-emigrating mammalian species as in female-resident species, (see Monard *et al.*, 1996), it can nevertheless be a good strategy to form relationships with related individuals when young if it can raise inclusive fitness later on. In general young females in female-emigrating species form strong relations with members of their matrilines — a behaviour which could later facilitate integration into new harems (Monard *et al.*, 1996). Such an argument is supported by the findings of Monard *et al.* (1996) who showed that dispersing mares approached groups in which there was a familiar and related young mare. Less is known about the males. Feh (1999) showed that the subordinate stallions that formed an alliance and kept a harem together had been close associates in their natal group, but they were not of close kin. It would be interesting to study the genetic relations between young dispersing males and associates in the new groups, which can be of different compositions (see Khalil & Kaesada, 1998 for explanation).

In the Icelandic herd, all the horses were familiar to each other so it is not possible to assess if they would prefer unfamiliar individuals with common genes (through the father line). The correlation between relatedness and affinitive relationships was not due to bonds between siblings through the mother line nor between mothers and daughters (see Table 1). What is of special interest is the fact that within both social groups, the horses chose to associate with more related horses of similar age. That is an interesting finding which might be a consequence of stallion absence. Therefore the question of kin recognition abilities and nepotism among horses is unanswered until more horse groups of different compositions are studied.

Summary and conclusions

The relationships that characterized the group in Iceland were dominance of the adult mares over the adult geldings and the sub-adults, mutual grooming

bonds between the adult mares and mutual grooming and play relationships amongst the other horses. Horses that were close in age formed affinitive relationships. So did adult mares also, which were close in rank. The more the horses are genetically related, the more they attach socially. Compared to groups including a stallion, the relationship pattern differ mostly in how much the adult mares bond with other adult mares and how little they associate with their sub-adult offspring (the exception being bonds between three mothers and their suckling yearlings). As a consequence of the adult mares' dominance and preferences, the herd was in effect divided into two social groups — the adult mares on one hand and the sub-adult plus adult geldings on the other. Interesting differences were found between these two social groups, reflecting more stable social structure in the adult mare group. For instance, the adult mares allogroomed with other adult mares of similar rank while no such correlation was found within the other group. Also, only within the the adult mare group was there a positive correlation between relatedness and being close in the dominance hierarchy.

Our finding that kinship affects bonding within a group of horses outside the family unit has, to our knowledge, not been reported before. Both adult mares and the sub-adults and the adult geldings are more likely to be spatially associated with more related peers and they also allogroom and play (*i.e.* the sub-adults and the adult geldings) more with more related individuals.

There was a good agreement between standing close to each other and affinitive relationships, which is in agreement with many other studies (Clutton-Brock *et al.*, 1976; Jezierski & Gebler, 1984; Wood-Gush & Galbraith, 1987; Whitehead & Dufault, 1999).

Adult mares (pregnant and barren) showed a typical stallion-like behaviour when they mounted other mares. Such a behaviour has rarely been described for adult mares among horses (Feist & McCullough, 1979; Waring, 2002), but seems to be common in female donkeys, which by the way do not form permanent bonds with the males (McDonnel, 2000). The mounting behaviour might be a sign of dominance as was witnessed amongst the stallions in Wyoming when they mounted immature stallions (Feist & McCullough, 1979). Protection of a post-partum mare and her new born foal by another mare is another unusual behaviour. Perhaps, the herd members take up the protective role of the stallion in his absence.

Some new and some unusual findings emerged in this study. Our results suggest that stallions may suppress adult mares in natural bands by preventing them to move between bands and hence disrupt their allogrooming

preferences. It is also likely that stallions suppress the youngsters in their group by intervening in play and allogrooming with youngsters from other harems and even within their own harem. More research is needed in this area; especially studies on play preferences amongst sub-adults in groups of different compositions and on stallion interventions in allogrooming and play.

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was, however, no difference in the number of lambs born per ewe lambed (1·92).

Pregnancy diagnosis in cows: Use of progestagen concentration in milk

R. B. HEAP, J. L. LINZELL, ARC Institute of Animal Physiology, Babraham, Cambridge and J. A. LAING, Royal Veterinary College, University of London, Bolton Parks, Potters Bar

IN previous studies (*J. agric. Sci. Camb.* **81**, 151, 1973) we reported that during the reproductive cycle the progesterone concentration of first milk taken at morning or afternoon milking followed a pattern similar to that found in plasma, though the values were generally higher. During a field trial on 204 cows in six farms a number of problems have become apparent. These include the time of sampling and the type of samples taken (first or bulk milk). In samples stored at −10° C, serious deterioration occurred. Furthermore, this field trial showed considerable variation between farms; the highest accuracy of pregnancy diagnosis was 83 per cent, and the lowest 51 per cent.

Approximately 25 per cent of progesterone in milk is bound to the casein-rich fraction prepared by high speed ultracentrifugation, while 75 per cent is found in milk fat. Casein binding interfered with the direct radioimmunoassay but was compensated for by the inclusion of casein in the progesterone calibration standards. The addition of common milk preservatives such as formalin or mercuric chloride and potassium dichromate, maintained the samples in good condition and was not detrimental to the assay. A semi-automated procedure has now been developed for a direct, rapid radioimmunoassay of progesterone in milk so that one operator can analyse over 100 samples in duplicate per 24 hours.

Observations in a herd of Jersey cows have shown that the progestagen concentration in first milk at morning milking was lower than that in morning bulk milk or in any afternoon samples. Experiments are in progress with this, and other herds, using the modified progestagen assay referred to above in order to evaluate the accuracy of pregnancy diagnosis from bulk milk samples. The first results show that when milk samples were taken at least 21 days after a fertile or infertile mating, or from cows later than day 21 of lactation anoestrus, 34 pregnant and six non-pregnant cows were diagnosed correctly, six pregnant and one non-pregnant were doubtful, and no animal was diagnosed incorrectly.

The work was carried out with the technical assistance of D. J. S. Cain, A. Henville, M. Gwyn, and S. Pakes.

Masculine behaviour in geldings

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THE occurrence of stallion-like behaviour in horses which have no visible or palpable testicles is relatively common. Most of these animals are cryptorchids, but a significant number are found to have been castrated. A study was made of these geldings to determine the cause of the behaviour.

Questionnaires were sent to the owners of 18 geldings showing stallion-like behaviour. The replies showed that surgical removal of the spermatic cords, which remained from the original castration, abolished sexual behaviour in most of the geldings and, in about 75 per cent of cases, aggressive behaviour was also controlled. This indicated that the spermatic cord might be responsible for the behaviour, possibly by producing androgen. This theory was tested by incubating the various tissues present in the spermatic cord with radioactive steroid precursors. No tissues were able to convert progesterone to androstenedione or testosterone. There was a low conversion of androstenedione to testosterone (less than 2 per cent) by tissues from three geldings with stallion-like behaviour and also by tissues from a normal gelding; but this activity was no greater than that shown by blood or liver.

The spermatic cord was not therefore considered to be a significant source of testosterone and was not responsible for the stallion-like behaviour seen in these geldings.

These cases probably represent stallions in which acquired sexual behaviour has persisted for longer than usual following castration, and it seems that the horse is less dependent on hormones than on cerebral mechanisms for the maintenance of sexual behaviour. However, an explanation has not been found for the loss of stallion-like behaviour following removal of the spermatic cords.

Pituitary control of testicular function in bulls

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THE purpose of this paper is to review some of our research on the control by anterior pituitary hormones of spermatogenesis and androgen production by bulls.

Sperm are first found in Holstein testes at about six months of age, and the number of sperm per gram of testis is near that typical of adults by one year. Blood LH increases markedly and pituitary FSH decreases during the same age-period. At one year in 41 Herefords, neither pituitary LH nor blood LH was related highly to sperm production, but pituitary FSH was related ($r = -0{\cdot}41$) to numbers of testicular sperm leading to the suggestion that release of FSH normally limits onset of spermatogenesis. An intramuscular injection of 200 μg gonadotrophin releasing hormone (GnRH) increased blood LH from 1 ± 1 to 23 ± 2 ng per ml within 45 minutes in bulls at two, four or six months of age. These increments of blood LH elevated blood testosterone to values typical of adults only in six-month bulls. Evidently the bull testis is not fully capable of responding to LH with testosterone secretion until near six months of age.

We failed to find a significant elevation of LH at five or 30 minutes after ejaculation in bulls. However, blood growth hormone and prolactin were increased three- and six-fold, respectively, within five minutes after ejaculation and blood testosterone was increased 50 per cent at five and at 30 minutes. Nevertheless, intravenous injections of prolactin failed to alter blood testosterone whereas injections of LH increased testosterone three- to four-fold in bulls and rabbits alike. Thus, LH is probably the principal androgenic hormone. A single intramuscular injection of 5000 IU of PMS resulted in a progressive

SEXUAL BEHAVIOR, SEMINAL pH AND ACCESSORY SEX GLAND WEIGHTS IN GELDINGS ADMINISTERED TESTOSTERONE AND(OR) ESTRADIOL-17β[1]

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Summary

Sixteen stallions were castrated and 30 days later assigned to one of four treatments: (1) testosterone propionate (175 μg/kg body weight), (2) 17β-estradiol-3-benzoate (44 μg/kg body weight), (3) a combination of both steroids or, (4) vehicle only. These dosages were administered every other day for 18 days. The dosages were then doubled and continued for 20 days. Concentrations of testosterone and estradiol in serum decreased rapidly after castration and stabilized within about 6 hours. Mean concentrations of testosterone and estradiol maintained by the steroids were 1.4 and 90 pg/ml, respectively, during treatment at the lower dosage, and 2.6 and 186 pg/ml during treatment at the higher dosage. Libido and the ability to ejaculate were gradually lost after castration. Testosterone restored both aspects of sexual behavior within 2 weeks. Estradiol effectively restored libido at the higher dosage, but was less effective in restoring the ability to ejaculate. The pH of gel-free semen increased after castration and was subsequently decreased by treatment with testosterone or the combination of both steroids. Estradiol had no effect on seminal pH. Weights of the seminal vesicles, ampullae and prostate were greater in geldings treated with testosterone or with both steroids than in estradiol-treated or control geldings. Since estradiol alone was able to restore libido in geldings, it is possible that the maintenance of libido in intact stallions involves either estradiol secreted directly by the testes or estradiol arising from aromatization in peripheral tissues. Treatment with estradiol at either concentration, did not appear to affect the size or function of sex glands in geldings.
(**Key Words:** Gelding, Testosterone, Estradiol, Libido, Accessory Sex Glands.)

Introduction

Sexual behavior in castrated male rats (Davidson, 1969; Sodersten, 1973), rabbits (Foote *et al.*, 1977), guinea pigs (Antliff and Young, 1956) and red deer (Fletcher and Short, 1974) can be at least partically restored and maintained by treatment with estrogens. Naftolin *et al.* (1971) suggested that the effects of androgens on male sexual behavior may be mediated by aromatization of the androgens to estrogens in target neurons.

Accessory sex glands in males are generally androgen-sensitive (Brandes, 1974). High doses of estrogen inhibit the function of accessory sex organs in chickens and dogs (Hoskins and Koch, 1939; Huggins and Clark, 1940), while low concentrations stimulate the musculature and connective tissue of accessory sex glands in rabbits (Bern, 1949; Beyer *et al.*, 1975; Foote *et al.*, 1977). Estrogens are concentrated in semen and accessory sex organs of guinea pigs (Belis *et al.*, 1977), rats (Saksena *et al.*, 1978) and bulls (Eiler and Graves, 1977).

Stallions produce and excrete large quantities of estrogens compared to those produced by males of most other mammalian species (Velle, 1966). These estrogens are produced by the testes (Nyman *et al.*, 1959; Bedrak and Samuels, 1969). Mean concentrations of estradiol-17β (estradiol) are seasonal in stallions and are parallel to the mean concentrations of luteinizing hormone (LH) and testosterone (Thompson *et al.*, 1977, 1978). The present experiment was designed to determine the roles of testosterone and estradiol in the regulation of sexual behavior and accessory sex gland

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function in stallions through examination of their effects in geldings.

Materials and Methods

Sixteen stallions of light-horse breeds, between 3 and 17 years of age, were trained to seminal collection via an artificial vagina (Pickett and Back, 1973). Semen was collected from each stallion every other day for at least 12 days before castration. On the day of castration, stallions were tranquilized with xylazine[3] and anesthetized with ketamine[4], and the testes were removed. On the fifth or sixth day after castration, seminal collections were resumed and continued on a twice-a-week basis throughout the experiment.

The experiment was conducted in four replicates, with each replicate including four animals, one in each treatment group. The castration dates for the four replicates were November 5 and 8 and December 1 and 4.

A 16 mg/ml solution of testosterone propionate (TP), a 4 mg/ml solution of 17β-estradiol-3-benzoate (EB) and a solution of both steroids (TE) at these concentrations were made with safflower seed oil. On the 30th day after castration, each of the four geldings received one of four treatments: 175 μg of TP/kg of body weight; 44 μg of EB/kg of body weight; TE at these dosages, or oil only. These dosages were administered subcutaneously every other day for 18 days (level 1). On the 48th day after castration, the dosages were doubled (level 2) and were continued every other day until sacrifice. Seminal collections were terminated on the 68th day after castration, and the geldings were sacrificed approximately 1 week later.

Thirty-milliliter blood samples were drawn before and after castration and during treatment for determination of the concentrations of testosterone and estradiol in serum. The samples were taken at −2, −1, 0, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 60 hr relative to the time of castration and to the initial injection at level 1 (day 30) and level 2 (day 48). In addition, blood was drawn at 0800 and 1600 hr on days 5, 10, 15, 20, 25 and 29 and then every third day until day 68. For frequent blood sampling, 10.2-cm, 18-guage indwelling catheters were placed in a jugular vein. Blood was allowed to clot for 30 to 60 min at 20 C, stored overnight at 5 C and then centrifuged at 10,000 × g for 10 minutes. Serum was stored at −15 C. Testosterone from extracts of .5- or 1.0-ml aliquots of serum was quantified by radioimmunoassay as described by Berndtson *et al.* (1974). Estradiol from extracts of 1.0 ml of serum was quantified as described by Thompson *et al.* (1978). Concentrations of testosterone and estradiol in serum were not corrected for assay blanks of 41 and 3.6 pg/ml, respectively.

Because of the expected loss of libido after castration, limits were placed on the time and effort allotted to each animal for seminal collection. Each gelding was allowed a maximum of 30 min with the teaser mare(s) to attain an erection. If erection was attained, the gelding was allowed a maximum of 60 min or 10 mounts with intromission into the artificial vagina, whichever occurred first. If the gelding did not ejaculate within these limits, he was returned to his pen until the following collection day.

For quantification of libido, a sexual behavior score was developed. At each attempted seminal collection, the geldings were scored as follows: (1) 0 = no sexual interest; (2) 4 = attainment of an erection without subsequent ejaculation and fewer than 10 mounts within the 1-hr time limit; (3) 6 = an erection followed by 10 mounts within 1 hr, without ejaculation, and (4) 10 = erection and subsequent ejaculation.

The pH of gel-free semen was determined immediately after collection. Subsamples of the semen were stored at −15 C for determination of spermatozoal concentration. Motility and gross appearance of spermatozoa were determined. At sacrifice, the accessory sex organs were removed, trimmed and weighed after removal of luminal fluids.

Data for sexual behavioral score and weights of accessory sex organs were analyzed by analyses of variance in a split-plot design. Means were compared by Duncan's multiple-range test (Steel and Torrie, 1960). Because of unequal numbers of ejaculates per treatment, data for seminal pH were analyzed by least-squares analysis of variance in a split-plot design after grouping of the data into five defined periods.

Results

The effect of castration on concentrations

[3] Rompun, Haver-Lockhart, Bayvet, Shawnee, KS.
[4] Vetalar, Parke-Davis and Co., Detroit, MI.

of testosterone and estradiol in serum is presented in figure 1. Mean concentrations of testosterone decreased ($P<.05$) rapidly during the first hour and then more gradually from 1 to 6 hr postcastration. After 6 hr, no further decrease was observed. Concentrations of estradiol also decreased ($P<.05$) rapidly during the first hour after castration, and 2-hr mean concentrations did not differ from those at subsequent intervals.

The release of testosterone or estradiol after the subcutaneous injection of the respective ester was gradual over a 6- to 12-hr period, and no transient abrupt increases were observed. Mean concentrations of both steroids remained higher than preinjection concentrations in treated geldings for up to 48 hr after injection.

During the level 1 treatment period, testosterone in the serum of TP- and TE-treated geldings averaged 1.4 and 1.3 ng/ml; during the level 2 treatment phase, concentrations averaged 2.7 and 2.5 ng/ml, respectively (figure 2). Corresponding values for estradiol in the serum of EB- and TE-treated geldings were 81 and 98 pg/ml during the level 1 treatment period and 163 and 210 pg/ml during the level 2 phase (figure 3).

Estradiol in TP-treated geldings averaged 6 pg/ml at the higher dosage, compared with 2 pg/ml ($P<.05$) in control geldings (figure 3). There was no difference in concentrations of

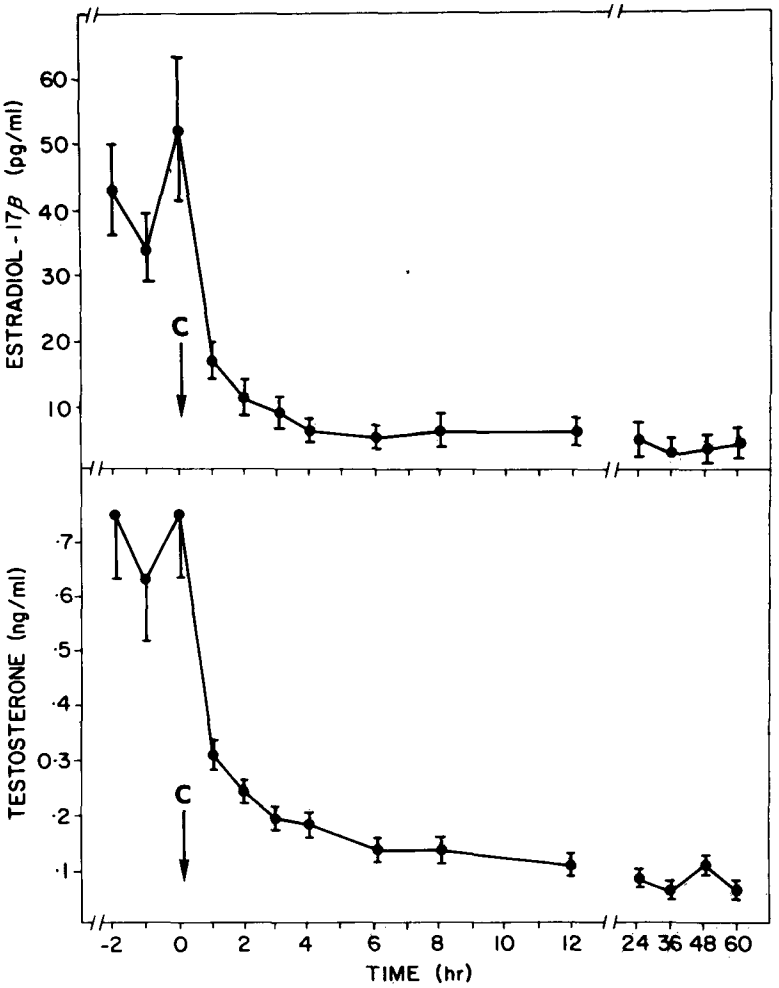


Figure 1. The effect of castration on concentrations of estradiol-17β and testosterone in serum of 16 stallions. Time of castration is denoted by the arrow. The vertical lines indicate the SEM.

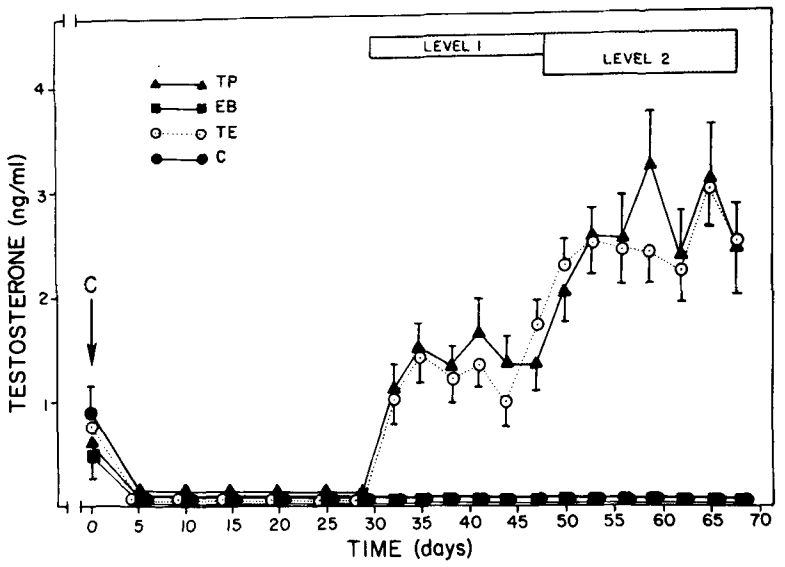


Figure 2. Concentrations of testosterone in peripheral serum of control and steroid-treated geldings after castration and during treatment. Time of castration is denoted by the arrow, and the periods of treatment at the low dose (level 1) and high dose (level 2) of steroid are indicated by the bars. The vertical lines indicate the SEM.

testosterone between EB-treated and control geldings during treatment.

Sexual behavioral scores for the four groups are presented in figure 4. Mean scores for all geldings decreased ($P<.05$) gradually after castration, and those for control geldings continued to decrease through day 56. Treatment with TP resulted in an improvement ($P<.05$) in score within 2 weeks. During the level 1 treatment phase, sexual behavioral scores of

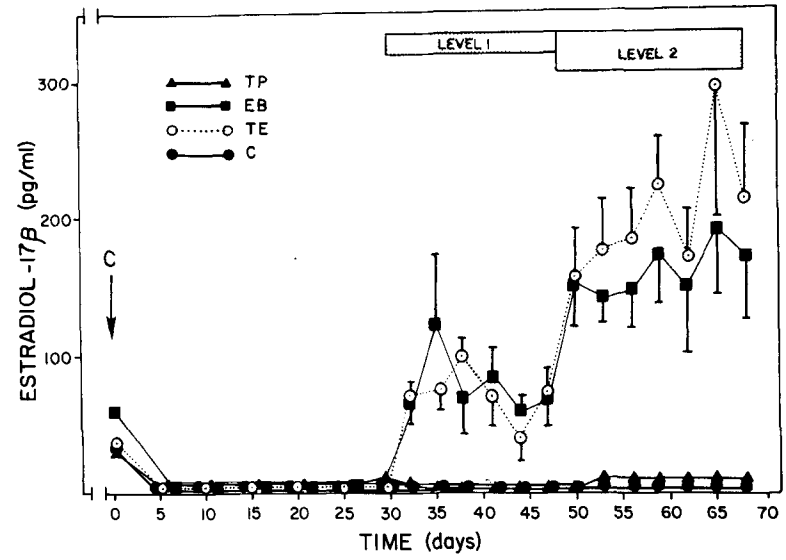


Figure 3. Concentrations of estradiol-17β in peripheral serum of control and steroid-treated geldings after castration and during treatment. Time of castration is denoted by the arrow, and the periods of treatment at the low dose (level 1) and high dose (level 2) of steroid are indicated by the bars. The vertical lines indicate the SEM.

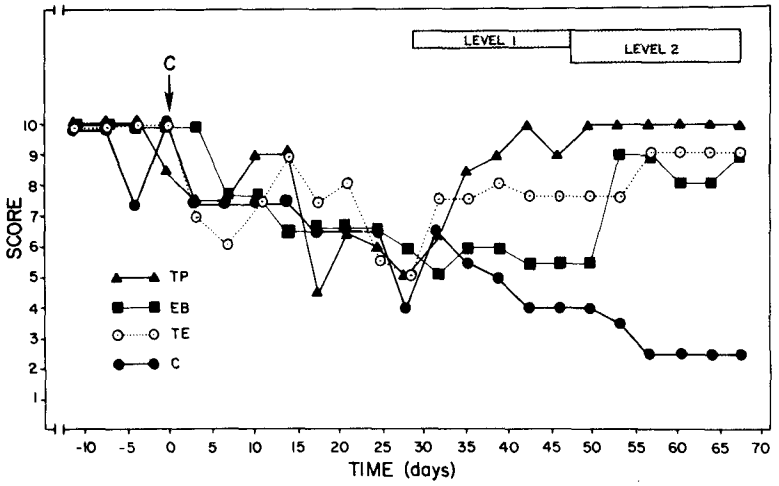


Figure 4. The effect of castration (arrow) and steroid treatment on sexual behavioral scores. The periods of treatment at the low dose (level 1) and high dose (level 2) of steroid are indicated by the bars. The pooled SEM calculated from the error mean square from the ANOVA was 1.12.

EB-treated geldings did not differ from those of the control geldings. By the second collection day during the level 2 phase, the scores of EB-treated geldings were greater ($P<.05$) than those of controls, and they remained similar to those of TP-treated geldings until the end of the experiment. The mean score for TE-treated geldings was greater ($P<.05$) than that for control geldings from the 42nd day after castration.

When the first ejaculate was collected 5 days after castration, the semen of one gelding contained a few spermatozoa that exhibited weak flagellations, but this was the only observation of live spermatozoa in the ejaculates from any of the geldings. Concentration of spermatozoa decreased rapidly from a pre-castration mean of 129.0×10^6/ml to 2.52 and $.78 \times 10^6$/ml in ejaculates collected 5 and 9 days after castration, respectively. In those animals that continued to ejaculate until treatment began, spermatozoal concentration decreased gradually until only a few, if any, spermatozoa were observable in a smear of raw semen at a magnification of $\times 200$. Such spermatozoa were generally tailless. During the first few collection days after the initiation of treatment, there was a dramatic increase in the number of spermatozoa per milliliter in the ejaculates of TP- and TE-treated geldings. This response was not observed in EB-treated or control geldings.

The pH of gel-free semen tended to increase after castration (table 1). In TP-treated geldings, seminal pH decreased ($P<.05$) during treatment to values similar to those observed before castration. The same tendency was observed in TE-treated geldings.

Volumes of gel and gel-free semen varied among animals and from one collection day to the next (data not shown). In general, seminal volumes tended to decrease after castration and were restored by TP and TE treatment. Treatment with EB had no effect on seminal volumes.

Weights of the seminal vesicles and ampullae in TP- and TE-treated geldings were approximately two times as great ($P<.05$) as those in EB-treated and control geldings (table 2). Weight of the prostate, but not of the Cowper's glands, also tended to be greater ($P<.07$) in TP- and TE-treated geldings than in EB-treated and control geldings. Estradiol did not stimulate the accessory sex organs, nor did it prevent the testosterone-induced stimulation.

Discussion

Concentrations of testosterone and estradiol in serum decreased rapidly after castration. Half-lives calculated from plots of the natural logarithm *versus* time were 48 and 37 min for testosterone and estradiol, respectively, during the first hour after castration; the half-lives of the second component of the decay curve were 173 and 130 minutes. The two components of the decay curve may reflect differences in

TABLE 1. MEAN PH OF GEL-FREE SEMEN BEFORE AND AFTER CASTRATION AND STEROID TREATMENT[a]

Interval[c]	Group[b] TP	EB	TE	C
Before castration	7.42 (15[d])[e,f]	7.35 (16)[e]	7.40 (15)[e]	7.46 (14)[e,f]
After castration:				
Weeks 1 and 2	7.57 (11)[f,g]	7.39 (12)[e]	7.54 (8)[e]	7.39 (11)[e]
Weeks 3 and 4	7.61 (4)[g]	7.52 (8)[e,f]	7.51 (5)[e]	7.37 (5)[e]
After treatment:				
Level 1	7.46 (15)[e,f,g]	7.64 (7)[f,g]	7.52 (10)[e]	7.63 (6)[f,g]
Level 2	7.38 (24)[e]	7.75 (13)[g]	7.38 (16)[e]	7.66 (6)[g]

[a]Means are least-squares estimates; the pooled standard error of the means was .055.

[b]Each group consisted of four geldings that received testosterone propionate (TP), 17β-estradiol-3-benzoate (EB), both steroids (TE) or vehicle only (C).

[c]The maximum numbers of ejaculates possible per group during the five successive intervals were 16, 16, 16, 20 and 24, respectively.

[d]Numbers in parentheses are the numbers of ejaculates included in the mean. All means are derived from data on at least two animals, except that for the C group during level 2, for which data on only one gelding were available.

[e,f,g]Means within columns with different superscripts are different ($P<.05$; Duncan's multiple-range test).

clearance rates due to binding of the steroids to albumin (loosely bound, cleared most rapidly) *versus* binding to sex-hormone binding globulin (more tightly bound, cleared more slowly). An alternative explanation is that the slower component of the decay curve was due to gradual loss of steroids from body fat and other body pools following castration.

Administration of the esters of testosterone and estradiol as subcutaneous injections in oil provided for slow and sustained release of the steroids into the pheripheral circulation. Mean concentrations of testosterone and estradiol in the serum of treated geldings during the level 1 treatment period were similar to those reported for intact stallions (Berndtson *et al.*, 1974; Thompson *et al.*, 1977, 1978). Because abrupt increases in concentrations of testosterone occur approximately seven times daily in stallions (Squires *et al.*, 1977), the mean concentrations of testosterone reported previously for stallions must have been an average

TABLE 2. EFFECTS OF STEROID TREATMENT ON WEIGHTS OF ACCESSORY SEX ORGANS OF GELDINGS[a]

Organ, paired	Group[b] TP	TE	EB	C
	g			
Seminal vesicles	54.4[c]	52.8[c]	26.0[d]	23.2[d]
Ampullae	57.6[c]	66.2[c]	27.7[d]	20.5[d]
Prostate	41.5[e,f]	45.0[e]	31.8[f,g]	28.2[g]
Cowper's glands	13.3	15.1	13.3	12.4

[a]The pooled standard errors of the means were 6.1, 5.0, 4.4 and 1.7 g for the seminal vesicles, ampullae, prostate and Cowper's glands, respectively.

[b]Each group consisted of four geldings that received testosterone propionate (TP), 17β-estradiol-3-benzoate (EB), both steroids (EB), both steroids (TE) or vehicle only (C).

[c,d]Means within rows with different superscripts are different ($P<.05$; Duncan's multiple-range test).

[e,f,g]Means within rows with different superscripts are different ($.05<P<.10$; Duncan's multiple-range test).

of baseline and spike concentrations. Since these mean concentrations were maintained continuously in the geldings in the present experiment, the amount of steroid available to target tissues may have been different from that normally available in intact stallions. This point was discussed by Smith *et al.* (1977), who found that mean concentrations of testosterone, maintained in castrated male rats by polydimethylsiloxane implants, suppressed LH release more effectively than did comparable endogenous concentrations of testosterone in intact males.

Normal sexual behavior was dramatically altered by castration. Two aspects of normal sexual behavior that appeared to be somewhat independent were the animal's desire to mount and thrust and his ability to ejaculate. In general, the desire to mount was retained for a longer period after castration than was the ability to ejaculate. The attainment of an erection was generally the last aspect of normal sexual behavior to disappear after castration. This behavioral pattern has been observed in impotent stallions and in those with other forms of abnormal sexual behavior (Pickett *et al.*, 1977).

Both libido and the ability to ejaculate were restored in TP-treated geldings. Restoration of these responses in TE-treated geldings was not as rapid, even though concentrations of testosterone in the serum were similar. It is unlikely that this delayed response of TE-treated geldings was caused by the estradiol in combination with testosterone, since estradiol alone was stimulatory.

Treatment with EB resulted in a significant increase in sexual behavioral score, but only at the higher dosage. In addition, libido appeared to be restored in these geldings to a much greater extent than was the ability to ejaculate. For example, EB-treated geldings that had lost their ability to ejaculate after castration showed only transient periods of improvement in that characteristic, whereas their desire to mount was improved consistently. This observation is consistent with the hypothesis that estradiol stimulates the central nervous centers controlling sexual behavior but has little effect on the functions of accessory sex organs and peripheral neurons involved with ejaculation (Luttge *et al.*, 1975). The response of EB-treated geldings was similar to that described previously for rats in which the glans penis had been anesthetized, (Carlsson and Larsson, 1964; Adler and Bermant, 1966); i.e., the animal exhibited frequent mounting and thrusting without ejaculation.

The restoration of libido by estradiol in these geldings is consistent with the hypothesis that the effects of testosterone on sexual behavior in males are mediated by aromatization to estradiol within the target neurons (Naftolin *et al.*, 1971). Peripheral aromatization of testosterone did occur in the TP-treated geldings, since mean concentrations of estradiol during the level 2 treatment period were higher in these animals than in control geldings. The possibility that testosterone *per se* stimulates libido in male horses can not be excluded.

Testosterone caused a dramatic increase in the number of spermatozoa in the first few ejaculates after the initiation of treatment. Testosterone has been reported to increase (Da Silva *et al.*, 1974) and decrease (Martins and Valle, 1939) the spontaneous contractility of the cauda epididymidis and vas deferens. The data in the present experiment appear to be consistent with the former report. Another possibility is that testosterone stimulated fluid secretions from the vasa deferentia and ampullae, thereby flushing residual spermatozoa from these sites. Since testosterone restored the ability to ejaculate in these animals, it is possible that the neurons involved in emission were also stimulated, resulting in a more efficient process (Cross and Glover, 1958).

The pH of gel-free semen appeared to be an androgen-responsive characteristic, with testosterone effectively returning the increased postcastrational pH values to normal. The pH of gel-free semen in intact stallions is affected by ejaculatory frequency (Pickett *et al.*, 1975, 1976) and season (Pickett *et al.*, 1976; Thompson *et al.*, 1977). The seasonal variation in pH of gel-free semen could be due to the seasonal variation in circulating concentrations of testosterone (Berndtson *et al.*, 1974) or to the seasonal variation in the relative contributions of the epididymides and accessory sex glands to an ejaculate (Pickett *et al.*, 1976).

Testosterone was stimulatory to the seminal vesicles and ampullae, but its effects on the prostate was not as dramatic. Estradiol at the concentrations maintained in TE-treated geldings did not inhibit the stimulatory effect of testosterone, as has been reported to occur when large doses have been administered to other species (Liao *et al.*, 1974). The stimula-

tory effect of testosterone on the seminal vesicles and prostate was also evident in the degree of vascularization of the glandular epithelium. There was no indication of stimulation of the Cowper's glands, and it is doubtful that these glands contribute much volume to the ejaculate of the normal stallion. The weights of accessory sex organs in TP- and TE-treated geldings agreed closely with those reported for stallions during the breeding season (Gebauer *et al.*, 1974).

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Effects of surgically imposed sterility on free-ranging rabbit populations

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Summary

1. Demographic changes in response to surgically imposed female sterility were monitored in 12 free-ranging rabbit populations in south-western Australia over a 4-year period. This was part of a research programme aimed at examining the potential for virally vectored immunocontraception to limit the abundance of rabbits (e.g. using a recombinant myxoma virus) and other mammalian pests. Sterility levels were 0%, 40%, 60% and 80% of all females in year 1, with a similar proportion of female recruits sterilized surgically in subsequent years.

2. There was a significant decrease in rabbit productivity with increasing sterility level. This was overcome by increased survival of kittens and adults on the high-sterility sites, such that the base-level numbers of rabbits were maintained, and mean annual rates of increase (r) were near zero for all treatments in all years. However, in the high-sterility populations this compensation was insufficient to overcome the effects of sterility totally, and there was a marked decrease in the seasonal peaks in rabbit abundance for these treatments.

3. Survival and recruitment were dependent upon the level of sterility, and consequently the density of rabbits, with greatest survival of adult rabbits occurring on the 80% sites. Survival of sterile females was greater than that of other adults, probably because of their increased ability to maintain body condition during times of low pasture biomass (summer drought). Thus two density-dependent processes were identified: the first was operating through increased survival of juvenile rabbits, the second through increased adult survival, particularly sterilized females.

4. Because the proportional impact of immigration was greater (i.e. immigrants constituted a greater proportion of the population) and emigration was less, from the 80% sites, the effects of sterility may have been underestimated on these sites.

5. The abundance of European rabbit fleas, a vector of myxomatosis, was significantly lower on the 80% sites, but this did not appear to affect the transmission of myxoma. Myxomatosis occurred as an annual epizootic in three of four years, with $> 90\%$ of rabbits on site after each epizootic testing positive for myxoma antibodies.

6. To achieve a sustained long-term reduction in rabbit abundance, 60–80% of female rabbits would need to be prevented from breeding. This could be achieved by a recombinant strain of myxoma provided the strain retained good transmissibility and all infected rabbits became sterile for life.

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Key-words: compensation, density-dependence, fertility control, productivity, rabbits.

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Introduction

Introduced vertebrates and other overabundant species have had a devastating impact on agricultural production and wildlife conservation world-wide. In Australia, the main culprits include the European rabbit *Oryctolagus cuniculus* (L.), the red fox *Vulpes vulpes* (L.) and the house mouse *Mus domesticus* (L.). (Wilson *et al.* 1992; Saunders *et al.* 1995; Williams *et al.* 1995; Choquenot, McIlroy & Korn 1996). A variety of control strategies, which include poison baits, shooting and trapping, are used in attempts to alleviate this impact (Robinson & Wheeler 1983; Saunders *et al.* 1995; Williams *et al.* 1995; Choquenot, McIlroy & Korn 1996). However, most are costly and the accrued benefits can be uncertain (Hone 1994); thus alternatives are being sought.

The potential for controlling the fertility of these pests is one such approach being examined. It requires the development of target-specific virally vectored sterility agents aimed at inducing an immune response in target species against specific proteins that are vital for reproductive success (Tyndale-Biscoe 1994). A recombinant myxoma virus that acts as a virally vectored immunocontraceptive is being considered for European rabbits in Australia (Tyndale-Biscoe 1994). However, there has been considerable debate concerning the merits of using fertility control of vertebrate pest populations as a long-term control strategy (Bomford 1990; Caughley, Pech & Grice 1992; Barlow, Kean & Briggs 1997). Barlow, Kean & Briggs (1997) suggest that previous models may have overestimated the effects of sterility as they have only considered a monogamous mating system, and that the long-term benefits will depend upon the sterilization regime and mating system. Sterility may even lead to an increase in abundance of the target species under some circumstances (Bomford 1990; Caughley, Pech & Grice 1992). Fertility control of vertebrates is predicted to work best in populations with high mortality that results in high population turnover (Stenseth 1981; Hone 1992). Target populations may, however, compensate for imposed infertility through an increase in the fecundity of fertile females, or through increased survival and recruitment of offspring produced, and increased immigration (Bomford 1990).

Chemosterilants have been successful in reducing the fecundity of some free-ranging vertebrates in North America (Kirkpatrick, Liu & Turner 1990; Turner, Liu & Kirkpatrick 1992), and injected porcine zona pellucida has been an effective immunocontraceptive in enclosed deer *Odocoileus virginianus* (Miller) populations (McShea *et al.* 1997). However, both these practices can be labour intensive, require re-exposure to the sterilizing compound, and therefore are not a practical option for wide-scale control programmes such as those required in Australia. Moreover, there is little information on the sterility level required to have a lasting effect on target populations. Three major outcomes are possible. Fertility control may result in an economic benefit by causing a decrease in the seasonal abundance of pest species, and hence a decrease in associated damage; or, alternatively, the more challenging objective of reducing pest densities overall may be achieved; or infertility will have little overall effect on target populations.

The determination of the level of infertility required to cause a sustained reduction in the abundance of target species is therefore important. Here we report on the effects of imposing different levels of female sterility in 12 free-ranging rabbit populations in south-western Australia. As European rabbits have a polygamous mating system (Bull 1956), the effects of male sterility have not been considered in our study because they are unlikely to be effective in reducing population size (Bomford 1990; Barlow, Kean & Briggs 1997). We compared productivity (kittens produced), survival, recruitment and immigration between the different sterility levels, and related these to the overall impact of sterility on the rabbit populations. The effects of sterility on the abundance of the European rabbit flea, an important vector of the myxoma virus, and on the epidemiology of myxomatosis were considered. We also discussed the likely regional impact of a fertility control agent on Australian rabbit populations. In addition, these experiments provided a unique opportunity to determine if any density-dependent processes were operating in the rabbit populations.

Methods

STUDY SITES AND DESIGN

The study was located at Wellstead in southern Western Australia (34°31′S, 118°35′E; Fig. 1) which is within 25 km of the coast. Although it can be subject to localized coastal influences, the climate at Wellstead is typically Mediterranean with an average annual rainfall of 500 mm. However, rainfall in this region can be highly unpredictable. Opening rains usually occur around April but they can be as

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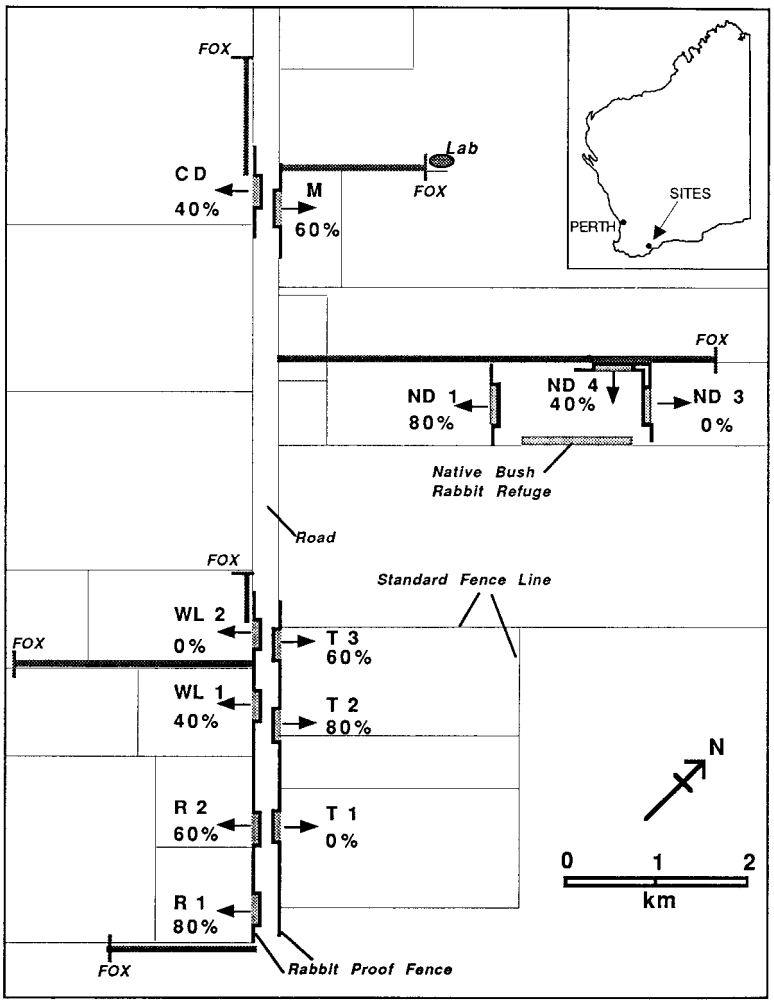


Fig. 1. The location of the Wellstead, Western Australia, study sites with the additional areas included in the fox transects shown. Fox transects also included the area in front of each experimental unit. Abbreviated site names are also shown by property.

late as July. Soils are generally aeolian sands over laterite cap rock. Remnants of native vegetation also persist. Merino sheep, beef cattle, cereal grain and oilseed production are the major agricultural enterprises. The basic biology and ecology of rabbits in this region are described in Twigg *et al.* (1998a).

Twelve discrete rabbit populations were established in 1992 using a combination of rabbit-proof fencing and buffer zones that contained relatively few rabbits (Fig. 1). Each experimental unit contained at least 320 × 60 m (range 320–420 m × 60–100 m) of natural bush refuge adjacent to the improved pasture where the rabbits fed (Fig. 2). All the sites were located within a 5-km radius, and the fences were constructed such that they only restricted the movement of rabbits in one compass direction (Fig. 1). Basically this was a linear system where the breeding stops/warrens were confined to the bush refuges. The only source of free-standing surface water was natural rainfall. Sites were assigned a level of female sterility of either 0%, 40%, 60% or 80% (three replicates of each) using a 'stratified randomized' design (three groups). The 'stratification' used to allocate treatment replicates was based upon the location, original numbers of rabbits, the amount of pasture biomass, and farming practices. These groups, which were not true statistical blocks, were used to avoid all three replicates of a particular treatment level being situated on the same farm/area by chance. In the summer (December–February) of 1992/93, we randomly assigned all females on each site to either surgical tubal ligation, sham operation or no surgery (left intact) in appropriate proportions to achieve the desired level of sterile females. For subsequent years (1994–96), the same proportions of new female recruits (previous offspring plus known immigrants) were allocated to the appropriate level of sterility. Eighty per cent of females always received some form of surgery.

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ASSUMPTIONS

Our experiment was assumed to mimic a single annual exposure to a virally vectored immunocontraceptive, namely a recombinant strain of myxoma virus. In most years myxomatosis occurred as an annual epizootic at the sites (Twigg *et al.* 1998a). We assumed that the sterility agent acted on, at least, a regional level and that infected animals were either sterile or resistant for life. However, for practical reasons the mortality effect of the myxoma virus was not always coupled with the time when sterility was imposed.

Differences in survival between sterile and fertile females were considered part of any compensatory processes and sterility levels were therefore not 'reset' each year. That is, after the experiments were established only the female recruits were considered when setting the level of sterility in subsequent years. Kittens that attained sexual maturity, and bred, during the year of their birth were also considered part of any compensatory mechanisms. Immigrants entering the populations were assumed to have come from populations exposed to the same sterility agent and were assigned an appropriate sterility status, but only if they could be distinguished from rabbits born on site (based on calculated birth dates from time of capture). Thus newly caught adult rabbits classified as immigrants over the months of January through to July/August were included in that year's sterility cohort.

MAINTENANCE OF BUFFER AREAS AND FOX CONTROL

Rabbit control by landholders was mainly through 1080 (sodium fluoroacetate) baiting, with some shooting, and took place at the sites until 1992. After this, all rabbit and fox control within 1–2 km of the sites became our responsibility until July 1996. None of the experimental rabbit populations was subjected to any rabbit control procedures during the trial period, but buffer areas were maintained relatively free of rabbits using a combination of 1080 baiting and shooting. A formal monetary lease agreement was developed with the landholders for the duration of the trials so that we could regulate farming practices across all sites.

Fox densities in agricultural areas in southern Australia are high, ranging from 0·5 to 1·0 foxes km^{-2}. These foxes have large home ranges ($> 25\,km^2$) that usually cover several farms, and daily movements often exceed 15 km (P. Thomson, personal communication). Fox control was undertaken by landholders outside our trial area, and regional fox shoots and baiting campaigns also occurred once or twice each year. Foxes can also be poisoned secondarily during 1080 rabbit-baiting campaigns (Algar & Kinnear 1996). Thus it was difficult to always maintain a consistent level of fox control at the field sites. Consequently, we implemented a minimum fox control strategy where each site was simultaneously baited between 1 and three times per year depending upon fox abundance. Dried meat baits containing 1080 poison ($4{\cdot}5\,mg\ bait^{-1}$) were used. Some fox control was necessary because we had increased fox predation on our experimental rabbit populations by removing most rabbits from the surrounding buffer areas.

PREDATOR COUNTS

Foxes were the main predator of rabbits at our sites. Standardized fox counts commenced in August 1993, and were conducted together with the spotlight counts for rabbits (see below). The fox transects included the rabbit treatments, the areas in between, and additional farm tracks (Fig. 1). The total fox transect length was 22·2 km and this was travelled over three consecutive nights during each monthly monitoring period. We counted both foxes and feral cats *Felis catus* (L.). To avoid double counting, animals were only counted for either the outward or inward journey and odometer readings were noted for each sighting. The number of foxes seen and the transect distance over the three nights was summed, and fox abundance was standardized as foxes seen per 100 km travelled. Commencing in January 1993, incidental sightings of foxes were also recorded, but these were excluded from the formal counts. Visitation rates for each of the 12 sites were estimated using only those foxes formally recorded along the 1-km rabbit transect in the front of each site (Fig. 2).

Diurnal predators of rabbits seen at the study sites included monitor lizards *Varanus rosenbergi* (Mertens) and wedge-tailed eagles *Aquila audax* (Latham) but we did not monitor their abundance.

POPULATION MONITORING OF RABBITS

Preliminary trapping of rabbits commenced in 1992, and from January 1993 all sites were monitored at 3–6-week intervals using a combination of live-trapping and formalized spotlight counts. All trapped

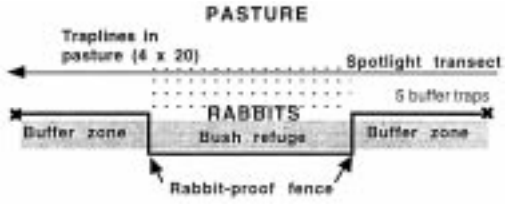


Fig. 2. The layout of an individual unit with the trap grid, buffer traps and spotlight transect shown.

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rabbits were marked with two uniquely numbered ear tags: a plastic Leader (swivel tag; sheep) primary tag and a brass fish fingerling secondary tag in the opposite ear. Primary tags were a unique colour for each site and had one of four scotchlite colours attached to facilitate identification of migrant rabbits during spotlighting. Males and females were tagged in opposite ears. A third tag with a fifth scotchlite colour was also given to all rabbits caught during each period when sterility was imposed (January–March). Thus, except for each year's cohort of young, all rabbits were tagged with two reflective ear tags as 100% of rabbits were caught over each sterility period (Twigg, Grifffin & O'Reilly 1996).

Conventional trap grids were a 4×20 grid with approximately 20-m spacing between traps, with an additional five traps at the end of each 200–300 m buffer zone, giving 90 traps per site as our standard trapping regime (Fig. 2). Wire cage live-traps with a treadle trigger were used with diced carrot as bait (Twigg, Grifffin & O'Reilly 1996). The first line of traps generally commenced within 1–5 m of the bush refuge. Trapping was undertaken for four consecutive nights with three sites trapped simultaneously.

At the end of the summer–autumn period (January–March) when the sterilizations were undertaken, a rabbit-proof electric fence was used as a trapping aid, which together with spotlight counts of tagged rabbits enabled us to determine that all rabbits on each site had in fact been caught during this period (Twigg, Griffin & O'Reilly 1996). We used the electric fence to confine rabbits to their bush refuge, thus denying them access to their usual feeding areas. This encouraged rabbits to enter the 150–250 cage live-traps distributed within the refuge. Electric-fence trapping was undertaken for three to five consecutive nights until less than three 'new' rabbits were caught on any night.

Spotlight counts were undertaken for three consecutive nights over a permanently marked transect (approximately 900–1000 m in length and 50 m from the bush refuge) in the pasture in front of each site (Fig. 2) using a 100-watt spotlight. The same vehicle, counter(s) and driver were used within each session. Vehicle speed was generally 15 km h^{-1}, depending upon terrain. Tagged, untagged, adult and kitten rabbits were counted. For practical reasons, we used four main counters but drivers often varied between sessions (Twigg *et al.* 1998b). Taking the highest count for each session, rabbit numbers were standardized to rabbits seen per km based on the length of the actual refuge where they resided. This provided an additional population index that was independent of mark and recapture.

Two field teams were used throughout the study; team 1 trapped six sites and completed the spotlight counts for the remaining six sites. Team 2 reversed this pattern, enabling all sites to be monitored without trapping and spotlighting interfering with each other. This approach also enabled spotlighting and trapping to be undertaken within 10 days of each other for each site. Approximately 20 days were required to complete one full monitoring session.

At their first capture within a trap session, rabbits were held in numbered calico bags and taken to a central field laboratory for processing. Recaptures for a current session were recorded and released at their point of capture. The grid location, sex, breeding status, mass, head length, number of rabbit fleas, myxomatosis status, times caught, tag status (lost tag, given new tag), fate of rabbit (i.e. alive, died in trap), capture class (i.e. within or between trap sessions or new), packed cell volume [haematocrit (%)] and plasma protein (refractometer g $100\,ml^{-1}$) were recorded for most/all rabbits during a trap session. Each rabbit remained in its calico bag during processing, and these bags were laundered before being reused on other sites to reduce the chance of spreading disease and/or fleas between sites.

MYXOMATOSIS AND EUROPEAN RABBIT FLEA ABUNDANCE

Clinical signs of myxomatosis and the abundance of European rabbit fleas *Spilopsyllus cuniculi* (Dale) were recorded for all rabbits for all trap sessions. Five categories for myxomatosis were recognized: no evidence, mild current lesions, considerable (heavy) current lesions, recovering old lesions, recovered old lesions. Fleas were counted on the head, ears and in the calico bag, with a maximum 1 min search time allowed. The fur on the head and ears was ruffled to facilitate counting. Categories were 0–10 (low), 11–50 (moderate), 51–100 (high) and > 100 (very high) fleas present.

SURGICAL PROCEDURES

A permanent three-room field laboratory was established at Wellstead with one room designated solely for surgery. All rabbits were processed in this laboratory. Xylazil 20 (5 mg kg^{-1}, i.m.; Troy Laboratories) was used as a premed; rabbits were anaesthetized with Zoletil 100 (50% Zolazepam and 50% Tiletamine, 15–20 mg kg^{-1}, i.v.; Virbac) and an antagonist to Xylazil, Antagozil S.A. (0·1 mg kg^{-1}, i.v.; Troy Laboratories), was administered at the completion of surgery to speed recovery. Alamycin L.A. (10 mg kg^{-1}, i.m.; Novartis) was also given as a prophylactic. Fallopian tubes were both tied and fulgurated through a mid-line incision. Sham operations involved anaesthesia and surgery, but the fallopian tubes were left entire. Virtually all rabbits that received surgery were released on the

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day of capture, except when surgery continued late into the afternoon which necessitated a few rabbits being released early the following morning. Once we became confident that we could capture all rabbits during the first sterility period, any kittens (< 700 g) and rabbits with active myxoma infection were not assigned to a sterility class until they were recaught at a later date.

DEMOGRAPHICS OF RABBIT POPULATIONS

The trapping data were used to determine changes in demography for each population. It was assumed that, unless there was evidence to the contrary, all rabbits were alive on site for the period between their first and last capture, regardless of whether they had been caught over this time. This enabled us to establish the minimum number of rabbits present for each time period. These data are referred to as 'assumed rabbit numbers' and were used to determine the proportion of the various age groups in the populations, rates of increase, and survival. Productivity (number of kittens produced) was estimated from the number of kittens trapped above ground. Offsite trapping grids were also established in areas immediately adjacent to some of the sites so that litter size for rabbits in the study area could be determined by autopsy. Age classes were based on body mass, with rabbits becoming sexually mature at 1200 g (King & Wheeler 1985). Categories were: kittens 25–699 g, subadults 700–1199 g, adults > 1199 g.

The observed instantaneous per capita rate of increase (r) was calculated using the trapping data and regression analysis to calculate a mean rate for each time period examined. This method is recommended by Caughley (1977) when using population estimates. Rates of increase were calculated using several different time periods to determine short- and long-term trends. First, rates were determined for each 12-month period using an average of the values calculated from the total number of rabbits assumed to be on site for each month and from one year to the next for each treatment level (i.e. average of: total numbers from January 1993 to January 1994, from February 1993 to February 1994, and so on). This gave an annual rate for each year. Exponential rates of increase were also calculated for breeding (April–November) and non-breeding (late November–March) periods.

MIGRANT RABBITS

We could usually identify migrant rabbits because of our tagging system and the frequency of trapping. Migrants were classified as those rabbits permanently leaving or entering a population and included known dispersal movements by adult and subadult rabbits. Short-term moves by rabbits within their home range were referred to as exploratory movements (Twigg *et al.* 1998b). To differentiate true emigration from exploratory moves, a given rabbit had to be captured on three occasions offsite before it was classified as a true migrant. If a rabbit did not meet this criterion (i.e. for their first and second offsite capture), it was returned to its site of origin.

RECOVERY OF OFFSITE RABBITS

Every effort was made to recover offsite tagged rabbits by shooting prior to rabbit control in the buffer zones surrounding the experimental populations. Destructive sampling was also used for the last trapping period of the trials to recover all rabbits in the immediate area; trapping and shooting continued until no tagged rabbits were known to be on or near each site. An extensive search of the thick scrub in the bush refuges was also undertaken towards the end of the trials (September–November 1995) to recover tags from any animals that had died. The presence or absence of an accompanying carcass was recorded for each tag found.

PASTURE BIOMASS AND PHYSICAL ATTRIBUTES OF BUSH REFUGE

Due to the often pronounced and shifting rabbit graze line away from the bush refuge, we used a stratified approach to determine pasture biomass; zones were 0–100 m and 101–300 m from the rabbit refuge habitat. A mean of these two zones was used as an estimate of biomass. Fifty quadrats were placed at random within each zone and the biomass scored as one of 15 categories using a series of standardized reference photographs. These scores were converted to kg dry weight ha^{-1} using calibration curves generated from cut samples after they had been visually scored and then dried for 48 h in an oven at 80 °C. A new calibration curve was generated for each sampling period. Pasture biomass was measured on three occasions each year: late autumn (scarcity), midwinter (average) and the end of spring (times of plenty).

Two measures of the physical attributes of the bush refuge were determined for each site in late 1995; depth of sand (potentially affects rabbit breeding capacity) and horizontal vegetation cover (predation). Using the trap grid positions as a reference, these measures were undertaken at similar positions on each site so that they were representative of the sites. Depth of sand was determined up to 1·9 m with a hand auger. The percentage vegetation cover was determined for the 0–20 cm and 50–70 cm layers using cover board estimates taken at two perpendicular compass bearings (Twigg & Fox 1990). Results were expressed as means for each site.

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STATISTICAL ANALYSES

Analyses were undertaken using SYSTAT (1992) or Statistica (1994). After using a one-way ANOVA to establish that there were no significant differences between subgroups, the response of intact and sham-operated females was combined as 'fertile females' for analyses (e.g. body mass, packed cell volumes, plasma protein). For ANOVA, the homogeneity of variance and sphericity were tested as appropriate, and any violations corrected by appropriate data transformations (e.g. arcsin, square root, log) before undertaking final analyses. Where graphical presentation of raw data indicated a linear trend, or a linear trend was suspected (e.g. productivity, recruitment), the relevant treatment degrees of freedom were partitioned into treatment, linear (1 d.f.) and quadratic (non-linear; 1 d.f.) components for each relevant ANOVA (i.e. orthogonal polynomials were used to test effects; SYSTAT 1992; Scheiner & Gurevitch 1993).

Because we believed many of the parameters we compared did not strictly represent a repeated measures design (e.g. recruitment by year involves different cohorts from the same population), a repeated-measures ANOVA was only used in place of a multifactor ANOVA if there was a significant correlation between years in the raw data. While it has been suggested that significance levels can be relaxed to 10–15% when using ANOVAs to analyse ecological data, provided the test assumptions are met (Scheiner & Gurevitch 1993), we chose the conservative option of using 0·05 alpha levels.

All rabbit survival rates were calculated using the staggered-entry progressively censored analytical technique of Pollock *et al.* (1989), which allows new animals to be added to those groups under consideration. Assumptions for this procedure include: sex and age classes need to be sampled randomly, survival times are independent for different animals, recapturing an animal does not influence its future survival, and precision is poor unless > 20 animals are tagged at a particular time (Pollock *et al.* 1989). All rabbits were assumed to be alive on site between their first and last capture. Because these survival estimates are not normally distributed, the Kruskal–Wallis H Statistic (non-parametric ANOVA) was used to compare kitten verses adult survival during each breeding season, and also the survival of each sterility cohort (Scheiner & Gurevitch 1993; SYSTAT 1992). Individual ANOVAs were used to compare the effects of sterility level, rabbit age and year on adult and kitten survival, and to compare the 34-day survival rates of adult males and fertile and sterile females throughout the trials. The non-parametric log-rank test, including the modifications suggested by Cox & Oakes (1984: 105), was also used to test whether survival curves differed, with one minor modification. Cox & Oakes (1984) provide three different modifications that differ in their levels of conservatism. Rather than use one of these, all three were calculated and the mean of these critical values was used to determine *P*-values. To ensure group sizes were adequate (Pollock *et al.* 1989), the three replicates for each treatment were pooled for each year.

Results

SITE CHARACTERISTICS AND GENERAL ECOLOGY

There was considerable variation in both the amount of rainfall and pasture biomass during the study (Fig. 3). Pasture biomass levels ranged from 1037 to 5108 kg dry weight ha^{-1}, with the greatest amounts occurring at the end of 1993/early 1994, and there were no significant differences between treatments (all years pooled; $F = 0{\cdot}81$; d.f. = 3, 9; $P = 0{\cdot}496$). In this region, pasture biomass levels below 750 kg dry weight ha^{-1} at the end of summer are considered extremely low. The main pasture constituents were the legumes *Trifolium*, *Lotus* and *Ornithopus*, and the grasses *Bromus*, *Hordeum*, *Danthonia*, *Ehrharta*, *Lolium* and *Poa*. The main weed genera were *Arctotheca*, *Hypochoeris*, *Erodium*, *Romulea*, *Rumex* and *Citrullus*. The depth of sand and the horizontal vegetation cover of the bush refuges were found to be similar across the treatments. Mean ($\pm$ SD) depth of sand was: 0% sites, 105 ± 18 cm; 40% sites, 69 ± 28 cm; 60% sites, 110 ± 23 cm; 80% sites, 79 ± 28 cm ($n = 3 \times 7$). Mean ($\pm$ SD) percentage cover in the 0–20 cm vegetation layer was: 0% sites, $91 \pm 8\%$; 40% sites, $83 \pm 14\%$; 60% sites, $92 \pm 17\%$; 80% sites, $85 \pm 15\%$ ($n = 3 \times 7$). Vegetation was less dense (mean cover ranged from 58% to 73%) in the 50–70 cm layer, but gave similar trends.

Preliminary trapping was undertaken in September or November in 1992 and, assuming equal trappability between sites, mean ($\pm$ SD) rabbit numbers ranged from: 0% sites, $29{\cdot}3 \pm 8{\cdot}5$; 40% sites, $34{\cdot}7 \pm 17{\cdot}9$; 60% sites, $38{\cdot}3 \pm 21{\cdot}2$; 80% sites, $41{\cdot}3 \pm 19{\cdot}3$ ($F = 0{\cdot}26$; d.f. = 3, 8; $P = 0{\cdot}850$). Treatments were imposed in the summer of 1992/93. Rabbit numbers were relatively high throughout the study, ranging from 30 to more than 150 rabbits ha^{-1} of bush refuge. Rabbit numbers peaked at the end of the breeding season in 1994, which was preceded by high pasture availability in late 1993/early 1994 (Figs 3 and 4). Rabbit numbers then partially crashed by mid-1995, particularly on the 0% sites. Pasture biomass also declined over much of 1995, due mainly to the timing of the low and variable rainfall in the first 6 months of the year (Fig. 3). Peak rabbit numbers were approximately twofold lower on the 60% and 80% sites than on the other two treatment sites (Fig. 4; $F = 8{\cdot}02$; d.f. = 3, 9;

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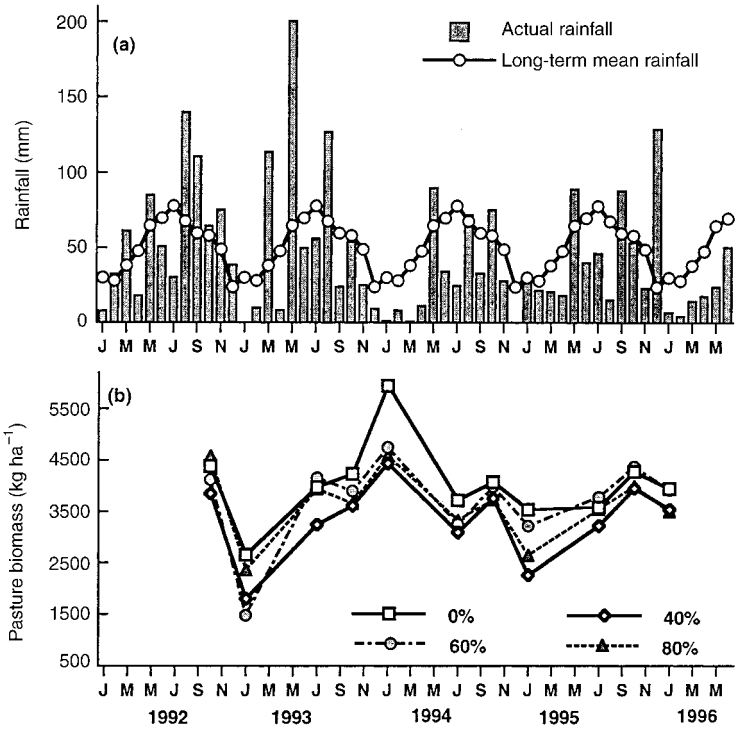


Fig. 3. (a) Rainfall (mm) with long-term mean and (b) mean pasture biomass (n = 3 sites) over the duration of the trials at Wellstead, Western Australia. Rainfall data were for Mettler, which is within 5 km of the study area, and were provided by the WA Bureau of Meteorology. Pasture biomass (kg dry weight ha^{-1}) was measured in summer, winter and spring of each year. Standard errors, which overlapped, have been omitted for clarity.

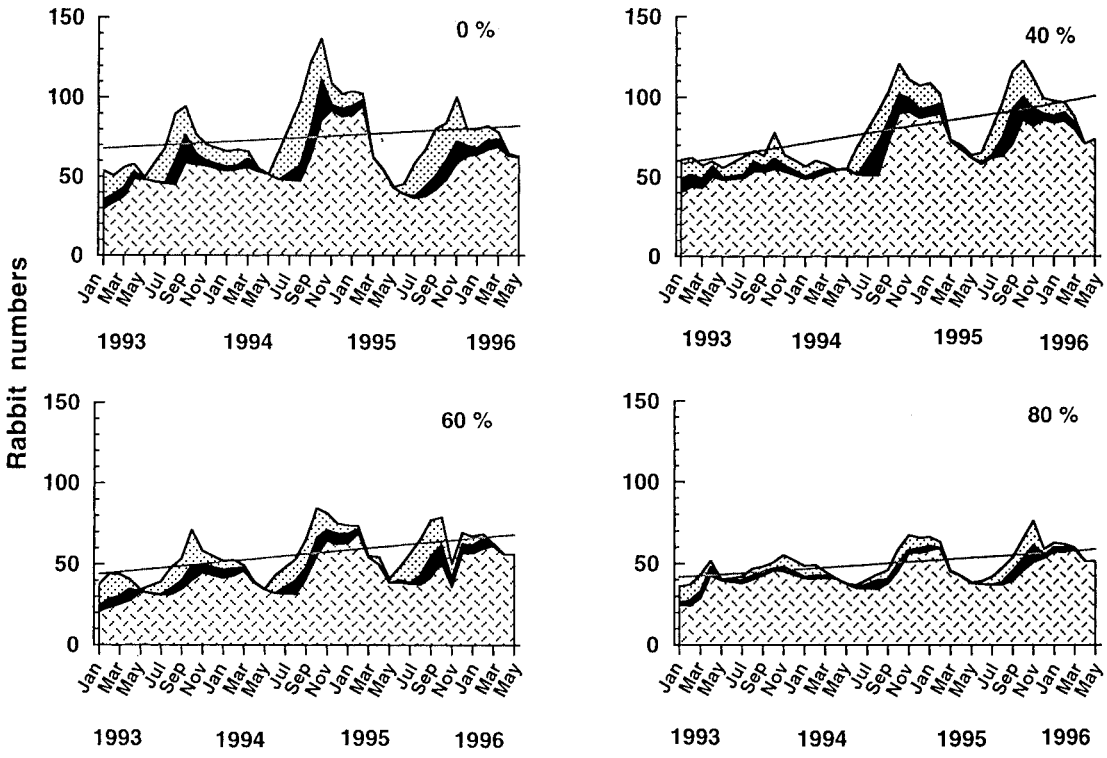


Fig. 4. Mean changes (n = 3 sites) in the proportion of adult (> 1199 g; hatching), subadult (700–1199 g; solid) and kitten (25–699 g; stippled) rabbits for each of the female sterility levels over the 46-month study at Wellstead, Western Australia. Linear regression lines are also given. Rabbit numbers are based on trapping data and assume all rabbits were alive on site between their first and last capture.

$P < 0{\cdot}001$). Trends generated from the spotlight counts of rabbits were identical to the general population changes seen from the analysis of the trapping data (Twigg *et al*. 1998a).

The main breeding season was prolonged and, based upon calculated conception dates, extended from March to November in 1993, and from April to November in 1994 and 1995. Pregnant females were present, although in low numbers (0·1–1·0% of fertile adult females), in all months outside the breeding season in all years except January 1994.

PREDATORS

There were 111 records of feral cats during the study, with low numbers seen in most months. This compares with 562 records for foxes, or 14·1 records ($\pm$ SD, 8·3) per month ($n = 40$). Foxes were seen in all months. Although not monitored, wedge-tailed eagles and monitor lizards were also common over the summer months. The number of foxes per 100 km of spotlight transect throughout the study ranged from 5 to 39. While there were 'hot-spots' of fox activity, these appeared to be related to available fox habitat/refuge rather than to visitation by foxes to sites of a particular sterility level (Fig. 5). There was no difference in visitation rates by foxes to the four treatments (all seasons pooled; $F = 0{\cdot}33$; d.f. = 3, 8; $P = 0{\cdot}802$).

RATES OF INCREASE

While rabbit abundance fluctuated considerably during a year (Fig. 4), the overall abundance of rabbits changed little over a 12-month period with exponential rates of increase (r) showing only marginal change (Table 1). The 0% and 40% sites were the only treatments that displayed a negative mean rate of increase over a given 12-month period (Table 1). However, most rates were low and were around zero, indicating that compensation was occurring in those populations where the sterility level was high.

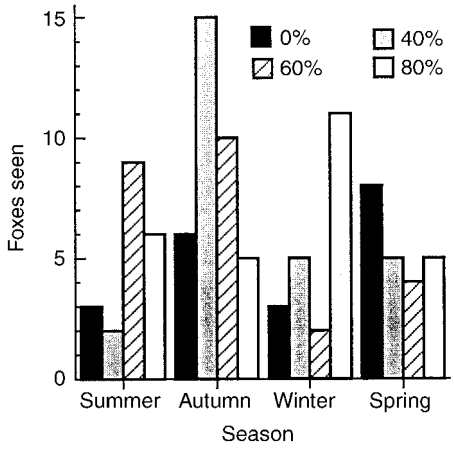


Fig. 5. The total number of foxes seen on the individual treatment sites only, by season and sterility level, during the trials at Wellstead, Western Australia. Sightings were recorded from January 1993 to June 1996.

Interestingly, at the end of our 4-year study, mean rabbit numbers were found to be similar ($F = 0{\cdot}59$; d.f. = 3, 8; $P = 0{\cdot}639$; Fig. 4) among treatments (destructive sampling was used to retrieve all rabbits during the last monitoring session). Mean ($\pm$ SD) numbers were: 0% sites, $62{\cdot}7 \pm 17{\cdot}4$; 40%, $73{\cdot}7 \pm 32{\cdot}6$; 60%, $56 \pm 19{\cdot}1$; 80%, $51{\cdot}3 \pm 13{\cdot}6$.

Mean exponential rates of increase (r) for the breeding season (generally April–November) and non-breeding periods were calculated for each year and then standardized to a 6-month period. All mean rates were positive during the breeding season, and ranged from 0% sites, 0·13–0·30; 40%, 0·06–0·25; 60%, 0·15–0·29; 80%, 0·06–0·10, for the three consecutive breeding seasons considered. However, the rate of increase was negative for all non-breeding periods, ranging from 0% sites, $-0{\cdot}14$ to $-0{\cdot}05$; 40%, $-0{\cdot}09$ to $-0{\cdot}06$; 60%, $-0{\cdot}10$ to $-0{\cdot}05$; 80%, $-0{\cdot}05$ to $-0{\cdot}02$. Rates of increase were greatest during the 1994 breeding season [0·10

Table 1. Mean exponential rate of increase (r) for each time period by the level of female sterility for rabbit populations at Wellstead, Western Australia. Average rates were based upon the available trapping data for relevant months from one year to the next. A rate for January 1993 to January 1996 is also included for comparison

	Sterility level							
	0%		40%		60%		80%	
Period	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1993/94	−0·002	0·008	−0·012	0·047	0·013	0·017	0·005	0·032
1994/95	−0·008	0·004	0·017	0·003	0·017	0·011	0·009	0·008
1995/96*	0·013	0·009	0·028	0·034	0·012	0·014	0·018	0·012
January 1993 to January 1996	0·001	0·001	0·014	0·004	0·011	0·011	0·007	0·009

*Data only available until May 1996.

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(80% sites) to 0·30 (0% sites)], and the highest rate of decrease occurred during the summer/autumn 'crash' of 1995 (0·14–0·04), particularly on the 0% sites. These trends were evident regardless of whether known immigrants were included or not. The 80% sites showed the least fluctuation, and the 0% sites the most.

RABBIT PRODUCTIVITY

Significantly fewer kittens were produced on the high-sterility sites (Fig. 6; $F = 13{\cdot}57$; d.f. = 3, 6; $P < 0{\cdot}001$). Overall, there was a strong linear effect due to the level of female sterility ($F = 39{\cdot}99$; d.f. = 1, 24; $P < 0{\cdot}001$) but non-linear (quadratic) effects were not apparent ($F = 0{\cdot}73$; d.f. = 1, 24; $P = 0{\cdot}402$). Differences between the 80% and both the 0% and 40% treatments were significant (Tukey; $P = 0{\cdot}014$; $P = 0{\cdot}050$), but the 80% and 60% treatments were similar ($P = 0{\cdot}353$). The 0% and 40% sites produced a similar number of kittens over the trials ($P = 0{\cdot}789$) and did not differ significantly from the 60% treatment ($P = 0{\cdot}161$; $P = 0{\cdot}513$, respectively). There were also significant differences in the number of kittens produced between years (repeated-measures ANOVA; $F = 5{\cdot}88$; d.f. = 2, 16; $P = 0{\cdot}012$) but the interaction of year and sterility was not significant ($F = 1{\cdot}82$; d.f. = 6, 16; $P = 0{\cdot}158$). Kitten production was lower on the 0% sites in 1995 due to these populations appearing to exceed carrying capacity by late 1994, such that there was a crash in rabbit numbers by the end of autumn 1995 (Figs 3 and 4). Pasture biomass levels were low on the 0% sites in the 1995 autumn (Fig. 3), and decreased even further thereafter due to lack of rain. The mean ($\pm$ SD) number of kittens produced per year during the trials was: 0%, 108 ± 105; 40%, 91 ± 76; 60%, 65 ± 47; 80%, 33 ± 42 ($n = 3$ sites $\times$ 3 years). As the survival of kittens varied among treatments (see below), it was not possible to obtain meaningful data for the number of kittens produced per fertile female (i.e. fecundity). Our data were also based upon the trappable rabbits and therefore included only emergent kittens. Pre-emergent mortality of kittens can be considerable (Gilbert *et al.* 1987; Twigg *et al.* 1998a).

There was no linear effect of sterility on the numbers of kittens attaining adult mass and breeding in the year of their birth ($F = 0{\cdot}61$; d.f. = 1, 24; $P = 0{\cdot}442$). Mean numbers ($\pm$ SD) were: 0%, $1{\cdot}8 \pm 1{\cdot}9$; 40%, $5{\cdot}7 \pm 3{\cdot}8$; 60%, $3{\cdot}0 \pm 2{\cdot}7$; 80%, $1{\cdot}7 \pm 2{\cdot}1$ ($n = 3$ sites $\times$ 3 years).

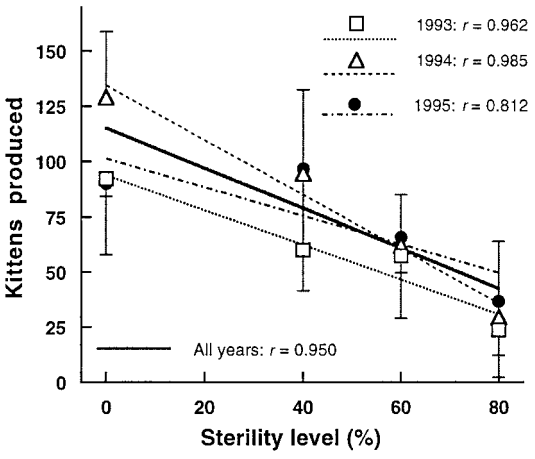


Fig. 6. Mean ($\pm$ SD) number of kittens produced during each breeding season (1993–95) at Wellstead, Western Australia. The mean of all years pooled (solid line, $n = 3$ sites $\times$ 3 years) is also given.

RECRUITMENT

In our context, recruitment refers to the number of offspring entering the adult population at the start of each breeding season. The mean proportion of kittens recruited into the adult population each year was positively correlated with sterility level and differed between sterility treatments (Fig. 7; arcsin; $F = 4{\cdot}61$; d.f. = 3, 24; $P < 0{\cdot}012$). There was a strong linear component due to sterility ($F = 4{\cdot}90$; d.f. = 1, 24; $P = 0{\cdot}037$) but no significant non-linear (quadratic) effects ($F = 0{\cdot}15$; d.f. = 1, 24; $P = 0{\cdot}702$), indicating that there was an overall linear increase in the proportion of kittens recruited with increasing sterility. The proportion of kittens recruited also appeared to decrease as the number of kittens produced increased ($r = -0{\cdot}313$; $P < 0{\cdot}100$; Fig. 7); that is, density-dependent processes became obvious following the imposition of sterility.

However, the number of kittens recruited was negatively correlated with sterility ($r = -0{\cdot}351$, $P = 0{\cdot}036$). Means ($\pm$ SD) were: 0% sites, $40{\cdot}2 \pm 14{\cdot}6$; 40%, $34{\cdot}9 \pm 19{\cdot}3$; 60%, $31{\cdot}2 \pm 17{\cdot}1$; 80%, $15{\cdot}9 \pm 14{\cdot}0$ ($n = 3 \times 3$), and were lower for the 80% sites than for any other treatment ($F = 4{\cdot}61$; d.f. = 3, 24; $P = 0{\cdot}011$; Tukey: 0%, $P = 0{\cdot}009$; 40%, $P = 0{\cdot}050$; 60%, $P = 0{\cdot}145$).

SURVIVAL

Mean 34-day survival curves for adults and post-emergent kittens over the three breeding seasons were computed based on the assumed rabbit numbers (trapping data) and the Pollock *et al.* (1989) procedure. Thirty-four days was the average time between conventional trap sessions, and each breeding season lasted approximately 136 days. Survival of post-emergent kittens was much lower than that of adult rabbits on all treatments (Fig. 8). Overall, survival of rabbits during the breeding season was better on the 80% sites than for any other treatment

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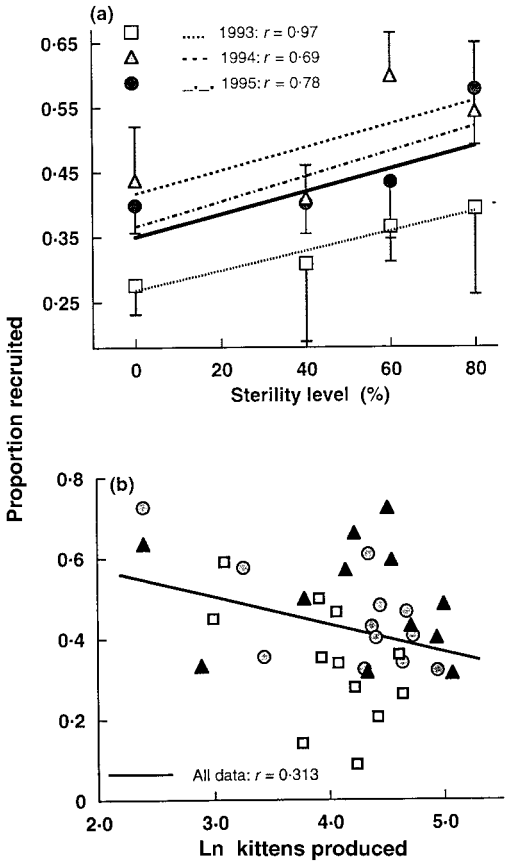


Fig. 7. Proportion of kittens recruited into the adult population following each breeding season (1993–95) at Wellstead, Western Australia: (a) Mean (SEM, $n = 3$ sites) proportion for each sterility level. The mean of all years pooled (solid line, $r = 0{\cdot}88$, $n = 3$ sites × 3 years) is also given; (b) The effect of kitten density (ln number of kittens produced on each site) on the proportion of kittens recruited ($n = 12$ sites × 3 years).

(Fig. 8). Survival was influenced by rabbit age (kitten vs. adult) (Kruskal–Wallis statistic, H = 4302·1; d.f. = 1; $P < 0{\cdot}001$) and sterility level (H = 27·1; d.f. = 3; $P < 0{\cdot}001$), but was similar among years (H = 3·6; d.f. = 2; $P = 0{\cdot}167$). The survival curves also differed between kittens and adults ($\chi^2 = 13{\cdot}0$–$81{\cdot}7$; d.f. = 1; all $P < 0{\cdot}001$), but this was less significant at the higher sterility levels.

Because of the length of time involved, the nature of the censored analytical technique for calculating survival (Pollock *et al.* 1989) and because the longevity of individual cohorts varied between sites, it was not possible to calculate meaningful average survivorship curves for the sterility cohorts. Consequently, sterility cohort-specific survival rates were calculated for adult males, and sterile and fertile females for each 34-day period, based upon the assumed rabbit numbers.

Although survival of sterile females was greater than that of the other rabbits, there was no evidence that this trend was consistently correlated with a particular sterility level (Fig. 9). Data for the 1995 and 1996 cohorts are not shown, but these trends were similar to those of the earlier two cohorts. Survival rates for the two sterility cohorts for which we had the most data, 1993 and 1994, were examined using the Kruskal–Wallis H Statistic. On both occasions, survival was influenced by the fertility status of rabbits (1993: H = 90·8; d.f. = 2; $P < 0{\cdot}001$; 1994: H = 29·8; d.f. = 2; $P < 0{\cdot}001$), with sterile females surviving best. In addition, using the 1993 cohort as an example, the log-rank tests indicated that the survival curves for both males ($\chi^2 = 8{\cdot}61$; d.f. = 1; $P < 0{\cdot}01$) and fertile females ($\chi^2 = 9{\cdot}03$; d.f. = 1; $P < 0{\cdot}01$) were different to that of sterile females, but the survival curves for males and fertile females were similar ($\chi^2 = 0{\cdot}71$; d.f. = 1; $P > 0{\cdot}05$). Some 1993 cohort rabbits were adults when first caught in 1992, indicating that rabbits at Wellstead can survive in excess of 5 years. On the basis of the number of tags found next to a carcass in the bush refuges (87%, i.e. 71 of 82 tags found), the observed behaviour of tagged rabbits during spotlight counts, and the strong site fidelity exhibited by rabbits (Twigg *et al.* 1998b), we believe the majority of rabbits died on site rather than emigrating.

LONGEVITY

A considerable number of sterile females were still alive at the end of the field trials (Fig. 9) and hence were excluded from the censored survival analytical technique of Pollock *et al.* (1989). Consequently, differences in longevity for each sterility cohort were also examined using the proportion of adults that were alive in January/March in subsequent years (Table 2). Each sterility cohort was analysed separately using arcsin transformations and a repeated-measures (time) ANOVA, and gave similar results for all cohorts. Using the 1993 cohort as an example, longevity was influenced by rabbit status (sex and/or sterility) ($F = 11{\cdot}44$; d.f. = 2, 30; $P < 0{\cdot}001$) and time ($F = 132{\cdot}79$; d.f. = 2, 60; $P < 0{\cdot}001$), and the interaction was also significant ($F = 4{\cdot}29$; d.f. = 4, 60; $P = 0{\cdot}004$), indicating that the different groups responded differently over time. The longevity of sterile females was greater than that of fertile females (Tukey; $P < 0{\cdot}001$) and adult males ($P = 0{\cdot}013$).

MIGRATION

There were 445 known immigrations during the study (Table 3), with the greatest numbers occurring around January and a smaller peak in March. Males immigrated significantly more often than females ($F = 7{\cdot}33$; d.f. = 1, 16; $P = 0{\cdot}016$; Twigg

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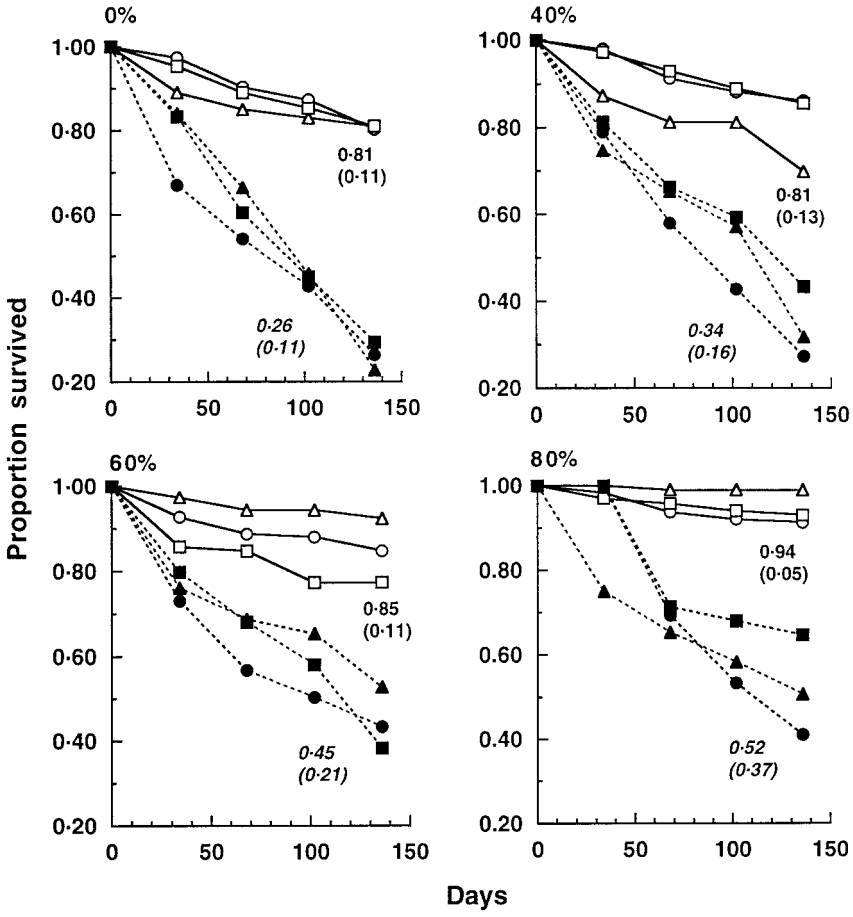


Fig. 8. Mean 34-day survival rates (Pollock *et al.* 1989) for post-emergent kittens (broken lines) and adult rabbits (solid lines) for each site during the three main breeding seasons at Wellstead, Western Australia (1993–95). Like-shaped symbols within each graph are the same site for each sterility level. Grand means (± SD; $n = 3$ sites × 3 years) for kitten and adult survival at the end of the breeding season (i.e. after 136 days) for all years are also shown. Day 0 corresponds to day 1 of kitten emergence for each site.

et al. 1998b; Table 3). The actual number of immigrants was similar between treatments ($F = 0{\cdot}28$; d.f. = 3, 16; $P = 0{\cdot}736$). However, the proportional impact of this immigration (i.e. the ratio of immigrant to resident rabbits) was greater on the 80% sterility treatments than on the 0% and 40% sites ($F = 3{\cdot}73$; d.f. = 3, 24; $P = 0{\cdot}025$; Tukey; all $P < 0{\cdot}05$) but not the 60% treatments ($P = 0{\cdot}103$). This did not differ among years ($F = 1{\cdot}21$; d.f. = 2, 24; $P = 0{\cdot}315$; Table 3).

Emigration was based upon the known movement of 147 tagged rabbits (Table 4). Significantly more males ($n = 102$) than females ($n = 45$) emigrated ($F = 11{\cdot}76$; d.f. = 1, 16; $P = 0{\cdot}003$; Twigg *et al.*

Table 2. The proportion of fertile female (FF), sterile female (SF) and male (M) rabbits present for the three main sterility cohorts in January/March of each year (all sites pooled) for rabbit populations at Wellstead, Western Australia

	1993 cohort			1994 cohort			1995 cohort		
Year	FF	SF	M	FF	SF	M	FF	SF	M
1993	225*	165*	435*	–	–	–	–	–	–
1994	0·404	0·618	0·443	109*	63*	267*	–	–	–
1995	0·227	0·515	0·282	0·405	0·571	0·439	252*	155*	382*
1996	0·133	0·418	0·165	0·239	0·363	0·221	0·210	0·314	0·209

*Number of rabbits assigned to relevant cohorts in January/March each year.

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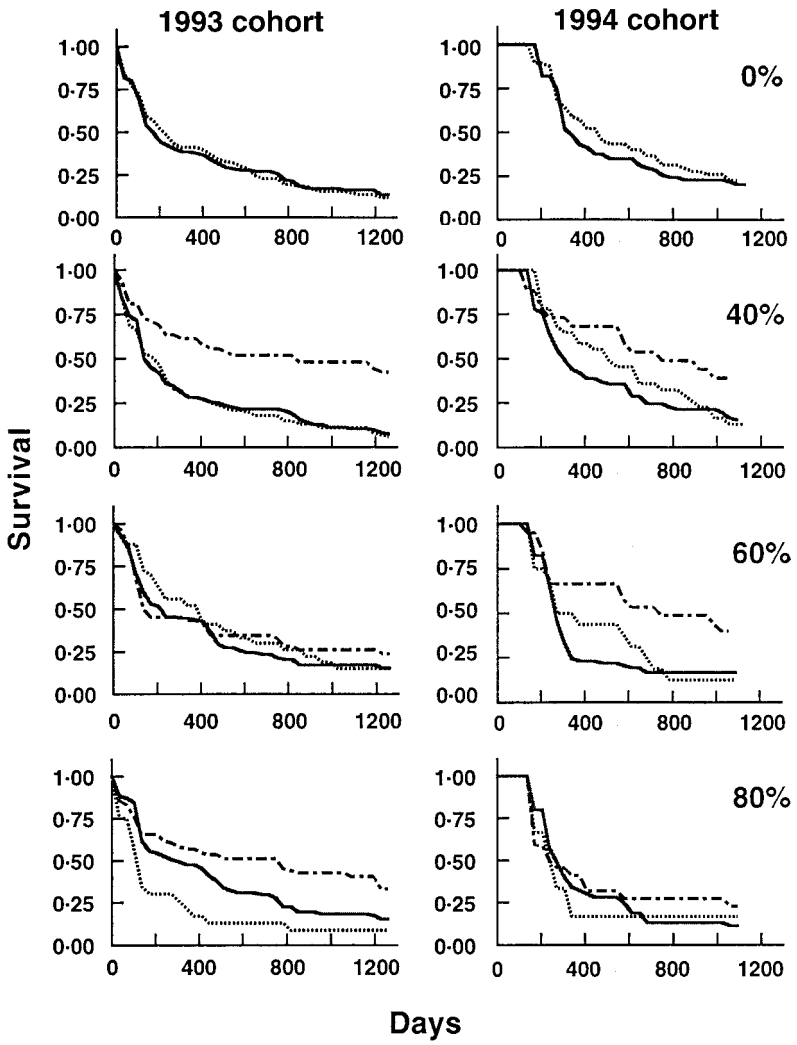


Fig. 9. Survival curves (Pollock *et al.* 1989) for adult male (solid line), sterile female (dashed line) and fertile female (dotted line) rabbits from the 1993 and 1994 sterility cohorts at Wellstead, Western Australia, by sterility level. Curves were calculated using 34-day intervals. Female sterility was imposed in January/March of each year (day 0).

1998b). Sterile females ($n = 11$) also emigrated less often than fertile females ($n = 29$) ($\chi^2 = 8{\cdot}10$; d.f. = 1; $P < 0{\cdot}01$). Emigration varied between sterility treatments ($F = 3{\cdot}45$; d.f. = 3, 16; $P = 0{\cdot}042$), with fewer rabbits emigrating from the 80% treatments than from the 0% (Tukey; $P = 0{\cdot}009$) and 40% ($P = 0{\cdot}023$) treatments, but not from the 60% ($P = 0{\cdot}170$) treatment (Table 4). Males moved further than females ($F = 5{\cdot}98$; d.f. = 1, 139; $P = 0{\cdot}016$).

BODY MASS

The mass of male and female (excluding mid- to late-stage pregnancies) adult rabbits varied between seasons (males: $F = 23{\cdot}56$; d.f. = 3, 3996; $P < 0{\cdot}001$; females: $F = 163{\cdot}84$; d.f. = 3, 3561; $P < 0{\cdot}001$), with rabbits heaviest in spring and lightest in autumn after the summer drought (Table 5). There were no differences between fertile females with and without surgery (i.e. shams and intacts; $F = 0{\cdot}01$; d.f. = 1, 1990; $P = 0{\cdot}938$). Compared to fertile females, sterile females were significantly heavier in summer and autumn, marginally heavier in winter, but not in spring (Table 5). Males and females were analysed separately using unique records only for each season.

BLOOD PARAMETERS

Using only unique records for each season, the mean values for packed cell volumes (PCV) and plasma protein (PP) levels of male and female adult rabbits were examined by sterility level and by season with all years pooled. For both males and females, PCV and PP levels were similar between

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Table 3. Summary of known immigration by male and female rabbits at Wellstead, Western Australia, during 1992–96. Mean (± SD) numbers, and the proportional impact of immigration, are given for each sterility level with all years pooled

			All rabbits	
Sterility level	Number of males	Number of females	Number*	Proportion†
0%				
Mean	14·0	4·7	9·3	0·106
SD	4·3	3·9	6·3	0·052
n	3 × 3	3 × 3	3 × 3	3 × 3
40%				
Mean	15·0	7·8	11·4	0·127
SD	12·8	7·3	10·6	0·102
n	3 × 3	3 × 3	3 × 3	3 × 3
60%				
Mean	11·8	4·5	8·2	0·132
SD	7·5	2·3	6·5	0·080
n	3 × 3	3 × 3	3 × 3	3 × 3
80%				
Mean	11·8	4·5	8·2	0·267
SD	7·7	3·7	6·9	0·167
n	3 × 3	3 × 3	3 × 3	3 × 3
All sites				
Mean	13·2	5·4	9·3	0·158
SD	8·2	4·6	7·7	0·122
n	12 × 3	12 × 3	12 × 3	12 × 3
Total rabbits	316	129	445	–

*Mean number of known immigrants per year.
†The proportion of all rabbits known to be immigrants on each site during the period when immigration occurred each year (December–March).

sterility treatments (Tukey, $P > 0{\cdot}05$). Mean (± SD) PCV ranged from 41·0 ± 4·1% (0% sites) to 41·6 ± 4·1% (60% sites) for males; 39·0 ± 3·6% (0% sites) to 39·8 ± 3·3% (80% sites) for fertile females; and 39·6 ± 3·8% (80% sites) to 40·1 ± 3·7% (60% sites) for sterile females. Mean (± SD) PP ranged from 5·40 ± 0·64 (0% sites) to 5·60 ± 0·73 g 100 ml^{-1} (60% sites) for males; 5·35 ± 0·62 (0% sites) to 5·57 ± 0·67 g 100 ml^{-1} (60% sites) for fertile females; and 5·49 ± 0·64 (80% sites) to 5·63 ± 0·73 g 100 ml^{-1} (60% sites) for sterile females. However, both PCV and PP levels varied significantly between seasons for both sexes, with lowest levels in summer/autumn and greatest levels in winter/spring (Table 5). When the data from all sites were pooled, PCV and PP levels were

Table 4. The mean (± SD) number and distance moved (m) by known male (M) and female (F) emigrants for each of the sterility levels (all years pooled) for rabbit populations at Wellstead, Western Australia, during 1992–96

	Sterility level													
	0%			40%			60%			80%			All sites	
	M	F	All	M	F	All	M	F	All	M	F	All	M	F
Number emigrated														
Mean	10·0	7·0	8·5	11·0	4·3	7·2	9·3	2·3	5·8	3·7	2·3	3·0	8·5	3·8
SD	4·4	5·3	4·6	4·5	2·3	5·1	0·6	2·1	4·1	0·6	2·1	1·5	4·0	3·3
n sites	3	3	3	3	3	3	3	3	3	3	3	3	12	12
Distance moved (m)														
Mean	593	364	499	911	525	820	791	604	754	1059	500	842	801	458
SD	616	381	540	628	362	596	840	178	778	1426	301	1144	805	378
n rabbits	30	21	51	33	10	43	28	7	35	11	7	18	102	45

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Table 5. Mean and standard deviation (± SD) of the mass, packed cell volumes (PCV) and plasma protein (PP) of adult rabbits for each season at Wellstead, Western Australia. All years pooled, with rabbits in mid- to late pregnancy excluded for that period for mass. Males and females were analysed separately using unique records only for each season

Parameter	Summer*	Autumn*	Winter*	Spring*	All seasons*
Mass (g)					
Males	1622 ± 224 ^{A}	1603 ± 188 ^{B}	1653 ± 155 ^{C}	1685 ± 153 ^{D}	1641 ± 150 ^{X}
n	1417	1499	700	384	4000
Fertile females	1575 ± 215 ^{E}	1529 ± 183 ^{G}	1671 ± 201 ^{I}	1838 ± 181 ^{J}	1653 ± 162 ^{X}
n	709	708	340	241	1995
Sterile females	1662 ± 262 ^{F}	1610 ± 208 ^{H}	1701 ± 184 ^{I}	1852 ± 161 ^{J}	1706 ± 171 ^{Y}
n	545	626	285	115	1571
All females	1613 ± 240 ^{K}	1567 ± 199 ^{L}	1684 ± 194 ^{M}	1842 ± 175 ^{N}	1677 ± 163
n	1254	1334	625	356	3569
PCV (%)					
Males	39·8 ± 3·8 ^{A}	40·4 ± 3·4 ^{B}	44·6 ± 3·0 ^{C}	43·7 ± 3·7 ^{D}	41·3 ± 4·0
n	1123	1387	649	358	3517
Fertile females	38·7 ± 3·7 ^{E}	39·0 ± 3·3 ^{E}	40·9 ± 3·6 ^{E}	38·7 ± 4·3 ^{E}	39·2 ± 3·7 ^{F}
n	619	647	328	234	1828
Sterile females	38·9 ± 3·4 ^{E}	39·2 ± 3·5 ^{E}	42·8 ± 3·1 ^{E}	41·3 ± 3·0 ^{E}	39·9 ± 3·7 ^{G}
n	468	594	271	112	1445
Significance for all females	H	H	I	J	
PP (g 100 ml^{-1})					
Males	5·45 ± 0·02 ^{A}	5·32 ± 0·65 ^{B}	5·83 ± 0·63 ^{C}	5·86 ± 0·57 ^{D}	5·51 ± 0·69^{-}
n	1044	1311	617	348	3320
Fertile females	5·39 ± 0·64 ^{E}	5·30 ± 0·63 ^{E}	5·72 ± 0·71 ^{E}	5·52 ± 0·62 ^{E}	5·44 ± 0·67 ^{F}
n	531	614	321	224	1690
Sterile females	5·48 ± 0·71 ^{E}	5·40 ± 0·58 ^{E}	5·86 ± 0·67 ^{E}	6·14 ± 0·56 ^{E}	5·57 ± 0·68 ^{G}
n	416	569	261	105	1351
Significance for all females	H	I	J	J	

*Different letters indicate significant differences for each parameter.
Mass: males by season, A–D Tukey, $P < 0{\cdot}01$. Sterile verses fertile females within seasons: Tukey, E–H $P < 0{\cdot}001$; I $P < 0{\cdot}100$; J $P > 0{\cdot}200$. All females by season: K–N Tukey, $P < 0{\cdot}0001$. All rabbits, all seasons: X–Y Tukey, $P < 0{\cdot}001$.
PCV and PP: males, A–D Tukey, $P < 0{\cdot}0009$. Sterile verses fertile females within seasons: E Tukey, $P > 0{\cdot}05$. All seasons, sterile verses fertile females: F–G Tukey, $P < 0{\cdot}0001$. All females by season: H–J Tukey, $P < 0{\cdot}016$.

also significantly higher in sterile than in fertile females (PCV: $F = 72{\cdot}25$; d.f. = 1, 3262; $P < 0{\cdot}001$; PP: $F = 76{\cdot}19$; d.f. = 1, 3030; $P < 0{\cdot}001$).

GROWTH RATE OF JUVENILES

Growth rates were determined using only rabbits that weighed < 900 g and which had > 19 days between their first and last capture. Juvenile growth rate was positively correlated with pasture biomass ($r = 0{\cdot}129$; d.f. = 733; $P < 0{\cdot}001$). A three-way ANOVA with year, sex and level of sterility as factors and with pasture biomass as a covariate revealed that growth rates were influenced by year ($F = 9{\cdot}74$; d.f. = 3, 702; $P < 0{\cdot}001$) and the level of sterility ($F = 8{\cdot}72$; d.f. = 3, 702; $P < 0{\cdot}001$) but not by sex ($F = 0{\cdot}93$; d.f. = 1, 702; $P = 0{\cdot}336$). However, the highest rate was recorded on the 40% treatments, and the linear component of the effect of sterility on growth rates was not significant ($F = 0{\cdot}22$; d.f. = 1, 702; $P = 0{\cdot}637$). Mean rates were: 0% sites, 9·68; 40%, 11·30; 60%, 10·17; 80%, 9·69 g/day.

FLEAS

Flea abundance was influenced by both season (two-way ANOVA; $F = 18{\cdot}47$; d.f. = 3, 164; $P < 0{\cdot}001$) and rabbit status ($F = 43{\cdot}67$; d.f. = 3, 164; $P < 0{\cdot}001$) (Fig. 10). More fleas were generally present in spring than in other seasons (Tukey; all $P < 0{\cdot}005$). Juvenile rabbits had the fewest fleas but were not significantly different from adult males ($P = 0{\cdot}309$). Both juveniles (Tukey; $P < 0{\cdot}001$) and adult males ($P < 0{\cdot}001$) had fewer fleas than adult females regardless of whether these were fertile or not. Within a treatment, the number of fleas on adult fertile and sterile females was similar ($P = 0{\cdot}275$). However, when considering all flea classes combined, and with sterile females excluded (none on 0% sites), there were less fleas present on the 60% and 80% sites than on the 0% and 40% sites (three-way ANOVA; $F = 6{\cdot}60$; d.f. = 3, 96; $P < 0{\cdot}001$; Tukey, all $P < 0{\cdot}015$). With the 0% sites excluded (no sterile females), the level of sterility also influenced the overall abundance of fleas on sterile females in the other three treatments (two-

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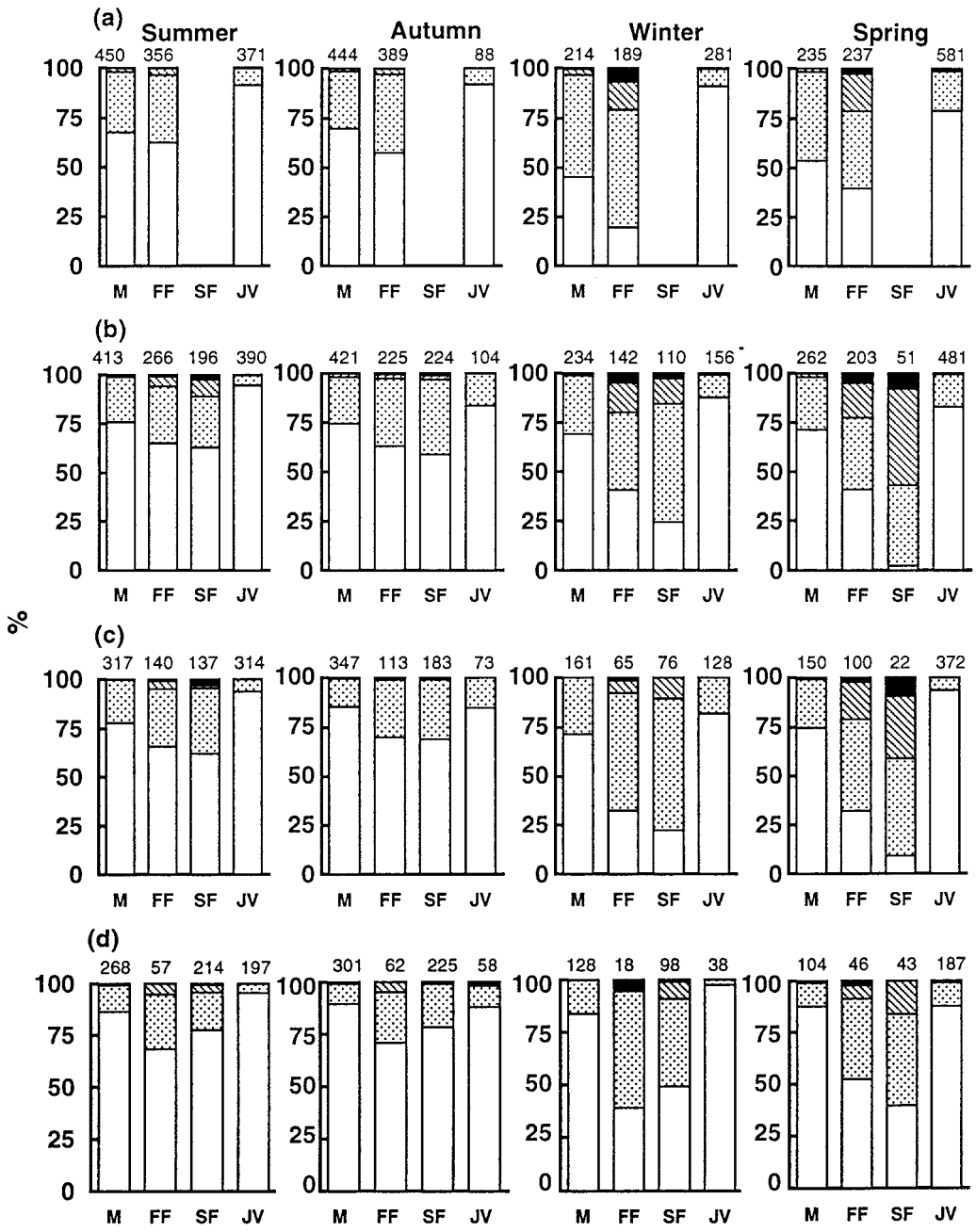


Fig. 10. The influence of season and the level of female sterility on European rabbit flea abundance for adult males (M), fertile females (FF), sterile females (SF) and juvenile rabbits (JV; < 1199 g) at Wellstead, Western Australia. All years pooled (1993–96) with only unique records for each season used. (a) 0% female sterility; (b) 40% sterility; (c) 60% sterility; (d) 80% sterility. Categories are: white, 0–10; stipled, 11–50; hatched, 51–100; solid, > 100 fleas present. Sample sizes at top of columns.

way ANOVA with season; $F = 4{\cdot}84$; d.f. = 2, 132; $P = 0{\cdot}009$). A smaller proportion of these females was recorded in the higher flea categories as the sterility level increased [80% = 60% ($P = 0{\cdot}982$) < 40% ($P < 0{\cdot}025$) treatments].

MYXOMATOSIS

The level of female sterility did not appear to affect the proportion of rabbits with signs of myxomatosis (Table 6; all rabbits, $F = 0{\cdot}19$; d.f. = 3, 32; $P = 0{\cdot}901$). Nor were there any apparent differences between the proportion of sterile females [mean ($\pm$ SD); $0{\cdot}31 \pm 0{\cdot}14$, $n = 3 \times 3 \times 4$] and fertile females ($0{\cdot}34 \pm 0{\cdot}18$, $n = 3 \times 3 \times 4$) with signs of the disease ($F = 0{\cdot}49$; d.f. = 1, 70; $P = 0{\cdot}489$). However, myxomatosis often appeared on site before sterility was imposed each year, and it was not always known exactly when some rabbits became infected. Thus, the results from the slowly spreading epizootic

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Table 6. The mean (± SD) proportion of juvenile (< 1199 g), adult male, sterile female (SF) and fertile female (FF) rabbits that were recorded with active signs of myxomatosis at Wellstead, Western Australia. Unique records only for each year with all sites pooled for each relevant factor (year, $n = 12 \times 4$; sterility, $n = 4 \times 3 \times 4$)

					Adult					
	All Rabbits		Juveniles		Males		SF		FF	
Factor	Mn	SD	Mn	SD	Mn	SD	Mn	SD	Mn	SD
Year										
1993	0·41	0·09	0·30	0·15	0·43	0·10	0·49	0·06	0·56	0·13
1994	0·16	0·09	0·15	0·14	0·18	0·16	0·20	0·10	0·30	0·16
1995	0·21	0·07	0·14	0·12	0·32	0·13	0·23	0·08	0·25	0·11
1996	0·29	0·08	0·38	0·26	0·24	0·14	0·33	0·07	0·24	0·11
*P**	0·001		0·0148		0·001		0·001		0·001	
Sterility										
0%	0·27	0·13	0·28	0·10	0·28	0·13	–	–	0·32	0·19
40%	0·26	0·15	0·22	0·28	0·31	0·15	0·21	0·16	0·38	0·22
60%	0·28	0·13	0·23	0·20	0·30	0·18	0·34	0·14	0·34	0·16
80%	0·26	0·11	0·23	0·20	0·30	0·20	0·30	0·12	0·31	0·17
*P**	0·901		0·508		0·973		0·200		0·543	

*Results of two-way ANOVA by year and sterility (arcsin transformation).

in 1995 (Twigg *et al.* 1998a), which clearly occurred after sterility had been imposed, were analysed separately. Using all 12 sites, a smaller proportion of rabbits on the 80% sites was recorded with signs of myxomatosis than on the 0% sites ($F = 3{\cdot}49$; d.f. = 3, 8; $P = 0{\cdot}070$; Tukey, $P = 0{\cdot}050$), and the linear component of the sterility effect was significant ($F = 9{\cdot}11$; d.f. = 1, 8; $P = 0{\cdot}017$). However, using only those sites with sterile females ($n = 9$), there was no difference between the numbers of sterile and fertile females displaying signs of myxomatosis ($F = 0{\cdot}02$; d.f. = 1, 12; $P = 0{\cdot}899$).

Discussion

THE DESIGN

The similarities among sites in the physical attributes of the bush refuge, and in the changes in pasture biomass that occurred during the trials, indicate that those environmental factors which were likely to influence the response of rabbits were similar between sites, at least at the treatment level. Moreover, if the design had not worked well, it would have been unlikely that the response of the demographic parameters, such as productivity and recruitment, to imposed sterility would have been linear.

The inclusion of the physical attributes for the sites as covariates (after reduction by factor analysis) did not improve any of the demographic analyses (e.g. productivity, recruitment), which further supports the notion that the design of the trial was appropriate. The overall patterns observed with respect to seasonal changes in rabbit abundance were also similar between treatments (Fig. 4). The levels of rabbit productivity (25–30 kittens $\text{doe}^{-1}\,\text{year}^{-1}$; Twigg *et al.* 1998a), survival and recruitment on our 0% sites were similar to those of rabbits from similar habitat in Western Australia (King & Wheeler 1985; Wheeler & King 1985a,b). There was no evidence that predators were preferentially hunting on sites of a particular sterility level. Thus we believe that the differences we observed in productivity, recruitment and survival were the result of the imposed sterility (Table 7).

THE RABBIT RESPONSE

The major findings of our study are summarized in Table 7. Annual exponential rates of increase were near zero regardless of the level of female sterility imposed. Moreover, the number of rabbits retrieved at the end of our trials was similar across sites. This indicates that compensatory demographic mechanisms were operating in the high-sterility treatments, and that these mechanisms were able to overcome the effects of sterility. The linear decline in productivity in response to increasing sterility demonstrates that fertile females were at maximum reproductive output and could not compensate by increasing litter size or the number of litters per year. It also suggests that all reproductive females were breeding in most years. This finding is not surprising as European rabbits usually operate at maximum reproductive capacity (King & Wheeler 1985; Wheeler & King 1985a; Williams *et al.* 1995; Twigg *et al.* 1998a). Thus, in our study, compensatory mechanisms were mainly operating through density-

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Table 7. Summary results for the major outcomes of the sterility trials conducted at Wellstead, Western Australia, from January 1993 to June 1996. Where appropriate data are means ± SD, and statistical tests are given in the Methods

	Level of female sterility				
	0%	40%	60%	80%	Significance
Site characteristics					
% vegetation cover, bush refuge (0–20 cm layer)	91 ± 8	83 ± 14	92 ± 17	85 ± 15	$P = 0{\cdot}464$
Depth of sand, bush refuge (cm)	105 ± 18	69 ± 28	110 ± 23	79 ± 28	$P = 0{\cdot}391$
Mean pasture biomass (kg dry weight ha^{-1}, all years pooled)	4236 ± 707	3792 ± 880	4095 ± 503	3988 ± 419	$P = 0{\cdot}496$
(a) Spring					
(b) Summer	4021 ± 1909	2896 ± 1249	3342 ± 1287	3356 ± 942	$P = 0{\cdot}087$
Predators (total foxes seen per treatment sites only, all years pooled)	6·7 ± 2·9	9·0 ± 2·0	8·3 ± 4·9	9·0 ± 2·6	$P = 0{\cdot}802$
Rabbit numbers and demography					
Initial rabbit numbers (spring 1992)	29·3 ± 8·5	34·7 ± 17·9	38·3 ± 21·2	41·4 ± 19·3	$P = 0{\cdot}850$
Final rabbit numbers (winter 1996)	62·7 ± 17·4	73·7 ± 32·6	56·0 ± 19·1	51·3 ± 13·6	$P = 0{\cdot}639$
Annual rate of increase (r)	0·001 ± 0·001	0·014 ± 0·004	0·011 ± 0·011	0·007 ± 0·009	$P > 0{\cdot}050$
Proportion of immigrants (both sexes 1992–96)	0·106 ± 0·052	0·127 ± 0·102	0·132 ± 0·080	0·267 ± 0·167	$P = 0{\cdot}025$
Number of emigrants (both sexes 1992–96)	8·5 ± 4·6	7·2 ± 5·1	5·8 ± 4·1	3·0 ± 1·5	$P = 0{\cdot}042$
Mean number of kittens produced $year^{-1}$	108 ± 105	91 ± 76	65 ± 47	33 ± 42	$P < 0{\cdot}001$
Mean proportion of kittens recruited $year^{-1}$	0·371 ± 0·116	0·373 ± 0·130	0·467 ± 0·148	0·505 ± 0·185	$P < 0{\cdot}012$
Breeding season survival – adults (proportion; all years)	0·81 ± 0·11	0·81 ± 0·13	0·85 ± 0·11	0·94 ± 0·05	$P < 0{\cdot}01$*
Breeding season survival – kittens (proportion; all years)	0·26 ± 0·11	0·34 ± 0·16	0·45 ± 0·21	0·52 ± 0·37	$P < 0{\cdot}001$*
Juvenile growth rates ($g\ day^{-1}$)	9·68 ± 0·11	11·30 ± 0·13	10·17 ± 0·11	9·69 ± 0·05	$P = 0{\cdot}637$†
Fleas and myxomatosis					
Flea abundance	High	High	Moderate	Moderate	$P < 0{\cdot}001$
Proportion with active myxomatosis (all rabbits)	0·27 ± 0·13	0·26 ± 0·15	0·28 ± 0·13	0·26 ± 0·11	$P = 0{\cdot}901$
Overall survival of adult rabbits‡	**Sterile females**	**Fertile females**	**Males**		
1993 Cohort (proportion alive, June 96; all sites pooled)	0·418	0.133	0·165		$P < 0{\cdot}01$

*Kitten survival was significantly less than that of adults for all treatments ($P < 0{\cdot}0001$).
†The 40% treatment was higher than the other treatments ($P < 0{\cdot}006$) but the linear component due to sterility was not significant.
‡Note that the body condition indicators (mass, PCV, PP) were generally higher in sterile females than in other adult rabbits.

dependent juvenile survival, and increased survival of sterile adult females (Table 7). At high levels of sterility (60–80%), however, these mechanisms were insufficient to overcome totally the effects of sterility, which ultimately resulted in a considerable reduction in the seasonal peaks in rabbit abundance (Fig. 4). Also, the survival of both adult and juvenile rabbits was density-dependent in all years, with one exception for adult rabbits (1994). In this year, pasture biomass levels were relatively high and probably contributed to the lower mortality of adult rabbits.

Immigrants constituted a larger proportion of rabbit populations on the 80% sites, but emigration from these sites was less than that which occurred on the other treatments. Thus we may have underestimated the effects of imposed sterility, and the impact of sterility may have been greater had we been able to sterilize rabbits over an entire region. We had several records of emigrating rabbits moving over 10 km (Twigg *et al.* 1998b). The distance moved by emigrants also tended to be greater for rabbits on the high-sterility sites (Twigg *et al.* 1998b). This behaviour would result in both advantages and disadvantages to control strategies. It would aid the transmission of a naturally spread sterility agent but, conversely, this trend could promote colonization of 'new' habitat, thereby potentially making rabbit control more difficult.

Sterile females had higher survival than other rabbits, and this trend was consistent across all sterility levels. Sterility levels were not 'reset' each year and thus, over time, increased survival of sterile females would result in a gradual increase in the overall level of sterility. In the long term, therefore, there are likely to be demographic shifts towards older, larger, sterile females that maintain their position in the social hierarchy because they are still hormonally intact. This would enhance the effects of a sterility agent. A similar increase in survival is known to occur in castrated feral male Soay sheep (Jewell 1986).

Similar compensatory mechanisms have long been suspected to occur, or are known to occur, in other animal populations subjected to fertility control using chemosterilants. Responses have included increases in survival, recruitment and immigration (Goulet & Sadleir 1974; Johnson & Tait 1983; Bomford 1990; Barlow, Kean & Briggs 1997). Because many of our fertile rabbits lived for at least 5 years, the effects of a sterility agent on individual rabbits would need to be long-lasting.

RABBIT CONDITION AND SURVIVAL

Body mass, plasma protein and packed cell volumes have all been used as indicators of body condition (Hanks 1981). All these measures for our rabbits indicated that sterile females were generally able to maintain better condition than any other rabbits throughout most of the year, particularly during periods of reduced food supply (summer/autumn). The ability of rabbits to maintain body condition is not only dictated by the availability and quality of their food, but it can also be affected by a restricted water intake (Hayward 1961), and this probably occurred in the Wellstead rabbits during the period of summer drought. The observed lag with respect to differences in body mass between sterile and fertile females until the summer/autumn is surprising, as you would expect this difference to be maximal at the end of the breeding season (i.e. end of spring). However, it probably reflects the abundance of green feed at this time. It is interesting that the condition and survival of adult males were more akin to that of fertile females, indicating that they also incur a reasonable physiological cost associated with breeding. Substantial breeding by European rabbits is dependent upon the availability of green feed (King & Wheeler 1985; Williams *et al.* 1995; Twigg *et al.* 1998a). The similarity in the body condition of rabbits from the different sites further indicates that the level of food supply was similar across sites.

Survival of animals is dictated by the interaction between their food supply, the level of predation, and the incidence of disease and pathogens. Although it is somewhat simplistic to examine these factors in isolation, this approach may nevertheless provide some insight into what may have caused the density-dependent processes seen as a result of the imposed sterility during our trials. Changes in pasture biomass at the sites were similar with respect to treatment. Therefore it is unlikely that the responses to imposed sterility were directly attributable to differences in food supply. However, during the dry summer/autumn months, the rabbit graze line shifted further and further away from the bush refuge, potentially increasing the risk of predation. This effect was most pronounced on the low-sterility sites, particularly early in 1995. There was no evidence that foxes or feral cats favoured sites of a particular sterility level (Table 7).

The impact of disease and pathogens on various demographic parameters of wild animal populations has been well documented (May 1988; Scott 1988; McCallum & Dobson 1995). The prevalence of coccidia (*Eimeria* spp.), a known rabbit pathogen, in faecal pellets collected from our rabbits over an 18-month period was very high, with 10 of the 12 species previously recorded in *Oryctolagus* present. The total parasite loads of known pathogens (e.g. *Eimeria*, *Trichostrongylus*) in our rabbits also appeared to be less in survivors than in non-survivors, although trends were less clear when these data are examined by individual parasite species and by the age of rabbits (Hobbs *et al.* 1999). It is possi-

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ble that such parasites may also predispose rabbits to other causes of mortality. For example, the microsporidian, *Encephalitozoon cuniculi*, a known pathogen in immunocompromised rabbits, occurs in approximately 25% of rabbits at our field sites (Thomas *et al.* 1997). The myxoma virus is a pox virus and as such, exerts many of its effects through immunosuppression (Fenner & Ratcliffe 1965). We believe, therefore, that either directly or indirectly myxomatosis was a major cause of rabbit mortality at Wellstead. As it usually occurs as an annual, or occasionally a biennial, epizootic at our sites, its impact will probably override the obvious effects of many other causes of mortality. Rabbit mortality at the sites, however, clearly resulted from an interaction between the effects of food supply, predation and disease.

RABBIT FLEAS AND MYXOMATOSIS

In Australia, the myxoma virus is mainly transmitted through direct contact, aerial vectors (mosquitoes) and the European rabbit flea (Fenner & Ratcliffe 1965). Reproduction by the European rabbit flea is dependent upon the provision of pregnancy hormones from female rabbits (Mead-Briggs 1964). Thus it is possible that a reduction in the number of pregnant rabbits could result in decreased transmission of the myxoma virus. There was a suggestion that this may have partially occurred during 1995 when mosquito numbers were low (Kerr *et al.* 1998), and viral transmission may have therefore been more reliant on rabbit fleas. In this year, it was suggested that the incidence of myxomatosis was lower on the 80% sites (Table 6), and the analysis of serum antibodies against the myxoma virus confirmed these results (Kerr *et al.* 1998). However, the rate of seroconversion was similar between fertile ($42{\cdot}5 \pm 2{\cdot}5\%$) and sterile females ($49{\cdot}6 \pm 2{\cdot}5\%$; $F = 0{\cdot}25$; d.f. = 1, 15; $P = 0{\cdot}622$). The abundance of rabbit fleas was also found to be lower on the high-sterility sites (Fig. 10) but there appeared to be sufficient vectors for the virus to be transmitted successfully at this time, as an epizootic still occurred on all sites even though imposed sterility may have reduced flea abundance on some treatments. Greater than 90% of susceptible rabbits were seropositive after each epizootic, indicating they had been exposed to, and survived, the disease. This begs the question of what is the minimum density of vectors required for the successful transmission of the myxoma virus?

The calicivirus (rabbit calicivirus disease, RCD; Cooke 1994) was recently introduced into Australia as a biological control agent for rabbits, and is having some impact on rabbit populations (W. Dobbie, personal communication). To maximize the effects of a fertility control agent, prior reduction in the target population by some lethal means (e.g. poisoning, RCD) should be undertaken, thereby allowing the sterility agent to then slow population recovery (Bomford 1990). In this respect a sterilizing strain of myxoma would be ideal, as it is likely to either kill or sterilize its target. However, with respect to RCD, this scenario needs careful consideration. It is possible that RCD and the myxoma virus will interact in such a way that the number of susceptible individuals is reduced below the threshold required to maintain each disease, thereby necessitating strategic reintroduction of both these agents. Conversely, the myxoma/RCD interaction may be such that a long-term decrease in rabbit numbers is achieved. Research in this area is urgently required.

WILL FERTILITY CONTROL WORK?

It has never been proposed that fertility control alone would result in the elimination of a target species, but rather that it would result in a significant decrease in abundance (Bomford 1990; Caughley, Pech & Grice 1992; Barlow, Kean & Briggs 1997). In this respect, high levels of infertility were effective in reducing seasonal peaks of rabbit abundance. However, importantly, the overall base-level number of rabbits on the sites after 4 years of imposed female sterility did not differ significantly between treatments (Table 7), which provides further indication that fertility control alone is unlikely to lead to the eradication of vertebrate pests. Two density-dependent processes, both operating through increases in survival, were mainly responsible for the observed demographic compensation. Nevertheless, at sterility levels of 60% to 80%, these compensatory mechanisms were insufficient to counteract the overall effect of sterility which ultimately resulted in substantially less fluctuation in the seasonal abundance of rabbits. This would result in considerable economic benefits.

A sterility level of 40% may have 'stabilized' rabbit numbers at relatively high levels (Fig. 4 and Table 1). Similarly, castration of a proportion of male lamb feral Soay sheep was also suspected to have led to an overall increase in sheep numbers (Jewell 1986). Thus, we believe that female sterility levels of 60–80% would have to be achieved to ensure long-term reductions using naturally spread sterility agents such as a recombinant myxoma virus or a bait-delivered sterility agent. This is not as daunting as it seems, at least for a recombinant myxoma, as > 90% of rabbits remaining on site after epizootics had myxoma antibodies, indicating they had been infected with the disease (Kerr *et al.* 1998). This scenario does, however, assume all exposed rabbits become sterile and that resulting infertility is permanent. It also assumes that the

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recombinant strain is, at least, as competitive as existing field strains of the virus.

The effects of fertility control and population control by culling (poisoning) on rate of increase (r) can be additive (Barlow, Kean & Briggs 1997) and therefore are likely to result in a synergistic effect, particularly where target species are promiscuous/polygamous. Our trials were deliberately undertaken in isolation from any other rabbit control procedures, and the effects of fertility control may be enhanced if it is part of an integrated rabbit control strategy. If the regional effects of rabbit infertility are similar to those achieved on our 80% sites, then there would be significant benefits to Australian agriculture. For example, if food consumption by 'surplus' rabbits (i.e. those not recruited) could be prevented on the 0% sites by decreasing rabbit productivity, and assuming each of these rabbits survives for 2 months and consumes 100 g day^{-1}, then a reduction in peak rabbit numbers similar to that of the 80% sites would have resulted in between 18 and 28 kg ha^{-1} of additional pasture during each growing season being available to other herbivores. However, the benefits to the pastoral industry, which has greater reliance on native plants, and to conservation are less clear. In arid and semi-arid Australia, small numbers of rabbits (4 ha^{-1}) can often prevent plant regeneration and can result in significant soil erosion (Cooke 1981, 1987; Foran, Low & Strong 1985). Rabbits are also an important (primary) prey item for foxes and feral cats in many areas of Australia, and significant decreases in their abundance may ultimately lead to less predation on native animals, particularly threatened species (Pech *et al*. 1992).

While fertility control of overabundant species is an appealing option, there is considerable public concern over the use of recombinant organisms, and this needs to be addressed as part of any new strategies for managing pest species. Requirements for protecting public and animal health, and environmental safety, need to be defined and adhered to (Tyndale-Biscoe 1994; Stohr & Meslin 1997).

Orally delivered immunocontraceptive vaccines are being considered for a range of target species, particularly predators such as the red fox (Bradley, Hinds & Bird 1997) and coyotes *Canis latrans* (Say) (Miller 1995). However, modelled outcomes for the red fox suggest that at least a 60–80% reduction in productivity is required to cause a sustained reduction in the abundance of foxes (Caughley, Pech & Grice 1992; Bradley, Hinds & Bird 1997). This estimate does not allow for demographic compensation such as that observed in our rabbit populations, and we suggest that even higher levels of infertility may be needed where demographic compensation occurs in target populations (see also Bomford 1990; Barlow, Kean & Briggs 1997). Bait-delivered immunocontraceptives may also require more than one immunocontraceptive bait to be consumed by each individual over a short period (weeks) to ensure an effective immune response. Therefore, the practicalities and cost of this need to be included in the development of such a strategy. Given the vast area over which rabbits need to be controlled in Australia, a bait-delivered immunocontraceptive is unlikely to be cost effective for Australian rabbits. However, immunocontraceptive baits may be an option for several target species (e.g. canids) where localized populations have been reduced by lethal means and immunocontraceptive baits are used to slow population recovery (Bomford 1990). In Australia, this could involve the localized protection of rare and threatened species managed for conservation purposes (Bradley, Hinds & Bird 1997).

DENSITY DEPENDENCE

Density dependence is of fundamental interest to ecologists (Nicholson 1933; Sinclair 1989); however, it has rarely been demonstrated experimentally in mammals. The two density-dependent processes identified during our study were operating through increased survival in both juvenile rabbits and sterilized females. While diseases such as myxomatosis were important in regulating rabbit numbers, decreased food supply over the summer 'drought', when little green feed was present and rabbits needed to forage further and further from the bush refuge, would have been the probable driving force behind these compensatory mechanisms, as both these factors were more pronounced on the low-sterility high-density sites (Twigg *et al*. 1998a). Rabbits were also likely to have been more prone to predation at this time. Evidence for this is suggested by the lower survival of kittens born towards the end of the breeding season and over the summer months (Twigg *et al*. 1998a). Although sheep were also grazing on our sites, most competition for the diminishing food supply would have been intraspecific. The abundance of feral donkeys *Equus asinus* (L.) in northern Australia is also known to be regulated by food-related juvenile mortality, which is associated with the need for quality forage, and the nutritional status of lactating females at the onset of the monsoonal wet season (Choquenot 1991). This supports Sinclair, Dublin & Borner's (1985) earlier demonstration that differences in mortality in Serengeti wildebeest *Connochaetes taurinus* (Lichtenstein) were related to density-dependent food shortages. Both emigration and immigration by rabbits in our study were also influenced by density, with immigration helping to maintain the high-sterility low-density populations (Twigg *et al*. 1998b).

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Conclusions

We were able to demonstrate that fertility control alone is unlikely to lead to the elimination of vertebrate pests. Even with high levels of infertility (80%), rabbit populations were able to persist, albeit with significant reductions in peak seasonal abundance. Although our trial was large and monitored the response of 12 discrete free-ranging rabbit populations over a 4-year period, it was none the less relatively small with respect to examining the nation-wide impact of a biological control agent. However, we are encouraged in that a similar study conducted in the tablelands of eastern Australia yielded findings similar to those of our study, at least with respect to broad trends (Twigg & Williams 1999). If a sterility agent was to operate at a regional level, then immigration would probably be reduced and the impact of such an agent could be enhanced. The response of the 40% treatments in possibly stabilizing rabbit numbers at relatively high levels indicates there is a need to monitor the long-term effects of any sterility agent after its release. It also indicates the need to take a holistic approach and integrate fertility control with other control strategies.

If a recombinant myxoma virus is to be used to control the fertility of rabbits then, among other things, it is important to determine how it would interact with existing strains of the virus. Is it readily transmitted, does it maintain adequate lethality, can it compete with existing strains, and how long does it persist? These are just some of the questions which need to be addressed before a recombinant myxoma virus could be released. Our data also suggest that any sterility agent, whether naturally spread or mechanically dispersed, would need to affect a high proportion of the breeding population where target species have relatively high fecundity, a polygamous mating system and are relatively long-lived. Its effects would also need to be long-lasting.

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I. INTRODUCTION

The Subject of Study

Much attention has been paid to isolated behaviour patterns of equids, such as grooming (Trumler 1959a), mating behaviour (Trumler 1958; Zeeb 1958; Zeeb & Kleinschmidt 1963) and in particular, facial expressions (Schneider 1930, 1931, 1932a, 1932b, 1934; Antonius 1937, 1939; Zeeb 1959; Schloeth 1956; Trumler 1958, 1959b). However, very few studies have been made of the general social organization of equids, except for the recent work of Klingel (1964a, 1964b, 1965, 1967, 1968, 1969; Klingel & Klingel 1966) on the steppe or Grant's zebra (*Equus quagga boehmi*), in the Serengeti; nor has much work been done on the mother–foal relationship in equids. Most available observations of their social life and the mother–foal relationships refer to captive animals in zoos.

In Britain there are nine breeds of semi-wild ponies: Connemara, Welsh Mountain, Shetland, Dales, Fell, Exmoor, Dartmoor, Highland and New Forest ponies. These breeds of native ponies are all owned and much controlled by man, particularly with regard to the stallions, but they do lead a wild existence in that most remain free throughout the year and forage for themselves with little extra food provided by their owners. Apart from their control by man, they provide an ideal subject for a general behavioural study. Most are accustomed to man's presence and will therefore permit close observations to be made of them; they are individually recognizable because of their great variety of colours, markings and sizes, and their previous family history can often be found from their owners, as can their ages. Thus the difficulties involved in studying truly wild animals are largely non-existent. There is no necessity to capture and mark the animals before observations can begin and there is little necessity for the use of binoculars.

The numbers of ponies of certain of these breeds, particularly those living in upland areas, are small; there are now also fewer Dartmoor ponies than there were 50 years ago because of the greater economic importance of sheep on the moors. However, despite the competition with domestic cattle, there is still a high density of ponies in the New Forest. High prices are paid for these animals both in Britain and on the Continent such as in Sweden and Holland, for their use mainly as children's riding ponies, and since the last war their numbers in the New Forest have increased.

In this general behavioural study of the New Forest ponies, observations were made of their behaviour when carrying out the various maintenance activities of grazing, resting, grooming and elimination, but particular attention was paid to their social organization: the structure and stability of the groups and the dominance–subordinance relationships within and between groups, and the changing relationship between mares and their foals and their older offspring.

The New Forest

The new Forest covers an area of 144 sq. miles of which 103 sq. miles are under public ownership, administered by the Forestry Commission. The Forest lies between the Solent and the Avon in the Hampshire Basin, a syncline in the chalk filled with tertiary sands, gravels and clays. Well drained gravel-capped plateaux of up to 400 ft support heathland with some birch and pine; older clays and loams exposed on the valley slopes support beech and oak woodland with an understorey of holly, hawthorn and yew, whereas the badly drained areas in the valley bottoms give rise to bog vegetation.

History of Ponies in the New Forest

Ponies were mentioned as being in the New Forest in the 'Constitutiones de Foresta', an early Norman Forest code. Nothing is known of their origin, but they are generally believed to be either descendants of small, wild horses indigenous to Britain, to have crossed from the

Continent before the channel was formed or to have been brought over by the Romans. Until the middle of the nineteenth century, due to the overriding importance of maintaining the deer population at a high level for the Royal hunts, strict laws were enforced to limit the grazing available to the ponies and other domestic stock. The stock was only allowed on the Forest for 5½ months in a year as it was excluded during the Winter Heyning from 22 November until 4 May, when food was scarce for deer, and during the Fence Month from 20 June until 20 July when deer were calving. This placed severe limits on the number of ponies because the smallholders could not provide sufficient food for them during these prohibited periods. After the 'Deer Removal Act' in 1851, when, in return for most of the deer being killed, 10 000 acres of the Forest were enclosed for timber production, the ponies could remain on the Forest throughout the year.

Present Day Status and Control by Man

The present population of over 2500 ponies have access to about 44 500 acres of unenclosed land. On payment of an annual marking fee, holders of common rights, which are attached to property and not to persons, can turn out ponies or cattle onto the Forest, and also pigs for a limited time in the autumn. Each pony must be branded and tail marked, the tails being cut to varying extents to produce distinctive shapes. Although many of the adult mares spend their whole lives in the Forest, having to fend for themselves and having unrestricted movement, man exerts much control over the young animals. Each autumn there are round-ups to catch most of the colt foals, which are then sold at the annual autumn sales, and to brand the fillies and the few colts that will remain on the Forest. Yearlings may also be removed at any time but the breeding mares remain relatively undisturbed. However, some may be taken off the Forest for the winter. Only about 120 adult males are allowed out on the Forest and each of these is a selected stallion, which must be passed as suitable each year by the Court of Verderers. This is an official body which functions to protect the rights and privileges of the commoners. The Verderers employ four agisters who are responsible for the day to day well-being of animals depastured on the Forest.

During the nineteenth century many attempts were made to 'improve' the breed by introducing pony stallions from other British wild breeds such as the Exmoor, Dartmoor or Welsh mountain ponies, and even by introducing several arab stallions. However, since 1935 no stallions, other than those registered in the New Forest studbook, have been allowed on the Forest and all scrub stallions have been removed. Every 2 to 3 years each stallion is moved to a different part of the Forest so that he does not serve his own offspring.

Observation and Recording Methods

One study area was chosen in October 1965 of 3 sq. miles of low-lying mixed woodland, heath, bog, streamside lawns, woodland clearings and two reseeded areas (see p. 90). A second area was chosen in February 1966 of 2 sq. miles, centred around a reseeded area at 340 ft above sea level with the surrounding woodland, heath and valley bogs. These two areas supported about 300 ponies but numbers fluctuated due to re-distribution by man, sales of the young animals and to the custom of taking some of the mares into the smallholdings and farms during the winter, as well as to the obvious causes of births and deaths.

All the ponies in the two study areas could be recognized individually by differences in colour, size and markings, and by their brand marks, although these were only visible in the summer when their coats were short. Initially a description was made of each pony on Royal College of Veterinary Surgeons forms and each animal was allotted one or more letters and numbers for future reference.

Most observations were made on foot from a distance of 10 to 50 yd but a pair of 8 × 30 and of 16 × 50 binoculars were also used for more distant observations. Many ponies could be

approached to within 5 yd but those used to cars and tourists would often then move towards the observer for food, and others would then show signs of alarm and move away. When the distance was kept to between 10 and 50 yd depending on the ponies, they usually looked up when the observer first approached but then showed apparent disregard and very quickly resumed the activity in which they had previously been engaged.

The position of each pony was plotted on a sketch map whenever any part of the study area was visited, so that relationships between ponies and the extent of their home ranges could be seen. All interactions and the animals involved were recorded.

When more than twenty ponies were under continuous observation from dawn to dusk, or for much of this period, counts were made at ½-hourly intervals of the numbers engaged in various activities such as grazing and resting.

Check sheets, each sheet recording various activities at ½-min intervals for 1 hr were completed for foals of known age. From analysis of these sheets changes in each activity with age, with time of day and any individual variation have been assessed. In the 1966 season check sheets were completed for many different foals at irregular intervals, but in 1967 they were only completed for twenty-four foals of which eight were fillies of primiparous mares, eight fillies of multiparous mares and eight colts of multiparous mares. Each of these foals was watched for 3 hr each week until their fourth week and then for only 3 hr every 2 weeks until their eighth week. After that they were only watched 3 hr per month until their ninth month and finally 3 hr every 2 months until they were 1 year old. The 3 hr were in the different time intervals: 06.00 to 10.00, 10.00 to 14.00 and 14.00 to 18.00 hours GMT. Unfortunately, three of the foals died before their fourth month and two more in their eleventh and twelfth months, and thirteen were taken from the Forest before they were 1 year old, so that for only six foals were records completed for a whole year.

The total number of hours of observation in the New Forest between October 1965 and September 1968 was 3948 of which 1552 hr were between October and March and 2396 between April and September. Any 2 hr of observation were not entirely comparable as some hours were spent watching only one or two individuals, others watching large numbers and others were spent moving from one area to another, only recording positions of ponies.

All times mentioned in this paper refer to GMT.

II. ECOLOGY AND POPULATION DYNAMICS

Habitats in the Study Areas

Woodland

Most of the woodland is deciduous with oak (*Quercus robur*) and beech (*Fagus sylvatica*) as the dominant trees, and some sweet chestnuts (*Castanea sativa*) and silver birch (*Betula pendula*). Yew (*Taxus baccata*) and holly (*Ilex aquifolium*) are common in the woods and hawthorn (*Crataegus monogyna*), blackthorn (*Prunus spinosa*) and brambles (*Rubus* spp.) near the edges and in scrub. There is a small amount of unenclosed coniferous wood in both study areas with pine (*Pinus* spp.) larch (*Larix decidua*) and spruce (*Picea excelsa*) and there are also several Forestry Commission enclosures from which ponies and cattle are excluded.

Heathland

Heathland makes up the greater part of both study areas. *Calluna vulgaris* and *Erica cinerea*, with *Erica tetralix* on wetter ground, and dwarf gorse (*Ulex minor*) are predominant. There is a sparse growth of purple moor grass (*Molinia caerulea*) and other species of grass, such as *Agrostis* spp. and *Nardus stricta* appear along the edges of tracks. There are also large patches of gorse (*Ulex europaeus*) which play an important part in the provision of food and shelter for the ponies, and bracken (*Pteridium aquilinum*) is prolific over large tracts of the heathland on valley slopes and near the edges of woods.

Grassland

Grassland occurs in the form of: (a) streamside lawns, (b) woodland clearings, (c) roadside verges, and (d) reseeded areas. There is an *Agrostis–Festuca* type of vegetation on the streamside lawns and woodland clearings, but many of the lawns and clearings show signs of invasion by scrub, or, the badly-drained 'lawns' by *Molinia*, *Juncus* spp. and *Erica tetralix*. The roadside verges and reseeded areas are particularly attractive to the ponies. Minerals leached out of basic materials used on the roads favour the growth of palatable grasses on the roadside verges (Hook 1964). The reseeded areas, which total about 830 acres in the Forest, are patches of heathland that were ploughed up towards the end of the last war, limed, enclosed and most of them then cultivated for 3 or 4 years before being reseeded with a mixture of clover and grasses such as *Lolium perenne*, *Dactylis glomerata*, *Cynosurus cristatus* and *Phleum pratense*. They are now of great importance in the behaviour of the ponies as, despite the small amount of food to be obtained on them, their high mineral content attracts large numbers of ponies, cattle and deer. They have not reverted to the original heath because they are heavily grazed throughout the year, although some have been invaded by gorse and on others recolonization by heather has begun. Bracken, too, has begun to invade several reseeded areas, but most of them remain stable. Minerals, especially phosphates, are returned from the *Molinia* in the bogs by way of faeces and urine (Scowen 1966).

Valley Bogs

Valley bogs provide the bulk of food for the stock on the Forest. Well developed *Molinia* hummocks and bog myrtle (*Myrica gale*) are characteristic of the bogs, with bogmoss (*Sphagnum* spp.), cottongrass (*Eriophorum* spp.), and deer grass (*Trichophorum caespitosum*), all abundant. Alder carrs (*Alnus glutinosus*) flourish along many of the slow-moving streams.

Open Water

There are numerous small streams in the two study areas and one larger river, and in addition, several man-made ponds and ditches. Much of the land, however, is badly drained and there is plenty of standing water, except after several weeks of drought in the summer months. Rushes (*Juncus* spp.), floating meadow grass (*Glyceria fluitans*) and bur-reed (*Sparganium* spp.) are amongst the species to be found in or near water in the Forest and all have a significant

effect on the grazing behaviour of some ponies

Relations with Other Animals

Mammals

Donkeys. Very few donkeys are pastured on the Forest but a pair was present for a year on one study area. The two donkeys moved about together and usually grazed separately from any pony groups. Only one interaction was seen between donkeys and ponies; a 10-month pony filly kicked out at the two donkeys when they were grooming near her.

Cattle. Before 1914 few cows were turned out on the Forest but since the last war, their numbers have increased to over 4000, well-exceeding the pony population. There is direct competition for food between the cattle and the ponies but most of the cattle, both dairy cows and beef animals, are generally taken onto the smallholdings and farms for the winter and supplied with food.

Ponies and cattle used the same grazing areas in daytime in the summer and sought shade together under the same trees or on the same hill (see p. 92). Adult ponies were however, dominant over the cattle and they tended to obtain the best sites in the shades. Foals were often threatened and chased by cows but some play was observed between foals and calves (see p. 171).

Deer. Of the four species of deer present in the Forest, the fallow deer (*Dama dama*) was the only species that occurred in large numbers on the two study areas. Competition for food with the ponies probably did occur as especially in the early mornings and evenings of winter and spring, deer, ponies and cattle could often be seen grazing together. The deer, however, had access to the Forestry Commission enclosures, which ponies and cattle had not. One interaction was observed between a pony and a fallow deer when a yearling filly threatened a doe that moved too close to her. Linsdale & Tomich (1953) also recorded mule deer (*Odocoileus hemionus columbianus*) grazing close to horses, but that they readily gave way when a horse approached too closely.

Dogs. Most ponies ignored dogs unless the dogs ran towards them; when this occurred the ponies took avoiding action and quickly moved away, kicking out at the dogs if they moved too close.

If a dog approached a young foal, the mother of the foal nickered (see p. 142). This alarm call caused the foal to stand up (if it was already lying down) and move towards its mother. At the same time the mother rushed to her foal and swung her body around so that she could direct a kick with her hind legs at the dog. If the dog did not then retreat, the mother and foal retreated together.

Birds

During the summer when insects were abundant around the ponies, starlings (*Sturnus vulgaris*), pied wagtails (*Motacilla alba*) and hirundines concentrated near them and caught insects on them or were disturbed by them.

Many birds, particularly starlings and corvids, collected hair from the ponies for nest material when the ponies were moulting in the spring. Jackdaws (*Corvus monedula*) and magpies (*Pica pica*) were observed perching on the ponies and actually plucking hair in the spring; at other times of the year they pecked around the mane and were probably feeding on ticks and lice. Little attention was paid by ponies towards these birds although very young foals showed some interest in their movement and mares sometimes seemed irritated by magpies on their backs. At first they kept turning their heads and biting at the birds but each time the magpies moved back out of reach until eventually the mares ignored them.

Invertebrates

Ticks (*Ixodes ricinus*) were abundant on the ponies from spring to autumn, especially around the head and neck.

Lice (*Damalinia equi*) also occurred in large numbers mostly in the ponies' manes in the winter.

Both lice and ticks caused ponies to scratch and rub against objects such as stumps and branches; and mutual grooming, where two ponies stand together and nibble and bite each other's neck and mane, may partly be a response to the irritation caused by them.

Diptera. *Hippoboscids or Keds: Hippobosca equina*, hatched from eggs in the spring and the adults spent the summer between the hind legs and around the vulvas of the ponies. Over forty of these New Forest flies were recorded on one mare. Adult ponies showed little concern but foals were much disturbed by their movements and spent long periods stamping, shaking, rolling and nibbling their hind legs in an effort to rid themselves of the keds.

Culicoids and Ceratopogonids: Mosquitoes and biting midges, *Culicoides* spp., were abundant along the bogs and streamsides in the spring and summer and were frequently seen taking blood meals from the ponies. Apart from head shaking and tail swishing, these flies caused little obvious behavioural effect although they probably influenced the ponies' summer pattern of movement by causing them to leave the sheltered valleys and move to open or high ground soon after dawn.

Simulids. One species of black fly (*Simulium equinum*) occurred and fed in and around the ears of the ponies in large numbers in the summer months, causing much local irritation and head shaking and rubbing.

Muscids and Tabanids: Many species of both biting and non-biting flies exerted a great effect on the behaviour of the ponies in summer. By mid-June the large numbers of flies active on hot, sunny and calm days, caused the ponies to stop grazing and move to higher ground or to other spots locally called 'shades'. There they stood close together, either head to tail with the tail of one constantly swishing against the head of another or with their heads against a tree or wall. Of eleven shades in the two study areas five were under groups of trees, three were in tunnels under railway lines and three out in the open. Of these three in the open, one was on a road on a ridge, one on a hill-top on grass, and the third on a patch of concrete by a building. All these 'shades' were spots where flies were less numerous, either in true shade or where air currents reduced the number of flies.

Small species of tabanids as *Haematopota pluvialis* and *Chrysops* spp. took blood meals from ponies but apart from an occasional twitch as the fly was biting, the ponies took little notice of them. The larger tabanids, notably *Tabanus verralli* and *T. distinguendus*, made a buzzing noise as they approached and their bites were presumably painful. The buzzing noise frightened the ponies and sometimes caused groups of ponies to gallop about wildly. When a large tabanid alighted on a pony, the pony stamped, twitched its skin at that point and tried to bite the fly.

Gasterophilus intestinalis, an oestrid fly, laid its eggs on hairs of the ponies, particularly on the fore legs and neck, from June to the end of September. This fly does not bite whilst laying its eggs but it makes a buzzing approach similar to that made by the large tabanids, and although harmless, it had a similar effect on the ponies in causing panics.

Nematodes. Due to high local densities in the favoured grazing areas many of the ponies, especially young animals carried high burdens of parasitic nematodes of the orders Strongyloidea and Ascaroidea. These affected the ponies indirectly by causing general loss of condition and possible death but more directly by causing irritation at certain stages in their life cycle. Adults of the ascaroid *Oxyuris equi*, identified from faeces of several ponies, attaches its eggs to the ponies' perineal region and causes local irritation and the affected animal to rub its tail region against trees, stumps or other fixed objects. The larvae of the oestrid fly *Gasterophilus intestinalis* also cause such rubbing as they emerge from the anus of ponies. *Strongyloides westeri*, eggs of which were commonly found in faeces, penetrates skin as infective

larvae causing skin irritation. *Strongylus* spp. have a migratory cycle within the body of their pony host and cause colic, and even death by damaging blood-vessels; rolling and restlessness being some of the chief symptoms. A count of 1000 worm eggs per gram of faeces is considered clinically significant (Soulsby 1965) but analyses of New Forest pony faeces gave a mean of 1500 eggs of strongyles per gram in yearlings. The mean for adult mares was only 776 but counts as high as 6950 eggs per gram were recorded. (J. Broughton, on behalf of New Forest Veterinary Surgeons Consultative Committee 1967.)

Population Dynamics

Age Composition of the Ponies in the Study Areas

Rough estimates of age can be made from the general body conformation such as length of tail, hollowing of the back, size of the stomach and degree of concavity of the maxillary bones, all these increasing with age. More accurate estimates can be made from the teeth, by their shape, the number of incisors and the nature of their opposing surfaces, but the examination of mouths of the ponies on the Forest proved to be impracticable due to the impossibility of their restraint and the danger involved. However, as a result of verbal and written enquiries ages of most ponies were obtained from their owners. Many of these ages were later verified by conversations with other owners, agisters and other interested parties and very few discrepancies resulted. The age composition of female ponies in the study areas in March of each of the 3 years, 1966, 1967 and 1968, is shown in Table I.

Table I. Age Composition of Female Ponies in Study Areas in March 1966 to March 1968

Age (years)	1966	1967	1968	Total
1	38	29	30	97
2	52	34	21	107
3	27	44	33	104
4–10	104	103	120	327
11–17	59	57	61	177
18+	8	7	5	20
Total	288	274	270	

Birth Rate

A small number of mares came into oestrus as yearlings but only one foaled when 2 years old out of 107 young mares of this age. Most mares foaled for the first time when 3 or 4 years old, but some not until they were 5 years old.

Many factors may contribute to the relatively low percentage of mares of 4 years and more which actually foaled. Hammond (1960) quoted a figure of 95 per cent fertility for pony stallions running with mares, but for New Forest ponies this was certainly wrong. Barren mares were rare as they were usually soon removed and sold but one mare in the study areas was 13 years old in 1966 and she had never foaled. Failure to copulate was probably not very important although young and subordinate mares were sometimes prevented from copulating by older mares. Adult stallions also tended to reject very young or small mares. Abortions seemed to be common during the late autumn, winter and early spring and may represent the major factor contributing to the low percentage of foals born. Nine aborted fetuses were seen by me or reported to me: one in September, one in October, two each in December and January and three in March. Many more must have remained unobserved.

Poor nutrition during the winter was probably largely responsible for the abortions but other causes such as infection may have resulted in some. Locally it is thought that mares tend to abort after periods of prolonged rain following heavy frosts. This could be true in that rain represents a great stress factor for the ponies. They are less able to maintain their body temperatures when cold is combined with rain because their coats then lose some of their insulating properties.

Mares often continued foaling until their death in old age. Only one mare over the age of 15 in 1966 had no foals between 1966 and 1968, whereas three other such old mares foaled in

each of the 3 years. One 24-year-old, found unable to climb out of a ditch in March, was rescued and supplied with hay and she then foaled within 2 months.

Frequency of Foaling

Very few of the 141 mares who were 3 years old or more in 1966 and still alive and under observation in 1968, foaled in each of the 3 years of study. Most only foaled in one or two of the three years and it was common for a mare to foal in alternate years.

No twins were recorded: these are rare in equids and if conceived are generally aborted at an early stage. A small number of foals may have missed being recorded if they were born dead or if they died soon after birth. However, this was not considered likely as most full term pregnant mares were conspicuously so and if any such mare was not seen for 1 or 2 days and then re-appeared with no foal but showing signs of having foaled, she was recorded as having done so.

Sex Ratio of Foals

In 1966 the ratio of colt foals to filly foals was close to equality (47 ♂: 52 ♀) but in 1967 there was an unusual distribution of the two sexes during the breeding season. Of the first fifty-five foals recorded 69·1 per cent were fillies and of the last fifty-four, there were still more fillies than colts (over the whole year 40 ♂: 69 ♀). In 1968 there was no significant difference in the numbers of colts and fillies (46 ♂: 38 ♀).

Although zebra colts have longer gestation periods than fillies, the difference is only 2 or 3 days (Wackernagel 1965) and this cannot account for the unequal ratio of the sexes in 1967. Zwolinski (1960) claimed that there was a statistically proved dependence between the date of siring (mating) and the sex of offspring, and that siring in the second half of May and first half of June produced more colts, whereas fillies prevailed from sirings made at other times of the year. He suggested that this was due to changes in food during late May and early June, when grass was fresh and green, and to temperature changes then. As the gestation length of horses and ponies is on average 336 days with a range of 315 to 365 days (Rossdale 1967), foals resulting from late May and early June sirings would be born throughout May the following year. However, in 1966, the sex ratio of New Forest foals born in May was about equal, in 1967 fillies predominated and in 1968 colts predominated, and so Zwolinski's theory was not relevant.

Removal of Young Ponies by Man

Almost all the colt foals were taken from the Forest in their first, or less commonly, in their second autumn. Two were left with their mothers after this time to become stallions and several were gelded (castrated) and left for one or more years. There was an old gelding on one of the study areas throughout the 3 years and another, which was 3 years old in 1966, was eventually removed in the autumn of 1967. Four other young geldings were present for shorter periods.

A small number of fillies were removed and sold in their first autumn and many others were taken from their mothers in the autumn or winter. This practice was common with foals of very young or very old mares, so that the mares did not have to suckle their foals in the months when little food was available for themselves.

Mortality in Foals

Some foals were born dead, or died on their first day either by failing to stand after their birth or due to accidents such as falling into ditches and bogs and being unable to climb out. Three foals were found dead in ditches. One foal was born prematurely by about two weeks and it was not able to stand or suck. It was taken from its mother and bottle-fed but died after 2 days. Another foal was rejected by its mother but was rescued by its owner and survived. However, the reasons for many neonatal deaths were unknown. Mares, due to foal, were sometimes not seen for one or more days and then reappeared after obviously having given birth but with no foal accompanying them. Other mares were seen with newborn foals but the

following day, the foals were not with them. The relative importance of premature births, weakly foals, accidents and rejections of foals by their mothers, was therefore not known. Nine foals were thought to have died due to one of these reasons; two of them were seen on their first day but not again, and these must have fallen into bogs or ditches.

The death of foals after their first day, except in road accidents, was unusual, although six were probably killed by stallions. One was seen being mauled by a stallion in 1966 and five young foals from the same part of one study area disappeared within two weeks of each other in 1968. One was found dead, with injuries on the neck suggesting that it had been attacked by a stallion. Another foal from a different area was found dead when 3 months old but there was no sign of any injury on it; another disappeared in its eighth week. This animal was thought to have died after it failed to find its mother following a long period of separation because, prior to its disappearance, it had repeatedly been seen separated from its mother and searching for her. Another foal that died in her sixth month was found to have a ruptured diaphragm, perhaps due to a road accident; one 4-month-old colt became impaled on some iron railings and was later destroyed and an 11-month-old filly died in a bog.

Eighteen foals were known to have been killed, or injured and subsequently shot, as a result of road accidents before they were 6 months old; most of these died when 2 to 3 months old at night. Two more foals were killed before they were 1 year old. The second study area was safe from these accidents because the main road alongside it had been fenced in 1964; another fence was erected during 1966 along one of the roads passing through the first study area but the most dangerous road, a 3-mile stretch through thick woodland, remains unfenced.

Mortality in Older Ponies

Thirteen ponies older than 1 year were killed in road accidents during the 3 years of study. Other causes of death in adults were mainly due to their poor condition, usually towards the end of the winter and in early spring, after several months of malnutrition. Old mares, heavily pregnant mares and young mares of 3 to 4 years were particularly prone to die for this reason, probably partly because old mares had difficulty obtaining sufficient food because of their poor teeth; the young animals also had teething troubles. Some of these mares were too weak to stand again after they had lain down and soon died where they lay if not found before then; others failed to climb out of ditches or bogs. Twenty mares died from one of these causes during the 3 years and three others were rescued before they died and they survived. Of these twenty-three animals, fifteen were older than 12 years (65 per cent) and four under 4 years but older than 1 year; all were found dead or in difficulty between November and April and only two mares were found stuck in ditches or bogs during the summer months.

Seven other mares died during the 3 years on the study areas; one had difficulty foaling and was destroyed; another was said to have died from ragwort (*Senecio*) poisoning but the cause of death in the other five was unknown.

Many more mares would probably have died but some old and poor mares were taken off the Forest by their owners in late autumn and fed with hay during the winter.

III. MAINTENANCE BEHAVIOUR

Grazing

Introduction

No quantitative data have been obtained for the amount or type of food eaten, but most of the plants that were grazed or browsed were identified. The amount of time spent grazing during different seasons was estimated by making ¼-hourly or ½-hourly counts of the number of ponies grazing when more than twenty ponies were under observation throughout the day. Unfortunately it was not possible to make such counts during the hours of darkness as only a very small number of animals could be observed at any one time. As movement and grooming accounted for such a small percentage of total time, the percentage of time spent grazing was estimated as the total time minus that spent resting.

Food Plants

Large clumps of *Molinia caerulea* in the valley bogs and a sparser growth on moist heaths seemed to form the bulk of food throughout the summer; large amounts were eaten at nights, which many ponies spent in the valleys. Some patches of heathland were deliberately set on fire in early spring to stimulate an early growth of *Molinia* by burning off the old heather plants.

Agrostis spp. and *Festuca* spp., the dominant species of grass on many woodland and stream-side 'lawns' and on patches of 'grass heath', were grazed throughout the year, mostly during the daytime as were those species such as *Lolium perenne* and *Cynosurus cristatus* on the reseeded areas. However, the amount of food grazed during the summer day must have been small as the grass was kept very short by the high concentrations of ponies, cattle and deer on these lawns, reseeded areas and roadsides; most of the ponies' food must have been eaten at night when the animals were scattered over the valleys and woods.

Few other species of grass were of any importance except *Glyceria fluitans* and other *Glyceria* spp.; these were favoured by certain individuals who spent long periods wading in ponds and slow-moving ditches, then feeding almost exclusively on them. Shoots of rushes were selected by ponies and sedges, *Carex* spp., were grazed together with the grasses growing with them as were most small flowering plants with the exception of the poisonous ragwort, *Senecio jacobaea*, and *S. aquatica*, and some larger thistles; these were generally avoided but bur-reeds, *Sparganium* spp., growing in ponds and ditches, were actively selected.

Although the ponies were normally grazers, browsing was important during the winter and early spring. Gorse was of great importance both as shelter during strong wind and rain, and as a food plant at such times. The younger tips of the shoots, which are of high food value, were browsed throughout the winter and spring especially when snow covered the ground. Plants of dwarf gorse were also selected by the ponies when they were grazing over the heaths and they often pawed with their fore-feet at the gorse plants before browsing them. This was presumably to crush and soften some of the spines. After heath fires, ponies were observed on several occasions standing around the gorse bushes and browsing the burnt and charred branches.

Heather plants, *Calluna vulgaris* and *Erica* spp., seemed to be of relatively little importance in the ponies' diet, despite the large areas covered by these species. Some heather shoots were browsed in the winter and the young plants resulting from the clearing of older, woody plants were also eaten, but never to a large extent. Leaves and shoots of brambles were browsed between autumn and spring, when ponies also took both leaves and leafless shoots of oak and beech. Whenever an oak was felled in the winter, a few ponies soon collected around the fallen tree and nibbled the tips of bare branches. Leaves of holly together with gorse provided almost the only food when snow was on the

ground. Most leaves were removed to a height of about 8 ft up to which ponies could stretch their heads.

Acorns had a great effect on the movement of some of the ponies, completely disrupting their normal daily pattern. They remained under oak trees for much of the day in October grubbing for fallen acorns instead of moving out onto the open woodland clearing. It is known that individuals can become 'addicted' to acorns and that the ingestion of large numbers of prematurely fallen green acorns can cause poisoning and death (Garner & Papworth 1967). In 1964 the deaths of forty-two ponies in the New Forest were attributed to acorns (New Forest Veterinary Surgeons' Consultative Committee 1964) but no deaths were attributed to them in the study areas between 1966 and 1968.

Bracken was the only other plant that was recorded as being eaten in any quantity. During the summer day when ponies were grazing out on the open grasslands, they sometimes moved into clumps of bracken which they browsed for short periods of up to ½ hr. They then returned to the grass. Several such periods often occurred during a day.

Time Spent Grazing by Adults

In winter apart from the small amount of time spent resting (see p. 100) the ponies devoted the whole day to grazing and browsing. After May they spent less time grazing during the day as the resting time increased, and in June on days when flies were abundant they remained in the shades from about 09.00 to 14.00 hours GMT with few excursions to feed during that time. From the few observations that were made at night, it seemed that most of the hours of darkness in all seasons were spent feeding. Peak grazing activity in daylight occurred soon after dawn until about 09.00 hours and in the late afternoon and evening; at these times very few or no ponies were observed resting (Figs 1 to 4).

Grazing Behaviour

The daily and seasonal patterns of movement of ponies over their feeding areas is discussed on page 135. On any grazing area there was usually an uneven distribution of ponies. This could often be correlated with differences in vegetation on the area; on the large woodland clearing in the first study area there was an invasion of heather along one edge and ponies grazed mostly on the heather-free part. However, on the reseeded areas, differences in vegetation were not immediately obvious, although aerial photographs showed them as being made up of numerous dark and light patches. Ponies mainly grazed on the dark patches. Cages were placed over samples of these dark and light patches by L. Scowen so that they were free from the effect of grazing, and after several weeks the grass samples in the cages were cut, weighed and analysed. The mean yield of dry matter in cwt per acre of the dark patches was 13·0 compared with that for the light patches of 2·23. After about 4 weeks the grass samples were again cut and the mean yield per acre of the dark patches was 8·5 compared with that for the light patches of 3·4. A further cutting was made after another month and the mean yields were 6·3 cwt of dry matter per acre compared with 2·3 cwt per acre. The more productive plots had fewer species of grass than the low yielding light coloured plots, which were chiefly short of potash (L. Scowen, personal communication).

Weather conditions also affected the distribution of ponies on a grazing area. In wind or rain ponies at first grazed with their backs to the weather, or faced directly into the wind if it was not very strong, but prolonged rain and strong winds caused them to move to the shelter of trees where they stood with their backs to the trees and wind or rain.

Development of Grazing Behaviour in Foals

In their first week, even on their first day, foals were seen nibbling and chewing at grass and other vegetation. They ate a little but usually did not bite off the grass. Sometimes they did bite off grass but then let it drop from their mouths only to pick it up again and toss it into the air, often bucking and rearing as they did so. One foal on his first day nibbled at grass around

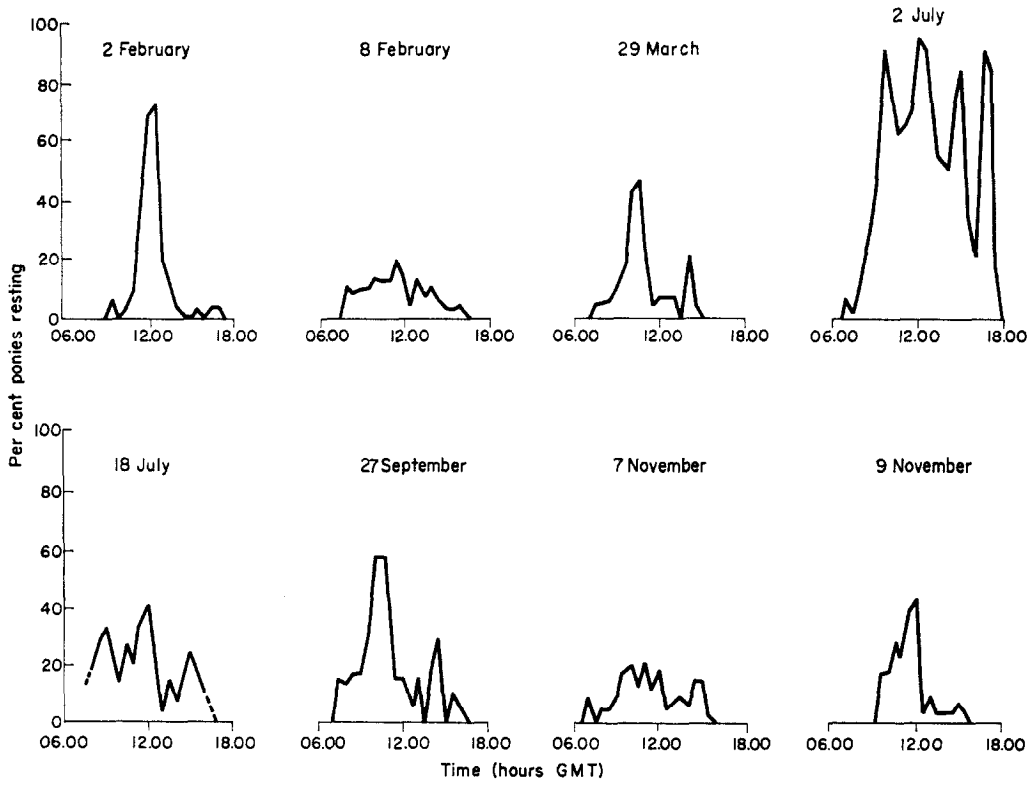


Fig. 1. Changes in frequency of resting with time of year and weather conditions (see p. 100). Figures for each date (1966) were based on observations of twenty ponies or more. Grazing time can be taken as 100 per cent — resting time.

his mother for over 15 min, whilst she was losing the afterbirth. However, the mean number of minutes per hour spent nibbling for first week foals was only 3·5 and the median was only 1·0 min/hr (Fig. 5). These young foals also nibbled at bark of trees and chewed twigs and branches as well as many unsuitable food objects such as pieces of paper. Before grazing foals frequently pawed the ground. Adults pawed gorse but rarely pawed at other vegetation although they did so in a variety of other contexts (see pp. 149 and p. 153 and Odberg 1972).

Until their third to fourth month, foals straddled their forelegs in order to reach the ground to graze because the length of the neck in young foals is relatively short. Foals often grazed on ridges or hummocks or on the banks of ditches, where they could more easily reach the vegetation, or they browsed brambles where they did not have this difficulty.

There was a significant increase in the amount of time foals spent grazing from their first week to the end of their first year ($P < 0{\cdot}01$) (Fig. 5). The increase was gradual between the first week and fourth month; the mean number of minutes per hour spent grazing increasing from 3·5 to 16·3; after the fourth month, the increase was more rapid and foals in their twelfth and thirteenth months spent a mean of 44·4 min per hr grazing. These figures were obtained from analyses of all 1966 and 1967 foal record sheets.

From analysis of the 1967 record sheets of first week to eighth month foals, it was found that the mean number of minutes per hour spent

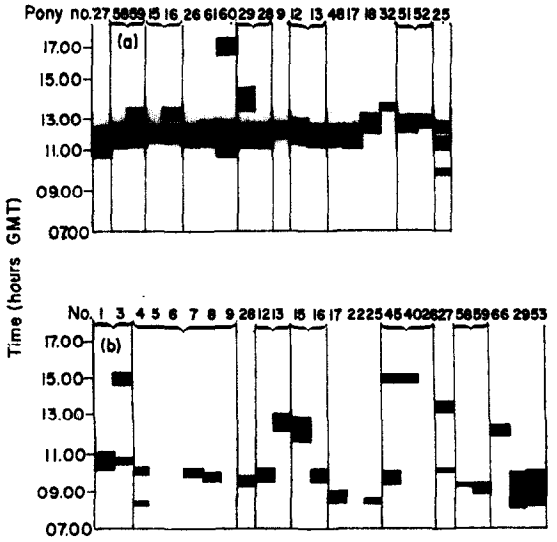


Fig. 2. Length of resting bouts and time of resting of a number of ponies of one population on 2 days towards the end of the winter. On 2 February 1966 (a) there was a peak of resting which occurred after the sun broke through the clouds. On 1 March 1966 (b) there was no peak and the sky was overcast all day. The ponies can be assumed to have been grazing when they were not resting.

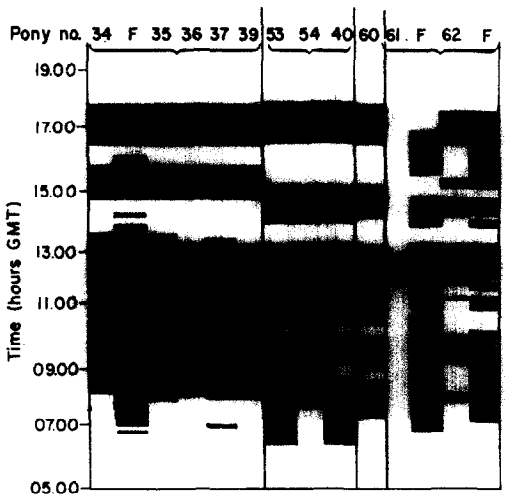


Fig. 3. Length and time of resting bouts of fourteen ponies of one population on 2 July 1966, showing co-ordination of resting between members of groups 34 to 39, 53 to 40 and 61 to 62F. F stands for foal. The ponies can be assumed to have been grazing when they were not resting.

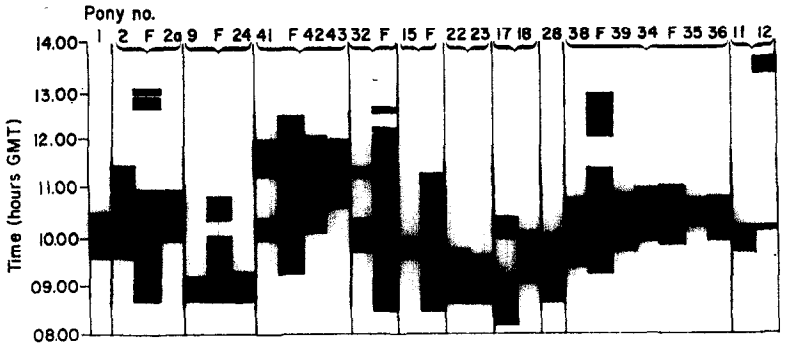


Fig. 4. Length of time of resting bouts of twenty-nine ponies of a population on 27 September 1967, showing social facilitation of resting between members of a group.

grazing by foals differed in the three time intervals: 06.00 to 10.00, 10.00 to 14.00 and 14.00 to 18.00 hours GMT (Table II). The means were significantly higher in the late afternoon and early evening period, 14.00 to 18.00 hours, than the means of the two earlier periods ($P < 0{\cdot}001$). There was no significant difference between the means of the early morning and mid morning periods ($P = 0{\cdot}5$).

Resting

Introduction

Ponies rested either standing or lying down; when lying down they were either on their sides with usually all four legs extended or they rested on their sternum with their forelegs and hind legs flexed below their body.

Table II. Mean Number of Minutes per Hour Spent Grazing by Foals with Time of Day

Age (weeks)	06.00–10.00	10.00–14.00	14.00–18.00
1–2	6·5	5·1	7·3
3–4	9·8	11·7	13·2
5–6	15·2	14·4	16·4
7–8	6·7	17·7	13·5
9–12	10·4	13·2	17·0
13–16	13·7	11·3	23·1
17–20	17·5	21·3	32·6
21–24	19·3	20·4	34·9
25–28	25·5	41·1	34·2
29–32	44·3	37·3	41·7

This was based on data from 1967 check sheets only. The mean no. of minutes spent grazing was significantly higher in the late afternoon period ($P<0·001$) than the means of the two earlier periods.

Yearlings (ponies between 1 and 2 years old) lay more frequently than did adults, who often lay to rest in the summer outside the shades but infrequently at other times of the year except when temperatures were high. Adults invariably stood when resting in the winter day-time although they were observed lying down close together on several occasions on cold winter nights.

Time Spent Resting

In winter. Each pony had one, or rarely two or no resting bouts during daylight hours. These bouts were short and their mean length for adults was 39·9 min (367 observations between October and March) and for young ponies in their first winter it was 43·9 min (122 observations).

Few ponies rested between dawn and about 09.00 hours but the numbers resting increased after this so that a peak often occurred when most of a population was resting at the same time. The time of the peak was dependent upon weather conditions. When the sky was overcast all day there was no obvious peak, but a slight increase in resting occurred after dawn and the

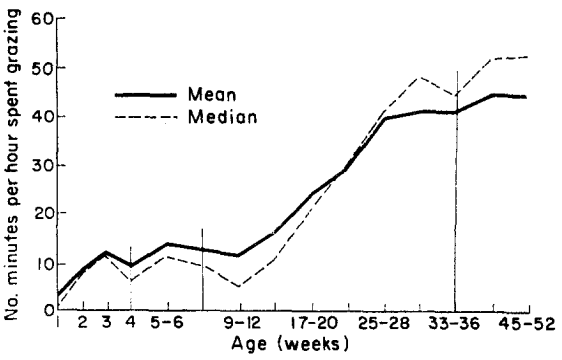


Fig. 5. Changes with age in the proportion of time foals spent grazing. Data from all 1966 and 1967 foal check sheets.

level then remained low throughout the morning and afternoon (Fig. 1, 8 February 1966 and 7 November 1966, and Fig. 2, 1 March). On days when the sun suddenly broke through the clouds, there was a corresponding sudden peak in resting which occurred at all times between 09.00 and 13.00 hours (Fig. 1, 2 February 1966 and 27 September 1966, and Fig. 2). When the sky was clear from dawn a definite peak still often occurred but it was not always as sharp as on days when the sun appeared for a limited time (Fig. 1, 29 March 1966 and 9 November 1966).

Although the sun's presence often determined the time when most of the ponies rested, they still usually all rested before 13.00 hours even when the cloud cover was total.

In summer. With an increase in available food in late April and May, ponies were able to spend more time resting and the length of their resting bouts also increased. However, as during the winter, few ponies rested during the 2 to 3 hours after dawn and few in the late afternoon and evening. From mid-June to September on hot, calm days ponies usually stood or lay out in the open for a short time after the postdawn grazing peak. They then moved quickly, often galloping, to the shades where they stood for up to 5 hr (Fig. 1, 2 July 1966 and Fig. 3). Occasionally they

moved from the shades to graze, drink, defaecate or urinate but quickly returned. Many different groups, stable social units (see p. 111), stood close together in the shades but ponies were usually closer to their group companions than to non-group companions so that the groups were often evident as separate units. Adult mares and stallions invariably stood rather than lay in the shades but foals and sometimes yearlings often lay near their mothers. The foals however, often kept jumping up and stamping, shaking and scratching. This was thought to be because of the activity of hippoboscids (see p. 92).

Shading behaviour was recorded from mid-June until the end of September in 1966 but in 1967 the 'shading' was first recorded on 1 July and it was seen on very few days in September. Wet and windy weather in late June and September 1967, which reduced the activity of flies, was presumably responsible for this difference. In 1968 the shades were little used for much of July and August when it was wet and cool. On wet, windy or cool days in the summer a similar pattern of resting occurred as in the winter (Fig. 1, 18 July). Strong wind or heavy rain at any time of the year caused ponies to seek the shelter of trees and gorse bushes, where they remained for long periods without grazing and young ponies often sheltered behind their mothers. When sheltering or even when in the open, ponies stood with their backs to the rain or wind but when they were grazing in light rain or wind, they usually faced the prevailing weather. When resting in the sun they orientated themselves so that they were at right angles to the sun's rays.

Rolling often occurred after and sometimes before resting bouts spent lying down and ponies also often yawned before and after resting.

Social Facilitation of Resting (see p. 132)

Members of a group rested close together and even in the shades, the various groups were to some extent segregated. When one pony lay down or stood resting, its group companions usually approached it and lay or stood near it. Similarly when one pony began to graze after a resting bout, the other ponies in the group then also soon stopped resting and began to graze (Figs 3 and 4.) The two groups of ponies, 34 to 39 and 53 to 40, shown in Fig. 3 were almost considered to be two sub-groups of one large group. The two Figs 3 and 4 illustrate the close co-ordination in resting between members of a group.

The data on the previous pages refer only to hours of daylight. Observations were made during parts of some nights but quantitative data for resting were difficult to obtain because ponies were very scattered at night and only a small number could be observed at any one time. On many occasions in the winter ponies were seen resting immediately before dawn. However, it seemed that they grazed for much of the night in all seasons but did have resting bouts during this time.

Resting in Foals

The mean number of minutes per hour spent resting during daylight hours remained remarkably constant for foals of 1 week to 3 months (Fig. 6). After the third month, the mean number of minutes per hour spent resting gradually decreased. This was correlated with the increase in age ($P < 0{\cdot}001$) but was presumably also due to the approach of winter and scarcity of food. There was also a rapid increase after the third month in the proportion of hours of observation in which foals did not rest at all (Fig. 8), again presumably largely correlated with the approach of winter and the associated necessity to spend more time grazing, as well as with the increase in age.

No well defined peak in resting of foals was evident during the day but the mean number of minutes per hour spent resting in the late afternoon period 14.00 to 18.00 hours, was significantly smaller than the means for the early and mid-morning periods 06.00 to 10.00 and 10.00 to 14.00 hours, in foals of 1 week to 8 months (Table III) ($0{\cdot}01 < P < 0{\cdot}02$). The means for the two morning periods were not significantly different ($0{\cdot}5 < P < 0{\cdot}7$). The relatively high mean for the early morning period was probably

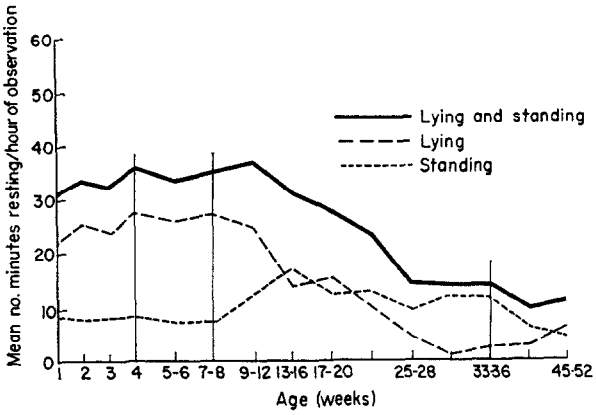


Fig. 6. Changes with age in mean number of minutes per hour of observation which foal spent resting. The mean for each age group is based on data from fifty-eight to 196 hourly check sheets. Vertical bars indicate a change in scale in time axis from 1-week intervals to 2-week, to 4 -week and then to 8-week intervals.

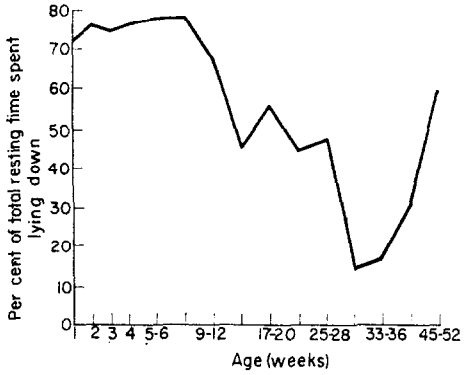


Fig. 7. Changes with age in the amount of resting time which foals spent lying down (replot of Fig. 6).

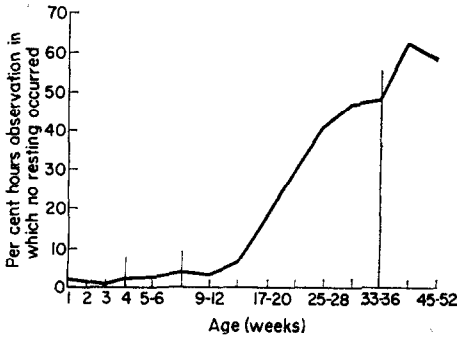


Fig. 8. Changes with age in the porportion of hours in which foals did not rest.

Table III. Mean Number of Minutes per Hour Spent Resting by Foals with the Time of Day

Age (weeks)	06.00–10.00	10.00–14.00	14.00–18.00
1	28·5	31·8	27·9
2	32·0	36·6	27·8
3	33·2	32·8	26·6
4	34·2	32·8	28·6
5–6	31·1	31·9	29·7
7–8	43·4	27·0	32·4
9–12	38·7	35·7	29·1
13–16	32·0	33·2	23·6
17–20	29·5	28·2	19·2
21–24	30·6	30·0	20·0
25–28	15·3	10·6	22·3
29–32	8·5	17·2	16·4

This was based on data from 1967 check sheets.

because the foals had spent the nights in the valley bogs or woods where the rough or wet nature of the ground prevented them from lying down and where their mothers kept moving from one spot to another; when they reached the open grasslands after dawn the foals were able to lie down

as their mothers then grazed in a small area for much of the day.

The ratio of time spent lying down to that spent standing remained constant in young foals (Fig. 7). They lay for 70 to 80 per cent of their total resting time up to the age of 2 months but the amount of resting time spent lying down then decreased with increasing age until the eighth month when it was 13·8 per cent. There was then an increase, which coincided with the spring and drier, warmer conditions which affected ponies of all ages, but the young foals still spent more time lying down than yearlings (Figs 6 and 7). Although in cold winds and rain foals tended to stand rather than lie down when resting, many other factors determined whether they stood or lay. They usually stood when they were on rough or hummocky ground and when the vegetation was long or dense as in the valleys; they also usually stood when their mothers were grazing on the heaths where they moved on several paces between each mouthful of food due to the sparse distribution of *Molinia* and dwarf gorse.

When young foals were lying down their mothers, if not resting by them, grazed repeatedly away from them and back towards them, or in circles around them. The distance a mare moved from her foal on its first day was very small, rarely more than 5 yd, but the distance rapidly increased with age. By the time a foal was about 2 months old, its mother sometimes did not graze back towards it, so that when the foal stood up again it had to search for her and long but temporary separations often occurred. The number of movements per hour made by a mare away from her foal was significantly smaller when it was lying down than when it was up until the seventh month and the number of movements made towards her foal was greater when it was lying down until its sixth month (Fig. 27 p. 163). After the seventh month foals spent such a small proportion of total time lying down, that analysis of movements per hour made by their mothers when they were lying down, gave meaningless results.

When a young foal was standing resting, it usually stood immediately behind its mother and almost each time she moved, it approached her so that the resting bout was frequently interrupted. Mares usually stood or lay close to their foals (when they were resting), even when the 'foals' were 1 year old, and it was common to see a mare, her foal and an older offspring resting together in a compact family group.

Sometimes foals rested together in small numbers even though their mothers often belonged to different groups. On one occasion a 4-month-old foal lay down and then another younger foal in the same group approached her and pawed at her for a few seconds before lying down beside her. Within 10 min a filly foal from another group moved over 30 yd from her mother towards these two foals, stood near them for several minutes and then lay close by them. Many similar occurrences were observed where foals seemed to seek out each other's company and then lay together.

Eliminative Behaviour

Eliminative Postures

The urinating posture of a mare was similar to that of a mare in oestrus. She stood with her hind legs straddled and tail raised and after urinating she kept her tail raised for several seconds and 'winked', the labia everting to expose the clitoris. Stallions often rushed excitedly to urinating mares as they did to mares in oestrus, but after sniffing them soon left. Stallions when urinating stood with their hind legs stretched backwards and apart, and tail slightly raised. When ponies defaecated they merely raised their tails for a few seconds and usually continued grazing whilst they defaecated, contrary to observations on thoroughbreds which stopped grazing to defaecate (Hafez, Williams & Wierzbowski 1962). When they urinated, ponies almost invariably stopped grazing to do so. They also usually stopped walking to defaecate or urinate unless they were alarmed (Table IV).

Taylor (1954) observed that horses stopped grazing and walked to special areas of their field

Table IV. Behaviour of Ponies when Urinating and Defaecating

	Per cent observations					
	Grazing		No. of observations	Walking		No. of observations
	Stop	Continue		Stop	Continue	
Urinating	59·0	41·0	368	100	0	231
Defaecating	6·3	93·7	890	95·7	4·3	378

When ponies were grazing or walking they either stopped before they urinated or defaecated or they continued grazing or walking whilst they urinated or defaecated.

before defaecating so that closely grazed patches developed where they grazed and rough grass patches where they defaecated. He suggested that this behaviour lowered the risk of worm infection as the horses avoided the egg-laden faeces, but he also pointed out that this coprophobia did not occur when cows or sheep shared the same pastures. In the New Forest where cattle are pastured with the ponies, there was no evidence that the ponies grazed and defaecated on separate areas; they defaecated as they grazed. The only indication that any special movement to defaecate was made by New Forest ponies was when they were standing close together in the shades. They often walked a few yards from the shade to defaecate and then returned. No special movements were made to urinate either, although in wind ponies did turn to face the wind before urinating.

Frequency of Urinating and Defaecating

Frequencies were estimated largely from records on the foal check sheets. Adult mares defaecated once on average in just over 2 hr and urinated once in every 4 to 4½ hr. No significant difference was found in the frequencies estimated for summer and winter. Although the frequencies are considered to be reasonably accurate, they are under-estimates rather than over-estimates as it was possible that the observer occasionally missed seeing a mare defaecate.

The frequency of urinating in young foals was high, almost once per hour, but it gradually dropped to a similar frequency as in adult mares in foals of about 1 year (Table V). The defaecating frequency was very low in young foals but this gradually increased with the foals' age (Table V). Foals started to strain to defaecate within an hour of birth and usually within 2 to 3 hours they had eliminated the meconium. However, during their first few days they often had difficulty in defaecating and strained to pass

Table V. Changes with Age in the Frequency of Urinating and Defaecating in Foals

Age (weeks)	Mean no. of hours in which foals: Urinated once	Defaecated once	No. hours observed
1	1·1	9·8	228
2	1·1	7·2	159
3	1·3	5·9	131
4	1·5	6·4	115
5–6	1·4	4·5	145
7–8	1·5	4·2	122
9–12	2·2	4·8	207
13–16	2·5	4·6	165
17–20	3·3	3·1	100
21–24	2·8	3·0	102
25–28	6·5	2·4	78
29–32	5·3	3·2	74
33–36	6·5	3·6	65
37–44	4·0	3·6	137
45–52	3·5	3·7	60

one or two hard pellets. When foals were about 1 week old, their faeces sometimes changed, becoming very loose. This coincided with the time that their mothers had their 'foal heat'. Zebra foals also had diarrhoea at the time of their mothers' 'foal heat' and Wackernagel (1965) attributed this to hormones from the mare affecting her milk.

Reaction of Ponies to Faeces

Stallions. Stallions frequently sniffed piles of faeces, walked forward a few steps and then defaecated, or less commonly urinated, on the faeces; they then often resniffed the faeces before walking away. This pattern of behaviour was sometimes repeated in quick succession on several piles; one stallion defaecated on three different piles of faeces and urinated on a fourth within 10 min. At other times stallions sniffed faeces but then either walked on, or stood over the faeces for a few seconds with a defaecating or urinating posture, but did not actually do either. Observations of the last two types were included in the category 'rejection of faeces' (Table VI).

Marking of faeces by stallions with their own faeces or urine occurred in the winter, but was most evident in the summer. However, it was not a reaction only to faeces of oestrus mares as Klingel (1969) believed it to be in zebras. It was commonly seen when a stallion arrived on a daytime grazing area in the morning. He walked from one pile of faeces to another, sometimes soon walking on but often stopping to defaecate or urinate on several different piles.

Marking also formed a regular part of the sequence of the brief fights between neighbouring stallions. During a fight the two rivals broke off from pawing and trying to bite each other and then both sniffed nearby piles of faeces before either continuing the fight or breaking away and returning to drive their own harems.

Directional quality in posture is one of three factors which Kleiman (1966) used to distinguish scent marking from true elimination. Marking in stallions was elicited by either the sight and/or smell of other piles of faeces and it was orientated to these piles. Moreover, when marking, they defaecated or urinated in very small amounts compared with their non-directional true elimination.

The function of this scent marking in stallions was not clear. It cannot have had a true territorial function as stallions did not have geographically defined territories; instead their harems constituted 'moving territories' as in reindeer *Rangifer tarandus* (Espmark 1964a). When a bull reindeer sees a rival bull during the rut, he tramps rapidly with his hind legs and urinates in small quantities, the urine falling on the lower part of his legs. Espmark believed it to be a form of territorial marking adapted to the fact that the bull is the centre of a moving territory and so he urinates on himself rather than on fixed objects. Espmark suggested that this behaviour might have a repelling effect on intruding rivals or might even have a congregating effect on the cows. However, pony stallions

Table VI. Comparison of the Reaction of Stallions, Foals and Mares to Faeces

	Ignores faeces after sniffing them %	Defeacates on faeces %	Urinates on faeces %	Eats faeces %	Urinates on faeces and eats them %	No. of observations
Stallions	18	65	0	17	0	118
Foals, 4 weeks	36	0	2	15	47	55
Foals, 4 weeks to 6 months	43	2	7	36	13	103
Mares	54	14	0	32	0	71

marked other faeces and not parts of themselves and there was no evidence from this study that marking exerted any repelling effect on rival stallions; nor was there evidence for any other functions, some of which have been suggested by Wynne-Edwards, Schenkel and Lyall Watson (see Kleiman 1966) for scent marking in other mammals.

Zebra stallions have an identical behaviour pattern of marking to ponies but they only mark faeces of mares in oestrus (Klingel 1969); Trumler (1958) assumed that its function in zebras is to hide the condition of the mare from rival stallions. However, Klingel observed that family stallions often ignored faeces and that even then other stallions did not react to them, and he believed that olfactory stimuli were of little importance. He suggested that scent marking in zebras is vestigial behaviour inherited from territorial ancestors. Probably scent marking in ponies is also such vestigial behaviour but it is of interest that both mares and foals were observed defaecating or urinating on other faeces. However, whereas stallions more commonly defaecated rather than urinated when marking, both mares and foals more commonly urinated; and foals only rarely defaecated on other faeces (Table VI). However, the frequency of defaecating in foals was very low anyway.

Other descriptions of the marking of faeces by stallions with their own faeces or urine have been made by Schloeth (1958) and Zeeb (1958). Dobroruka (1961) has also described it in the Przewalski's horse (*Equus przewalskii*), in which he claimed it had a territorial function. He observed that a stallion most commonly marked faeces on the borders of his territory but did not always mark those within his territory. However, these Przewalski's horses were observed in the zoological gardens in Prague in three small groups, and it is not known if this species in the wild is also territorial.

Mares. Although mares often sniffed faeces they usually rejected them, but sometimes they did urinate on them (Table VI) and this was a more frequent response in mares than in stallions. Defaecating on faeces was much less common. Mares were observed sniffing faeces when they were separated from their group companions, as were stallions; this presumably was to gain olfactory cues about whether their group companions had passed, as it seemed very likely that ponies could recognize faeces of their own group by smell.

Mares were never observed eating their young foals' faeces as Wackernagel (1965) recorded in zebras.

When one pony defaecated, one or more ponies standing near it sometimes looked up and then also defaecated. This was thought to be an example of social facilitation and not a response elicited by the smell of faeces, as the ponies involved were sometimes many yards apart. Social facilitation of urinating was also observed.

Foals. Coprophagy (eating faeces) was common in foals of up to 3 to 4 weeks but it was observed in foals of up to 14 weeks and even first day foals were seen nibbling at their mothers' faeces. The faeces eaten almost always belonged to the foals' mothers but on two occasions a foal was observed eating its own faeces. The young colts and fillies usually pawed at the faeces first and only ate parts of one or two pellets.

Foals very occasionally defaecated on faeces, although none under 4 weeks of age were seen doing this and in only two cases did foals between the age of 4 weeks and 6 months defaecate on faeces out of 103 observations of foals sniffing faeces. However, foals defaecated very infrequently compared with adults (Table V). Urinating on faeces became more common as foals grew older and was a fairly common response to faeces by both colts and fillies (Table VI).

Reaction of Ponies to Urine

Stallions. When stallions sniffed urine they often urinated, usually first showing flehmen posture (see p. 143). Often they showed flehmen posture only and did not urinate, although they sometimes attempted to do so. They usually orientated themselves so that they directed the

Table VII. Comparison of the Reaction of Stallions, Foals and Mares to Urine

	Ignores urine after sniffing it %	Defaecates on urine %	Urinates		Flehmen only %	No. of observations
			Urinates and shows flehmen posture %	Urinates only %		
Stallions	6	0	39	16	39	31
			55			
Foals under 6 months	1	1	53	33	12	76
			68			
Mares	8	8	15	54	15	13
			69			

jet of urine onto the urine that they had sniffed. Stallions were never seen marking urine with faeces, and marking of urine was less common than marking of faeces (Table VII). The number of observations of marking of urine was also low because those observations have been excluded where stallions, after they had copulated, sniffed the ground on which either urine or ejaculate from the mare had fallen. Urine marking was almost invariably directed to urine of mares in oestrus but on several occasions, the mares concerned showed no behavioural signs of oestrus. However, 'silent-heats', where no behavioural signs are evident, are not uncommon in horses, and it seems likely that these mares were probably in fact in oestrus.

Mares. Mares very rarely sniffed urine but when they did so they usually urinated, sometimes first showing flehmen posture, or showed flehmen posture and did not follow that by urinating. On one of the thirteen observations of a mare sniffing urine, she then defaecated (Table VII). When it was very windy, mares showed flehmen posture whilst urinating as the wind blew back the urine to their heads. Other ponies standing close to mares also showed flehmen posture at such times.

Foals. Foals were usually standing close to their mothers when these mares urinated and they almost invariably immediately sniffed the urine on the ground, showed flehmen posture and then stood over the urine and urinated. Only once out of seventy-six observations did a foal show no reaction after sniffing urine and only once did one defaecate on the urine rather than show flehmen posture or urinate (Table VII).

Grooming

The grooming patterns of equids have been fully described in zebras by Trumler (1959a) and in Przewalski's horse by Mazak (1961). All these patterns were shown in an identical form by the New Forest ponies. Trumler classified grooming as follows:

1. Localized muscle contractions.
2. Shaking.
3. Striking one part of the body against another or against the ground.
4. Rubbing (in which he includes rolling).
5. Scratching.
6. Nibbling.
7. Social, or mutual grooming.

Local Muscle Contraction

Local contraction of the superficial muscle, *Panniculus adiposus*, caused small areas of skin to twitch. When a tabanid or oestrid fly landed on a pony, twitches occurred at that point so that the fly was disturbed; twitches were particularly evident when hippoboscids were crawling over the flanks or back of a pony. This phenomenon may be included as a form of grooming if grooming includes those means used to relieve skin irritation.

Shaking

Shaking of either only the head or the whole body occurred. Head shaking was a frequent response to the presence of flies settling around the eyes and nose, but shaking of the whole body occurred more commonly after a pony had rolled on the ground, after a pony had been lying down or after rain. It served to rid the coat of dust, other foreign material or water.

Striking One Part of the Body Against Another or Against the Ground

When a fly landed on the shoulders or forelegs of a pony the pony often swung its head around or downwards to strike at that part of the body; when a fly landed on its belly, it often raised a hind leg and kicked against its belly, or if on the hindlegs, the animal stamped. Tail swishing fits into this category of grooming and was chiefly a response to the irritation caused by various insects. Little tail movement occurred during the winter but it was very much in evidence from May to September. Foals in late April to about July were particularly affected by the movement of hippoboscids on their bodies and they spent long periods stamping, kicking at their bellies, rolling, shaking and lashing their tails.

Rubbing

This also probably occurred largely as a response to irritation caused by insects, lice and ticks, but long periods spent rubbing were thought to be auto-erotic. Ponies briefly rubbed their heads against their flanks, rump or forelegs, or their hindlegs against each other, but they usually rubbed against fixed objects. In any area certain stumps, trunks and branches were in frequent use as rubbing posts. In several parts of the study areas there were wooden bridges over small streams, which were much used by ponies and it was unusual for any pony to cross the bridges without pausing to rub against one of the posts supporting a hand-rail. However, although most ponies had regular rubbing posts, they did use any suitable object, even other ponies. At the start of a rubbing bout, a pony moved its head and neck backwards and forwards against the post. It then often rubbed its flanks and finally turned away from the post and rubbed the region at the base of its tail. At the same time it swayed its head from side to side and wrinkled and moved its upper lip. The above sequence during a rubbing bout was common but any combinations or any one of these rubbing positions were also used, and frequently a pony just moved backwards and forwards under a low horizontal branch, rubbing its back. Possibly rubbing of the vulva and tail region in mares was a form of masturbation and when stallions were rubbing they often showed erection of the penis. Rubbing in horses and ponies is not thought to have a marking function as it has in deer.

Rolling where the back was rubbed against the ground, often occurred at the end of a resting bout spent lying down; it was also common on wet grass after rain and when ponies encountered patches of bare sand or sawdust but at other times rolling occurred on ground with no obvious special features. There were no special sites reserved for rolling in any area as Schloeth (1958) recorded in horse territories. Ponies frequently pawed the ground before rolling and social facilitation of rolling was common where two or three ponies near each other rolled in quick succession.

Scratching and Nibbling

Scratching of the head and neck with a hind hoof and nibbling with the teeth of various parts of the body such as the back, rump and legs were frequent forms of grooming.

All these patterns were observed in foals during their first week and even in the few hours after birth, although they were often imperfect. When a very young foal raised a hind leg to try to scratch its head or neck, it was usually unable to balance on three legs and soon fell or nearly did so.

Young foals of only 1 or 2 days spent long

periods rubbing, sometimes rubbing their bellies by walking back and forwards over low heather or gorse clumps for up to 15 min.

The above six categories of autogrooming all usually occurred in response to some temporary irritation, or to cleanse the coat of foreign material and loose hair, especially in the spring at the time of moult of the winter coats. Corvids and starlings also helped to clean the coat of both old hairs and ectoparasites and free living flies. Except for rubbing and tail swishing, forms of autogrooming were exhibited for very short periods at a time, usually only a few seconds. However, different forms were often shown in quick succession and repeatedly, as by foals annoyed by hippoboscids, and all were more frequent between late spring and autumn than in the winter.

Mutual Grooming

This probably had the same functions in part as autogrooming but it was also an important form of social contact between ponies. It occurred when two animals stood facing each other and nibbled at the neck, mane, forelegs and withers (the ridge between the shoulder-bones) of their partner, mostly parts of the body that would not be reached easily during autogrooming. In a long bout, the ponies continued nibbling beyond the head and neck, along the back to the rump. Occasionally a third animal joined in but such occurrences were rare and brief. If a foal or other young pony approached its mother when she was already mutually grooming, it sometimes started to nibble and groom her at the same time. The mother either ignored it and the foal then soon left, or she broke off from grooming with her partner and began to groom her foal.

The earliest age that a foal was observed mutually grooming with its mother was 6 days, and an 8-day-old foal was seen grooming with its yearling sister. From their first day, foals nibbled and chewed at various parts of their mother's bodies, particularly at the neck, mane and tail, but they rarely stood facing their mother when they did this and a mother was never seen to respond by nibbling the foal until the sixth day. After their third to fourth week foals began to spend long periods together mutually grooming and this often developed into play fights in colts (see p. 172). Mutual grooming with other foals was most common in their third to fourth months but many foals were removed from the Forest soon after this and the frequency of mutual grooming then dropped (Fig. 9).

All observed bouts of mutual grooming were recorded and the number of bouts per 50 hr of observation between ponies older than 1 year was estimated for each month. Although any 2 hr of observation were not comparable because widely different numbers of ponies were under observation during different hours, it was thought that any two periods of 50 hr were roughly comparable. There were significant differences in the frequency of mutual grooming during different months of the year ($0{\cdot}001 < P < 0{\cdot}01$) (Fig. 10). Mutual grooming was most frequent in April and July and most infrequent in September. The high incidence in April can be correlated with the time of moult, when ponies were shedding their long winter coats. This began in March and continued throughout April and into May. It lasted longer in yearlings, who retained part of their winter coats well into the summer. The frequency of mutual grooming

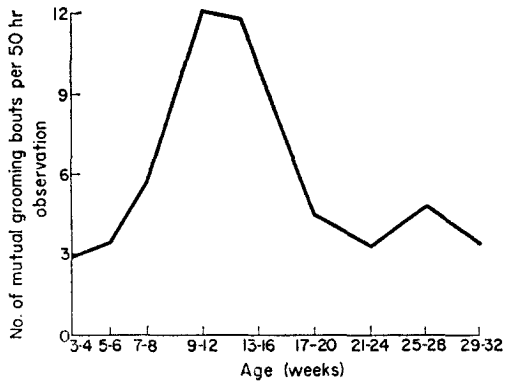


Fig. 9. Changes in the frequency of mutual grooming in foals from their third and fourth weeks to their eighth month. The minimum number of hours of observation for each group age was 72.



Fig. 10. Monthly variation in the frequency of mutual grooming in adults.

dropped in May and June, probably because many mares had very young foals during these 2 months and they spent much time close to them and correspondingly less time near other adults than before the birth of their new foals. The high frequency in July and August corresponded with the time that ponies spent much of the day close together in the shades. The number of interactions, both of an aggressive and non-aggressive nature, was markedly higher when ponies were shading. In September the frequency was again low as ponies spent more time grazing during the day and less resting in the shades. However, it increased gradually during the winter and this may have been because ectoparasites, particularly lice, increased in numbers during the winter, when the pony coats were at their thickest, and they then probably caused greater irritation.

Mutual grooming was therefore a response to irritation caused by lice or shedding hair but it was also a social phenomenon. Each pony had only a small number of other ponies which it used as regular grooming partners and of any two ponies, the same one invariably ended a grooming bout.

Non-mutual allogrooming was only observed between young foals and their mothers or siblings, and between stallions and mares in oestrus. After parturition a mare licked her foal almost continuously for 20 to 30 min (see p. 150) and then only for a few seconds at a time during the rest of the foal's first day, particularly when she suckled it. Occasionally during a foal's second or third day, its mother licked its rump as it sucked but after the third day, this was not observed. The behaviour of foals when they were chewing and pulling at their mother's mane and tail might also be interpreted as a form of allogrooming as can the licking and nibbling by stallions of the hind legs and rump of mares in oestrus, which was a main part of the pre-copulatory behaviour.

IV. GROUP BEHAVIOUR

Size and Composition of the Groups

During the day ponies were generally spread out grazing but observations in the evening, during the night and around dawn showed that certain ponies remained closer together. When ponies were resting or sheltering from wind or rain, or when they were alarmed, this close association of particular animals was also evident. By plotting the positions of every pony on a sketch map whenever part of the study areas was visited during the day, the association of ponies into small groups was firmly established even when many groups were grazing close together on the lawns and reseeded areas (Fig. 11).

Structure of Groups

Most of the groups were simple family units consisting of one adult mare and one or more of her own offspring; other groups consisted of two adult mares with a varying number of offspring and more rarely of three to six mares and their offspring (Table VIII). Many mares appeared to be completely on their own but this was because their foals had been killed or removed.

Young fillies occasionally left their mothers when only 1 year old but most remained with them until their second or more commonly their third or fourth year (Table IX and p. 168). Unfortunately very few mares on the study areas were left completely undisturbed by their owners, so that the total number of animals in each age group that were able to remain with their mothers was small (Table IX). However, from the little evidence available it was shown that of twenty-one animals, whose age was known when they left their mothers' groups, none left voluntarily when younger then 1 year and only two when younger than 2 years, and the majority left between the ages of 2 to 4 years.

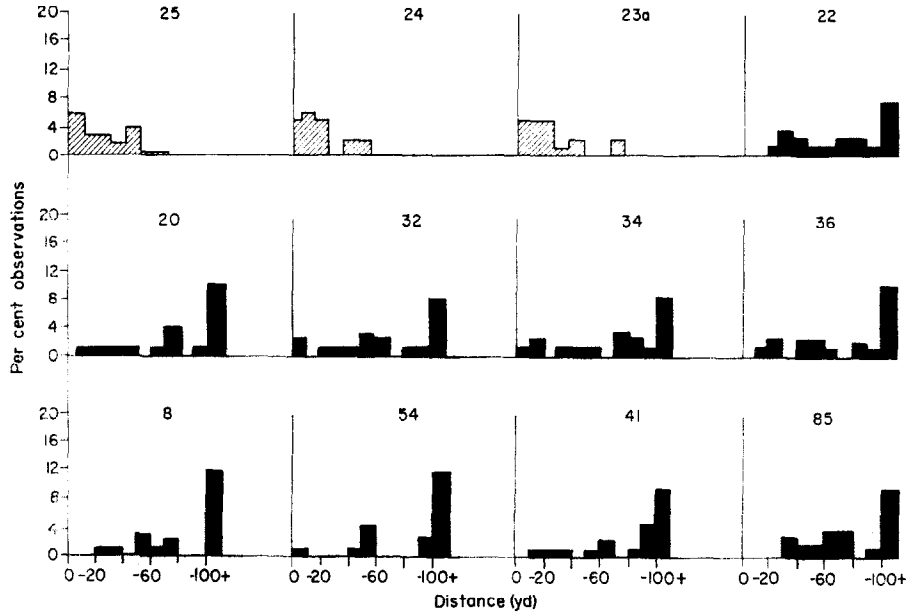


Fig. 11. The association of ponies into groups as shown by the number of observations out of twenty which twelve ponies spent within various distances of a particular pony (BH23). Three ponies (BH25, 24 and 23a) were group companions of BH23; the others belonged to different groups but the same population, and all grazed in the same reseeded area.

Table VIII. Composition of the Groups in the Study Areas in the Winters of 1965/66 to 1967/68

Winter	1 adult mare + offspring	2 adult mares + offspring	3 or more adult mares + offspring	Total no. of groups
1965/66	60·7	27·8	11·5	122
1966/67	59·0	28·2	12·9	124
1967/68	64·5	25·0	10·5	124

Due to the formation of false family groups (see p. 114) the true mother of some of the 'offspring' may be in doubt.

Table IX. The Length of Time During which Fillies Remained in Groups with their Mothers

Fillies	Year of birth				
	1967	1966	1964	1964	1963 or before
With mother autumn 1968					
No.	23	13	18	6	3
%	100	93	82	46	25
Left mother before autumn 1968					
No.	0	1	4	7	9
%	0	7	18	54	75
Known ages (years) at which foals left their mothers	<1	1–2	2–3	3–4	4–5+
No. of fillies	0	2	7	7	5
% of fillies	0	10	33	33	24

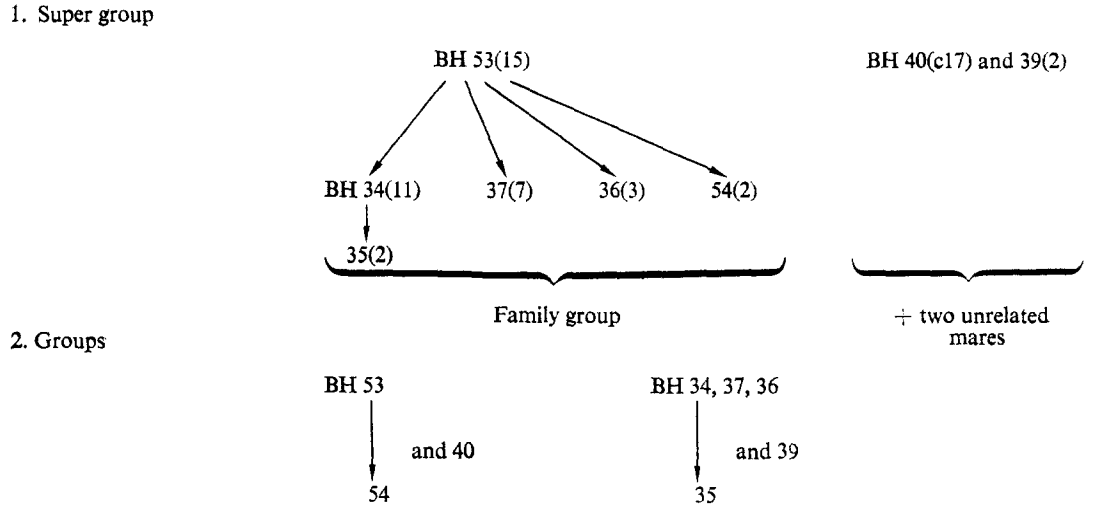
Colts were usually removed by their owners in their first or second autumn.

The break from a mother was sometimes abrupt but the fillies usually spent several days or weeks away and then returned. This was repeated with the period of separation gradually increasing in length. However, the relationship between mother and daughter was still often maintained long after the young mare had finally left her mother's group such as by mutual grooming bouts. The young mares, after leaving their mothers' group, either joined up with another group or with a single mare, not necessarily another young mare, or remained on their own so forming the basis of a new family group. In several instances two or more sisters broke away from their mother after she had given birth to a new foal, and the sisters remained together in a group. One large family group was present in the second study area in the winter of 1965 to 1966; this consisted of an old mare, her four daughters, a grand-daughter and an unrelated young mare. However, the old mare and her youngest daughter eventually formed a separate group from the other mares, although all frequently rested and grazed together throughout the three years (Table X). All foals produced by these mares were removed in their first autumn; in the winter 1967 to 1968 the old mare BH53 was separated from her daughter BH54 and taken off the Forest. However, she was returned in spring 1968, and BH54, who had temporarily joined up with an older mare and her foal, immediately rejoined her mother.

Four young mares remained with their mothers even after they had foals of their own. In 1965 to 1966 winter a 6-year-old mare was in the same group as her 11-year-old mother and they were still in the same group at the end of the study period although both had foaled in the intervening period.

Unfortunately most colts were removed from

Table X. Large Group of Eight Ponies in Winter 1965 to 1966 which Split into Two Sub-Groups during the Study Period



Age in years given in parentheses. Arrows indicate mothers and their offspring.

the Forest before their first or second winter, but all yearlings that were left remained with their mothers. One colt spent long periods in close association with his older sister but they both still remained with their mother. Only two colts were left in the study areas uncastrated until they were older than 2 years. One remained in close association with his mother and young sister and other members of his mother's group during his third summer and winter but in the following summer he was the only male in an area where there were over thirty mares. He then spent much time away from his mother and in his fourth winter he joined up with a small mare group. However, throughout the following 18 months he frequently approached and groomed with his mother. The other young colt was 2 years old in the spring of 1968 and although he spent much time with young mares during the summer, he was often seen grazing and resting with his mother.

Colts became sexually mature by the age of 2 years but were not treated as rivals by adult stallions until they were three. Both of the 2-year-old colts mentioned above were tolerated by other stallions present in the area but there were none present during the first colt's fourth summer. It is not known if the 3-year-old colt would have been allowed to remain in his mother's group had adult stallions been present but from observations of other adult and 3-year-old stallions it seemed likely that he would have been driven away. Therefore most colts left on the Forest probably leave their mothers' group when 3 years old, either of their own accord to establish 'harems' or because they are driven away by older males.

Position of Adult Males in the Group Structure

Many stallions were taken off the Forest and onto the farms and small-holdings for the winters but of sixteen individuals that were left out, twelve became closely associated with a particular mare group. This relationship was maintained for as long as the stallion was allowed to remain in that area; every 2 to 3 years the commoners moved their stallions to different areas to prevent them from copulating with their own daughters, although some stallions soon returned to their original range. During the summer when each stallion usually copulated with mares belonging to many different groups, the group

stallions always grazed closer by members of their own group during the day when no mare was in oestrus and they also spent most nights with their own group. The four non-group stallions remained single, or temporarily and briefly joined up with various mare groups.

Comparison of Social Organization of New Forest Ponies with that of Other Equids

Few wild equids live in a natural state today. The Camargue horses and various breeds of wild horses in North America are all much controlled by man, but horses on Sable Island off Canada are now left completely undisturbed with no control of the stallions. Each group of Sable Island horses consists of one stallion, a small number of mares and some of their offspring (Bruemmer 1967). This closely parallels the organization of steppe zebras (Klingel 1964a, 1964b, 1965) and mountain zebras *Equus zebra hartmannae* and *E. z. zebra* (Klingel 1968). A group of these zebras again consists of a stallion—the family stallion—one to six mares and their foals. This is similar to the situation seen in New Forest ponies but the lack of these pony stallions ensures that most groups consist only of mares and their offspring; the close association of the few stallions with mare groups suggests that their organization would more closely parallel that of Sable Island ponies and steppe zebras if the sex ratio of adults were more equal. Moreover, if a steppe zebra stallion is removed from his group, the mares of that group still remain together and are taken over as a unit by a new stallion, showing that the mares form a stable unit in themselves and are not kept together only by the family stallion. This again demonstrates the similarity between New Forest pony groups and the groups of these other equids.

Surplus and young males of both Sable Island ponies and steppe and mountain zebras form bachelor groups; the small number of males in the Forest prevents the existence of such all-male groups, although in 1967 a 2-year-old and a 3-year-old colt were turned out on the same area in the spring and they remained together as a group throughout the summer. Two adult stallions were present in that area and prevented the two young males from establishing 'harems'.

Steppe zebra colts leave their mothers between 1 and 4½ years but usually when they are about 3 years old. There is little evidence for the equivalent age of New Forest colts but it is suggested that they also usually leave when about 3 years old. Zebra fillies leave their mothers soon after their first oestrus periods when 1 to 2½ years old, because they are then abducted from their family stallion by other stallions (Klingel 1964a, 1964b, 1965). New Forest fillies usually remained in the groups with their mothers until older than this, but this difference could again be explained by the lack of stallions available to abduct them.

Detailed information on the organization of other equids is unfortunately lacking.

Stability of the Pony Groups

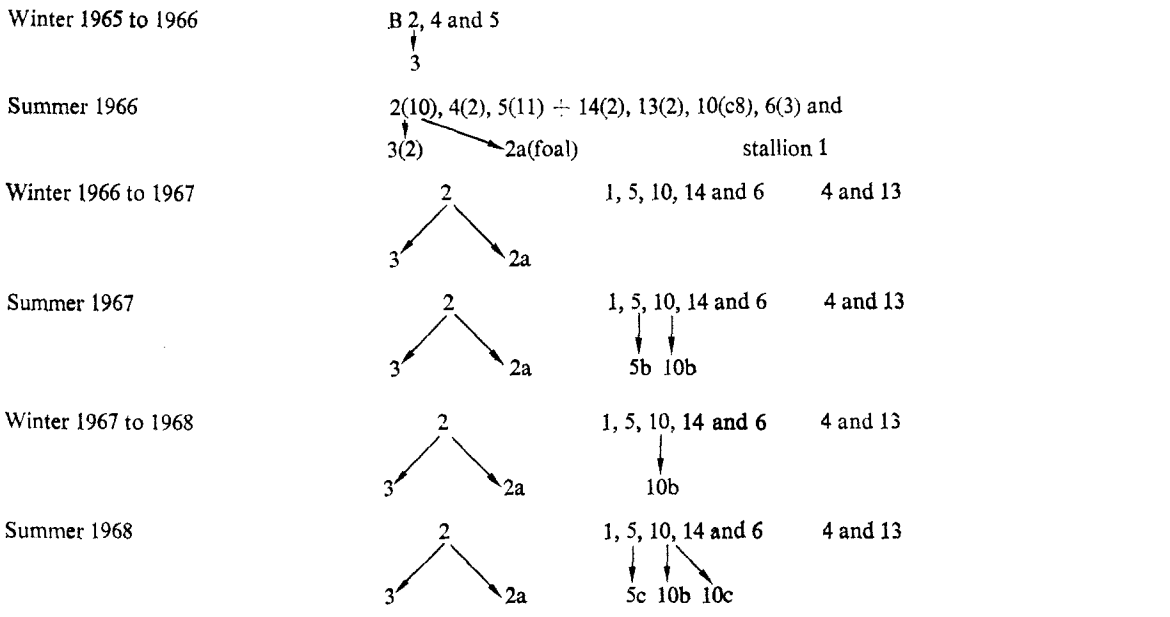
Changes in group were frequent among young mares. After leaving their mothers, they often joined up with another group or single mare for a short time, then left, joined up with another group and left again several times before a stable relationship developed. It is of interest that many of the young mares joined up with and remained permanently with older mares who already had a foal or yearling with them. This resulted in apparent family groups although they were not in fact so. Similarly when two mares died their offspring attached themselves to the dead mare's group companions and remained with them even after the mares had foaled, so that false family units were again recorded. Such groups complicated the observer's system of classifying them according to whether they consisted of one, two or more adults (Table VII), but those false family groups have been called family groups where the non-family member was under 3 years old. Groups of two sisters, even where one sister was under three, have however been included in the second category but only two such groups were present in the study areas.

Although large groups were uncommon, one group of five adult mares between the ages of 4 and 12 years in 1966, remained stable during the 3 years. One of the mares and her yearling filly were removed in the spring of 1968. Another 2-year-old mare became associated with the group in the summer of 1966 and remained with it up to the end of the study period and a filly produced by one of the mares in 1966 was with the group up to this time. Another born in 1966 was removed in the spring of 1968 and all colts were removed. One of the adult mares, a 6-year-old in 1966, was a daughter of the oldest mare in the group but the other mares were unrelated.

Only two other groups consisted of five adult mares but one was formed during the study period and soon split up (Table XI). The groups formed by the split were, however, stable for the remaining 2 years; they consisted of a family group, a group of two young mares and a larger group of a stallion (B1) and four adult mares. These three groups were often seen grazing and resting together, particularly during the summer months of 1967 and 1968, forming a super-group. During the winter, in early mornings and evenings and at other times the super-group separated into its three components. The other group of five mares was a group of three sisters, a daughter of one of them and a young unrelated mare (Table XI and XIII, Group D).

It is suggested that other instances where different groups frequently rested, grazed and groomed together might be taken as an indication that these groups had recently split up or alternatively that they were merging. During the study period two small groups of an adult mare and her daughter and two adult mares completely united. In two other cases two small groups became closely associated but they were still sometimes seen apart at the end of the study period. Many single ponies, especially 2 and 3 year olds, attached themselves to existing groups, so increasing the group size, or commonly, two single ponies united.

Table XI. Large Group in Summer of 1966 which Split into Three Groups



These three groups remained stable during the rest of the study period. Mares B2, 4, 5, 8, 10, 13 and 14 were unrelated. 3 and 2a were offspring of 2. Foals of other mares have been given letters (a, b or c) after their mothers' numbers. The age of the summer 1966 group is given in parentheses for each individual.

However, apart from the attachments of single, usually young mares, to one another or to groups, division and joining up of groups were relatively rare and most of the groups remained stable from the autumn of 1965 to that of 1968. The few changes amongst adult members of groups were largely due to deaths, removal of old mares and redistribution of some others by man. Sometimes a group of two mares was temporarily broken up when an owner took one of the animals off the Forest for the winter, but almost invariably, if this animal was returned to its original range in the spring, the two mares soon reunited. This even occurred when the remaining mare had joined up with another group during the winter.

Of six changes in group involving mares over 4 years old and due to causes other than those mentioned above, four occurred after one of the group members foaled. The mare then left her group and remained with her foal separate from it. This was unusual however, and most mares after foaling, continued their association with their original group companions or very temporarily remained apart from them. Two of the four mares which did leave their groups were primiparous mares and only 5 years old; they both left their mothers' groups.

Other temporary separations of group members occurred but members were rarely seen apart for any length of time and when separated they soon became anxious. They whinnied or nickered frequently (see p. 141) and they kept looking around and moving towards various distant mares until they recognized their own group companions. Olfactory means of communication seemed also to be of importance as 'lost' ponies frequently sniffed the ground, particularly piles of faeces. A stallion was found grazing on his own in a large area of woodland and, when disturbed by me, he looked up and whinnied several times. There was no audible reply but he then sniffed the ground and began to walk. He continued walking for over a mile, pausing at intervals to sniff faeces, until eventually he rejoined his mares. His path through the wood was very direct, even when he had a choice of several pony tracks. Visible and auditory means of communication can probably be ruled out because of the distance involved, leaving only olfactory means.

The only other observed temporary separations of group members were when one mare was in oestrus and no stallion was in the vicinity. The mare then left her group and even her home range (see p. 133) for several days. On another occasion a whole group of a mare and two daughters left their home range together when the mother was in oestrus.

Although the relationships between two or more adult and unrelated mares were so strong and stable, it is of interest that over half the groups were pure family groups and that even when a mare's offspring were all removed, the mare still often remained solitary.

Mutual Grooming (see p. 109)

Grooming Partners

Each pony had only a small number of partners with which it groomed. These partners were usually related animals, unrelated group companions or members of two closely associated groups. In small groups of three or four animals such as family groups, each pony usually groomed with every other pony in the group (Tables XII and XIII), but in larger groups each often had one to three preferred grooming partners as well as its own offspring, mother or sibling with which it also groomed (Tables XII and XIII). The mare BH16, however, groomed with four unrelated ponies as well as with her young daughter, 16B, and two other non-group companions (Table XII, Group B), but she groomed with the mare BH21 much more frequently than with other mares (Table XIII, Group B). Certain other mares were only observed grooming with their own offspring, e.g. BH19 and 22 (Tables XII and XIII, Group B). Similarly BH23 and 25 (Tables XII and XIII, Group C), although they were the only two adult mares in their group, were never observed mutually grooming together. They both only

Table XII. The Limited Number of Grooming Partners of Any One Pony as Represented by Members of Four Groups, A to D

Group	Pony	Age (years) 1966	Total	No. of grooming partners			
				Group companion		Non-group companion	
				Related	Unrelated	Related	Unrelated
A	BH32	8	2	1	1		
	BH33	1	5	1	1		3
	BH31	2	2		2		
B	BH16	6	6		4		2
	BH20	4	1		1		
	BH21	6	2		2		
	BH19	6	1	1			
	BH14	1	8	1	2		5
	BH22	10					
	BH47	1	2		1		1
C	BH23	8	1	1			
	BH24	1	4	1	1		2
	BH25	11	1	1			
	BH26	1	2	1	1		
D	BH40	c17	4		2	1	1
	BH53	14	3	2	1		
	BH54	2	3	2			1
	BH34	11	3	2	1		
	BH35	2	3	3			
	BH36	3	4	3			1
	BH37	7	9	4	2		3
	BH39	2	7		2		5

This shows that generally only young ponies groomed with unrelated animals from different groups.
Foals born in groups between 1966 and 1968 have been excluded from this table.
Bracketed pairs are related.

groomed with their own daughters, although their daughters, BH24 and 26, who were born in the same year, frequently groomed together until the death of BH26 in her third winter; their younger daughters BH23A and 25B also groomed together.

In the four groups shown in Tables XII and XIII, the mare, BH37, had the maximum number of grooming partners. She was observed grooming with six other mares in her group, four of them related to her, and with three unrelated mares from different groups. However, she was only seen grooming once with one of these non-group companions, whereas she groomed very much more frequently with the mare, BH36, than with any other. This large number of partners was exceptional amongst adult mares but more common with immature and young animals, e.g. BH33 (Table XII, Group A), BH14, a yearling colt (Table XII, Group B), BH24 (Table XII, Group C) and BH39 (Table XII, Group D). Moreover, these young animals more commonly groomed with non-group companions than did older mares. Foals and yearlings, especially colts and those in small groups, where there were no other animals of their own age, often groomed with young animals of other groups and the relationships so formed were sometimes maintained beyond their first or second year. However, the usual grooming partners of a foal were its mother, an older sibling or another foal in the group. When there was another yearling other than a sibling, this animal was also a frequent grooming partner, e.g. BH23A and 25B. The grooming partners of foals varied with their age (Fig. 12). Until their fourth week, they rarely groomed with other foals, but after this the number of bouts in which

Table XIII. The Variation in the Number of Grooming Bouts Observed Between Different Group Companions of the Four Groups A to D

Group A

32	33	31	Pony
	34	10	32
		14	33
			31

Group B

16	20	21	19	14	22	47	Pony
	9	38		4		2	16
							20
				1			21
				18			19
							14
							22
							47

Group A: 32 = mother of 33

31 joined group spring 1967

and was removed spring 1968

Group B: 22=mother of 19

19=mother of 14, a yearling removed spring 1967

Group C

23	24	25	26	Pony
	24			23
			16	24
			19	25
				26

Group D

40	53	54	34	35	36	37	39	Pony
	9							40
		17			2			53
						3		54
				13		8	2	34
					2	5		35
						28		36
							8	37
								39

Group C: 23=mother of 24

25=mother of 26

26 died winter 1967 to 1968

Group D: 53=mother of 34, 36, 37 and 54

34=mother of 35

40 was removed autumn 1967

The table shows that except in small group A, each pony did not groom with every other pony in its group, and that each pony groomed more frequently with one particular pony than with its other observed grooming partners.

other foals were partners rapidly increased and remained large until their fourth month. Many foals were removed from the Forest when about 4 months old and this undoubtedly largely explained the drop in the number of bouts in which foals were grooming partners to other foals older than 4 months. However, this coincided with the winter when different groups remained more separate than in the summer and this made the few foals that were left on the Forest even less available as grooming partners. The number of bouts in which a foal's mother was its grooming partner became then correspondingly larger (Fig. 12).

The occurrence of mutual grooming between adult mares of different groups sometimes suggested that the two groups may once have been a single group, or that they were in the process of splitting up or merging.

Grooming Bouts—Their Initiation and End

When initiating a mutual grooming bout, one pony usually approached another with its ears forward and its mouth occasionally open with

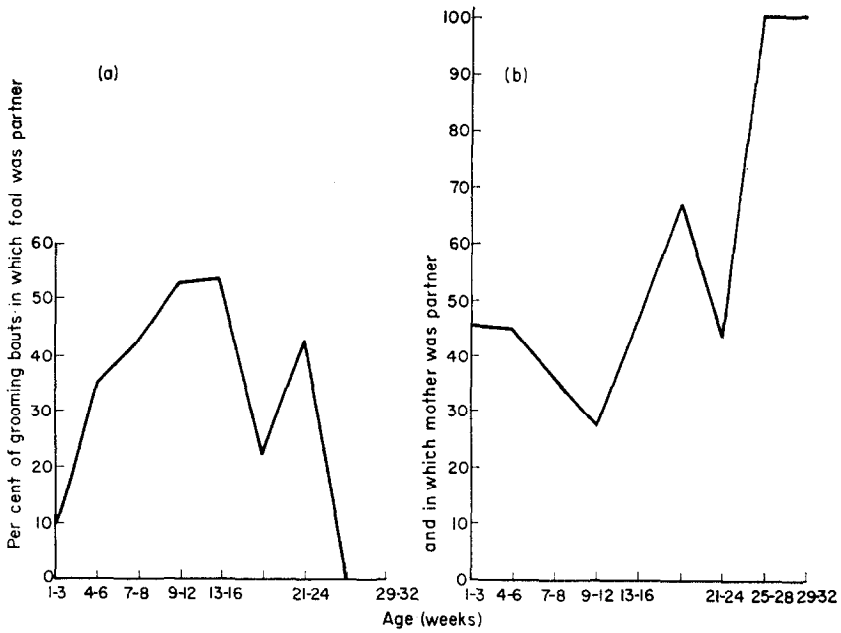


Fig. 12. Changes in the proportion of bouts in which foals of up to 8 months groomed with other foals (a) and with their own mothers (b). These figures were based on between eleven and fifty-one observations of mutual grooming for each age group.

the lower teeth slightly exposed; exposure of upper teeth and laying back of ears were signs of aggression (see p. 121). The expression often seen when a pony initiated a grooming bout was similar to the greeting expression described in equids by Trumler (1959a, b). However, when the pony initiating a bout was the dominant animal of the two, it often approached the subordinate one with its ears laid back. However, it then touched the subordinate's neck and the lower ranking pony reacted usually by nibbling the neck of the dominant. When a subordinate pony approached a more dominant one and showed the invitation behaviour of mutual grooming, the dominant pony either co-operated and began to groom with the subordinate, moved away or quickly laid back its ears and threatened to bite or even kick it. The subordinate pony then usually walked away but occasionally re-approached the dominant, stared at it and again invited mutual grooming. This was especially common when mares avoided grooming with their young foals. The foals repeatedly approached their mother and nibbled at their necks; the mares rarely threatened their foals but often kept moving away until their foals discontinued their attempts at mutual grooming.

The beginning of many mutual grooming bouts was unobserved but initiation was by the dominant pony of a pair in only 38 per cent of 162 cases where it was observed. Kummer (1956) and Rowell (1966) observed that in baboons *Papio hamadryas* and *P. anubis* it was the high ranking animal of a pair that usually initiated interactions, whereas it was the subordinate which determined the form that the interaction took, either by moving away from the dominant or staying. However, in the New Forest ponies, the subordinate animal of a pair initiated significantly more mutual grooming bouts than did the dominant ($0{\cdot}001 < P < 0{\cdot}01$) and the dominant usually determined what form the interaction took by moving away, threatening the subordinate or co-ordinating with it. When

the dominant pony of a pair initiated a grooming bout, the subordinate almost invariably remained and groomed with it, although it occasionally avoided the dominant.

It was often impossible to determine which of two ponies initiated a mutual grooming bout; frequently one pony walked towards another, who looked up when it was still several yards away. This second pony then often nickered and also walked to meet the first and they both began to nibble each other at the same time. Such observations were not included in the number of bouts in which initiation by one animal was recorded, although the animal that first moved towards the second pony might be taken as the initiator of the grooming bout.

During a bout either of the two ponies involved, sometimes lowered its head beneath or raised it above the head of the other and began to nibble the other side of its partner's neck. Where it was known which of the partners changed sides 139 observations were recorded and in 66 per cent of these cases, it was the dominant pony of the two which brought about the change. The difference in the numbers of changes of side brought about by dominant and subordinate ponies was significant ($P < 0{\cdot}001$). During some bouts several changes of side occurred but during others, the partners only groomed one side of each other's neck. Sometimes the dominant partner paused to scratch or nibble herself; the subordinate then also usually paused until the first animal continued grooming her. The subordinate animal was very rarely observed to temporarily break off during a grooming bout, except in the case of foals grooming with their mothers.

It was usually very obvious which of two grooming partners ended a bout, although it was less clear when the partners were a mother and foal. With the latter, even when the mother made a move away from her foal, the foal then often followed her and after nibbling her neck, the bout was resumed. At other times the foal broke off to stamp or scratch and the mare then walked away from it. Although the foal made the first move to stop the bout, this was often thought to be only a very temporary break and that it was the mare who really ended the bout. However, such observations were excluded from the 280 cases where it was obvious which animal ended the bout; 97 per cent were ended by the dominant partner usually by moving away from the subordinate partner or commonly by suddenly threatening it and biting it.

Snapping

When a foal or other young pony approached or was approached by an adult, it often responded by stretching out its head towards the head of the adult and by repeatedly opening and closing its jaws, with its lips retracted to expose the lower teeth (see p. 144). When the older pony touched the foal's neck and began to nibble it, the snapping movements made by the foal gradually passed into nibbling movements as it also began to groom the adult. Zeeb (1959) described this facial expression, which occurred only in immature or young animals, and referred to it as 'Unterlegenheitsgebärde'. It occurred in a variety of situations but most commonly when adults passed near foals and when foals were threatened by adults. Zeeb suggested that it may have originated as an intention movement of mutual grooming. This is also suggested by observations of the New Forest ponies, as a continuous sequence from snapping to grooming movements was often recorded. Zeeb also suggested that it acted as a submissive gesture when foals or other young animals were threatened by adults.

Length of Mutual Grooming Bouts

The number of mutual grooming bouts of known length was small because the beginning of many bouts was not observed, and these therefore could not be included. The number of bouts between stallions and mares was particularly small as there were so few stallions under observation.

Bout lengths were recorded in seconds and they ended when the partners separated. However, when one partner paused for only several

seconds perhaps to scratch, and then continued grooming after the pause, this was recorded as one bout and not as two separate bouts; the short pause was excluded from the total length of the bout.

The median length of 263 grooming bouts between mares older than 2 years was 56 s. The median length of 150 bouts between foals or yearlings and adult mares was 73 s, that between young animals of under 2 years was 78 s (60 bouts) and that between stallions and mares was 114 s (28 bouts). Although there were so few records of bouts of known length between stallions and mares, it is thought that such a long bout is typical because many bouts, whose beginning was missed, continued for 2 to 3 min after observation had begun. The longgest recorded bout was between a stallion and mare and that continued for over 30 min with only two to three very brief pauses. Copulation followed the grooming bout.

Despite the difference in median length of bouts in which the partners were two mares and of those in which they were a foal or yearling and an adult mare, the difference in the bout lengths was not statistically significant ($P > 0{\cdot}05$); nor was there a significant difference in lengths of bouts between immature animals and lengths of those between immature animals and adult mares ($P = 0{\cdot}5$). However, the lengths of bouts between stallions and mares were significantly longer than those between adult mares ($P < 0{\cdot}05$), as also were those of bouts between immature animals ($0{\cdot}02 < P < 0{\cdot}05$).

There was great variation in the frequency of mutual grooming in different months (Fig. 10) but there was no significant difference in the length of the grooming bouts in the summer, May until September, from those in the winter months, November until March ($P = 0{\cdot}3$).

Dominance–Subordinacy Relationships

Expressions of Dominance and the Nature of the Hierarchy

Expressions of dominance by the ponies took the form of threats to bite or kick, which were delivered when one animal came within another animal's individual distance, a term first used by Burkhardt (1944). It is a variable distance with no topographical reference (Conder 1949) whose size varies according to the rank of the animal.

The strength of threats to bite varied from very mild ones seen when a pony extended its head horizontally towards another and slightly laid back its ears, to stronger ones when a pony completely laid back its ears and opened its mouth slightly. It then sometimes even lunged out and bit the intruder usually on the neck or rump. These threat postures have been described by Trumler (1959b).

Threats to kick were much less common and were considered to be signs of greater aggression than were threats to bite. The aggressor laid back its ears and quickly turned its body so that its hind legs were directed towards the approaching pony; it then sometimes actually kicked out but kicks that made contact were rare except when two strange mares met and in fights between stallions. Kicking fights between mares were recorded on several occasions when a stranger was turned onto, or moved of her own accord onto a new area. When a resident mare was encountered, both the stranger and the resident sometimes threatened to bite each other. When neither moved away they dispensed with threats and both turned and repeatedly kicked out at each other with their hind legs. Several such fights, which continued for 2 to 3 min, were seen between two pairs of mares during a period of less than a week, after which one mare of each pair established her dominancy. Two other kicking fights were recorded between mares and it is thought that these indicated that mares were attempting to change their rank (see p. 129). Kicking with the forelegs was not observed in mares but was a common feature in the stallion fights.

Grzimek (1944) carried out some work on dominance–subordinacy relationships on two separate herds of nine yearling fillies and of twenty-nine yearling colts, and Montgomery (1957) on a small herd of four mares and seven

Table XIV. Aggressive Interactions between Thirteen Ponies of One Population During a Half-Hour Period when Hay was Supplied

No. threats delivered		No. of threats received (pony no.)													Total	Rank	Age
		59	43	5	53	34	37	13	39	20	36	35	54	10			
Pony no.	59		3	8	2	3	1	1	1		1			2	22	1	11
	43			4		2	2				2			1	11	2	13
	5				8	4	3	3	3		1				23	3	5
	53							2	2						4	4	15
	34						1								1	5	12
	37							2	1		3	1			7	6	8
	13								3					1	4	7	4
	39										1	1			2	8	3
	20										1			1	2	9	5
	36														0	10	4
	35													1	1	11	3
	54														0	12	3
	10														0	13	2
Totals received		0	3	12	10	9	7	8	10	0	9	3	0	6	77		

This table shows that the more dominant ponies delivered most threats to the ponies immediately below them in rank, and also shows the correlation between rank and age in the lower part of the hierarchy.
Ranks of the younger ponies were known from previously recorded interactions.
59 = stallion.

castrated males kept together in a field. Grzimek used the threat to bite as a primary criterion and the threat to kick as a secondary one in assessing rank orders, but Montgomery used four criteria: the kick, the bite, the head bump and passive reaction. However, his head bump corresponded to a mild threat to bite in the ponies, in which all degrees from slight inclination of the head towards another to an actual bite were recorded. Passive reactions, which occurred when a subordinate pony moved out of the way of a dominant pony without the latter showing any signs of aggression, were used in this study to asses rank orders, but passive avoidance could not often be recorded by numbers of interactions as could threats to bite or kick.

In this study all interactions between ponies were recorded and rank orders were assessed from these. During the winter, groups were spread out and few interactions were observed, except between members of a group, but in the summer when ponies concentrated in shades and at drinking places, interactions were more numerous between members of different groups. Hay was supplied during the winter months to provide competition. The individual distances of ponies were usually much larger during these hay feeds than during normal grazing, when competition was negligible, so that large numbers of threats could be recorded in a short time. Although by feeding ponies with hay, much data could be obtained for high ranking animals, the order of rank amongst low ranking ones was not always made clear. These subordinate animals avoided threats by remaining at a distance from the hay and making little or no attempt to

procure any. When hay was supplied in many separate piles, the rank order of these subordinates was still not made clear immediately. The large number of ponies present from many different groups quickly divided themselves up into their individual groups with one group to each pile of hay. The low ranking ponies then ate hay with their own group; threats were usually milder from members of their own group than from members of others.

Interactions were recorded as they occurred by noting the two ponies involved in each and the type of interaction. Data for each hay feeding period were subsequently recorded as in Table XV, the ponies being arranged in their order of rank. Although from the observation period shown in Table XV, the relationships between some animals cannot be seen, their rank in relation to one another was known from previous observations.

The rank orders eventually obtained for each population of mares from interactions recorded only during hay feeds, corresponded exactly with those rank orders obtained from natural interactions. As already mentioned natural aggressive interactions occurred during normal grazing behaviour and especially in the shades, when one animal overstepped the individual distance of another. They were also recorded at the beginning and end of some mutual grooming bouts. The rank orders of mares obtained from these various situations were identical and a mare dominant over a mare in one situation was never subordinate to the same mare in another situation. However, when stallions were included in assessing the rank order of a population, this was not so. A stallion was dominant over a mare in one situation but the same mare was dominant over the same stallion in another (see p. 127). This agrees with the conclusion of Rowell (1966); she found that in baboons (*Papio anubis*) the rank orders obtained from observations of priorities at food, of choice of resting places and of behaviour during routine colony management, were not entirely consistent. No single criterion could be used throughout the group of baboons to assess rank orders.

Although the hay feeding method of obtaining rank orders gave much data in a short time, it had many disadvantages:

(1) As described above the lower part of a rank order was not made clear because of passive avoidance on the part of the more subordinate animals. Many ponies were near the hay on 13 February 1967 (Table XV) other than those listed, but they stayed a few yards from it and did not either receive or deliver any threats.

(2) Some animals, usually the most dominant, remained near the hay until it was finished, whereas others left before that time. Some also

Table XV. Aggressive Interaction Between Ponies when Hay was Supplied

Threats delivered		Threats received (pony no.)					Total delivered	Rank	Age (years)
		18	70	63	29	35			
Pony no.	18		12	3	1	1	17	1	8
	70			11	5	1	17	2	5
	63				5	2	7	3	3
	29					2	2	4	4
	35						0	5	1
Total received		0	12	14	11	6	43		

Aggressive interaction between five ponies from different groups during a ½-hour period when hay was supplied, showing correlation between age and rank and that each pony delivered more threats to the pony immediately below it in rank than to the other ponies.

approached the hay sooner than did others so that a comparison of the number of threats received and delivered by each pony was not a true measure of its aggressiveness.

(3) A few groups never approached hay and they did not recognize it as food. They showed signs of alarm when they saw it and then quickly moved away.

(4) The number of interactions between group members was usually higher than that between ponies from different groups, because members of a group soon collected close together around any hay and because threats between members of a group were usually milder than those between members of different groups. Threats were also often milder between members of different groups that came into contact frequently than between those that only occasionally met. A pony reacted to a mild threat by usually only making a slight movement away from the aggressor and then soon moving back near it and the hay, whereas it reacted to a higher intensity threat by moving several yards away. Therefore a pony that received a mild threat from a group companion might soon receive several more, whereas a pony that received a higher intensity threat moved a sufficient distance from the aggressor so as not to immediately receive further threats from it. The individual distance of a pony was also usually greater when the 'trespasser' was a stranger, than when it was a familiar animal. On 26 September 1967 an adult mare repeatedly lunged out at and threatened to bite or kick a strange mare, her yearling filly and a yearling colt of the same group, whenever they approached within 10 yd of her, but she tolerated four mares from other groups well known to her within 2 to 3 yd of her. Such occurrences were not unusual.

(5) During a hay feeding period, the highest ranking ponies delivered more threats than ponies of lower rank, but a large proportion of threats delivered by each animal was directed to the animal immediately below it in rank. As shown in Table XV half the threats delivered by BH59 were directed to the two mares, 43 and 5, and nearly half of those delivered by 5 were directed to the mares 53 and 34, immediately below her in rank. Ponies 59, 43 and 5 were members of the same group, but even when all the ponies involved were from different groups, each still usually directed most of its threats to the animal just below it in rank (Table XV).

Apparent redirection of aggression was observed on several occasions. When a pony was threatened, it then immediately threatened a more subordinate pony nearby, which it had previously been tolerating at that distance.

There was no dominance by one group as a whole over another as all interactions were between two individuals. However, the presence of a very dominant pony in one group did deter whole other groups from approaching, so that the dominant pony's group was in effect dominant over others in situations such as the competition for hay. Thus when a group containing a very dominant mare approached some hay, other groups already there soon moved out of its way. Similarly a family group consisting only of a very dominant mare and her offspring was effectively dominant over groups of more subordinate mares, although when the offspring were separated from their mother they were threatened by these other mares.

The rank order of any one group of mares was a simple linear one (Table XVI), as was that when several groups were considered, but when a large number of animals from many different groups of a population was involved their rank order was complicated by various triangular relationships at any position in the hierarchy (Table XVII). Montgomery (1957) recorded one triangular relationship in his herd of horses but Grzimek (1944) found only a simple linear rank order in his two small herds. In Table XVII there are two triangular relationships both involving the two mares BH40 and 37.

Factors Governing Rank

The age of the ponies at first seemed to be of great importance in determining their rank and there was often a significant correlation between

Table XVI. Dominance–Subordinacy Interactions between Members of One Large, Stable Group of Mares, Showing Stability of the Hierarchy within the Group

Threats delivered		Threats received (pony no.) 22	21	19	16	20	47	Total delivered	Rank of mare	Age (years)
Pony no.	22		15	6	6	8	4	39	1	11
	21			11	16	8	5	40	2	7
	19				17	10	10	37	3	7
	16					15	6	21	4	7
	20						10	10	5	5
	47							0	6	2
Total received		0	15	17	39	41	35	147		

All threats observed between spring 1966 and spring 1968 are included in this table.

Table XVII. Rank Order

Age (years)	Ponies
12	BH 59
6	28
6	27
13	43
5	5
15	53
8	41
12	34
5	20
?	70
17	40
4	13
8	37
3	39
4	36
3	10
3	35
3	54
2	11

Rank order of nineteen ponies of one population showing correlation of rank with age in the lower part of the order, and two triangular relationships involving mares BH 40 and 37. 59=stallion.

age and rank in different populations, as for those ponies shown in Table XVII ($P < 0{\cdot}01$). However, this was most evident in the lower part of hierarchies (Tables XVII and XVIII) and

Table XVIII. The Rank Order, Age and Relative Body Size of Fifteen Ponies of One Population, Showing the Closer Correlation between Rank and Body Size than between Rank and Age in the Upper Part of the Hierarchy

Pony	Rank	Age (years)	Order of age	Order of size
BL 62	1	13	2	1
38	2	7	7	2
10	3	12	3·5	5
35	4	9	5	6
32	5	18	1	3
60	6	12	3·5	4
18	7	8	6	7
70	8	5	8	8
63	9	3	11	10
26	10	4	9·5	9
29	11	4	9·5	11
39	12	2	12	12
52	13	1½	13	13
35	14	1	14	14
6b	15	Foal	15	15

in the upper parts age was of less importance in determining rank. Thus there was no significant correlation between the two for the eight most dominant mares in Table XVII or Table XVIII.

Unfortunately weights of ponies were not recorded due to practical difficulties but the size of each pony of any population was estimated in relation to that of other ponies so that they could be placed in order of their size. This was obviously far from accurate as the orders given were based only on visible appearances and it was often difficult to decide which of two large animals was in fact the larger and heavier. Amongst young animals size was usually directly proportional to age and hence size was also directly correlated with rank. Amongst dominant ponies size was of more importance than age in determining their rank. For the eight most dominant mares in Table XVIII whose age was not correlated with their rank, there was a significant correlation between their size and rank ($P = 0{\cdot}01$). Similarly size and rank were correlated ($0{\cdot}01 < P < 0{\cdot}05$) for the eight dominant mares in Table XVII whereas age and rank were not.

Both Montgomery and Grzimek also believed that weight was the most important factor determining the position of a pony in the hierarchy. However, there were numerous examples of particular pairs of ponies where age did seem more important than size and weight. Thus the small old mare, BH40 (Table XVII) was dominant over a larger but younger mare, 37, and in many other groups a small old mare dominated a larger younger mare. This would occur when the young mares had been, since their birth or since they were immature, in the same group as the older mares, and when their original ranks in relation to one another had remained stable. The young mares had retained their initial subordinate position even though they were now larger than the older mares.

It was difficult to determine how large a part was played by the temperament of individual mares in determining rank orders. Neither Montgomery nor Grzimek considered temperament but in many cases size or age did not explain the high positions of some mares in hierarchies and these mares were often very aggressive; they commonly used the threat to kick, whereas other mares only rarely did so, and they usually had large individual distances at all times. Thus the very aggressive, old, dominant mare B17 (Table XIX) not only delivered over four times as many threats as did any of the other five ponies under observation, but nearly 25 per cent of these threats were threats to kick rather than threats to bite. Moreover, she rushed towards the other mares and threatened them when they were up to 10 yd from her, whereas other mares usually only delivered threats at hay feeds when a subordinate pony approached within 2 to 3 yd of them. Although size was correlated with the rank of the eight most dominant mares shown in Table XVII, when the five most dominant mares were considered, there was no such correlation. BH28, 27 and 5 were all comparatively young mares and BH5 in particular was a small mare and yet she was dominant over several larger, older mares. In numerous other cases small, young but aggressive mares were dominant over larger, older ones. In the triangular relationship shown in Table XVII BH37 dominated 39 but this 3-year-old, small but very aggressive mare dominated the old mare, BH40, who in turn dominated BH37.

The length of time that any mare had lived in a group or population was not important in determining her rank, as Montgomery recorded also. A very aggressive 5-year-old mare arrived in one area as a stranger in Spring 1967 and within 2 weeks she showed herself to be dominant over all other mares in that population.

In studies of dominance–subordinacy relationships of other ungulates, the closely related factors, weight, size and strength, were usually the most important factors determining rank. However, in reindeer, Espmark (1964b) found that age was the most important factor within each sex group, although size and strength were more important in determining rank between

Table XIX. Aggressive Interactions between Six Ponies during a Hay-Feed

Threats delivered	Threats received (pony no.)						Total delivered	Total no.		Age (years)
	17	5	2	6	3	4		Kicks	Bites	
Pony no. 17		12	40	7	16	5	90	22	68	18
5			11	3	5	1	20	1	19	10
2				6	1	7	14	0	14	9
16					0	1	1	0	1	3
3						0	0	0	0	2
4							0	0	0	2
Total received	0	12	51	26	22	14	125	23	102	

The very aggressive nature of the old, dominant mare is shown by the large number of threats that she delivered and also by the unusually large proportion of kick threats in this total number of threats.

the sexes. The presence and size of antlers in reindeer were also important in determining rank for when the antlers were cut from one animal, it quickly dropped in rank. Animals with small antlers were in general lower-ranked than those with large ones. Hierarchy in cows was similarly dependent on the size and shape of their horns and when a cow was dehorned she soon fell in rank (Woodbury 1941). When all cows in a herd were dehorned, strength and tenacity in pushing when in head to head combat were the most important factors. Strength was largely correlated with age but tenacity was not. Wagnon et al. (1966) also found that size was of importance in determining rank within a breed of cows but that it was not responsible for breed differences in dominance.

Position of Stallions in the Hierarchies

The factors determining rank as described above referred mainly to mares; data obtained about stallions were fragmentary because of their small numbers. In competition for food, such as hay, adult stallions were dominant over almost all mares. In one population of ponies a colt had been left with his mother after his second year. He was dominated by most mares older than himself in this third winter but in the following breeding season, when a 3-year-old, he was the only stallion present in the area with over thirty mares. He then showed a very sudden rise in dominance when competing for food and became dominant over the most dominant mare of that population until the next spring. A very aggressive mare, a year older than himself, then appeared in the area and quickly dominated not only all the mares but the young stallion too. Apart from this exception all stallions were dominant over all mares in food situations, whereas yearling and 2-year-old colts were treated as subordinates by adult mares as were fillies of the same age.

However, mares shown to be subordinate to a stallion when hay was provided, were observed threatening both to bite and kick him when they were in oestrus or when they had young foals whereas the rank orders of mares was similar in all observed situations. It is, therefore, perhaps better to consider separate hierarchies for mares and stallions rather than a mixed hierarchy, although data for relationships between adult males was insufficient to draw many conclusions; there was a maximum of only three males over 2 years of age in any population at the same time.

Adult males threatened immature and young colts in the manner described for mares but their reaction to other adult males was very different. Although more tolerant outside the breeding season, adult males usually not only threatened younger males but also chased them some

distance, often biting at their rumps and hind legs as they did so. These young males rarely fought with the older stallions and when the older stallions approached them, they usually quickly moved away as they were subordinate to them. Fights occurred between strange males of similar age, as between strange mares, but the stallions used their forelegs to strike at their opponents and they bit at each other's head, neck and forelegs, as well as kicking with their hind legs. Fights also occurred between stallions resident on the same area but they were usually very brief and were usually the result of a mare from the harem of one stallion moving close to the second stallion. There was often no evidence of complete dominance by one stallion over another on the same area as fights occurred throughout the breeding season, but the number of mares that each stallion kept together during this time was a measure of his strength. Large, strong and usually old stallions had large harems whereas young stallions on the same area as older males were either chased away or were allowed small harems of only one or two mare groups (see p. 177).

Thus although the small number of stallions prevented the formation of many conclusions about hierarchies amongst them, there was generally a stable rank order amongst the two or three males in any one population. This was especially evident where there was a large discrepancy in age or size between them. The dominant stallions, old and large animals, had larger harems than the subordinate males. However, many comparatively young stallions had large harems because they had no rivals due to the artificial distribution of stallions on the Forest by man.

Position of Castrated Males in the Hierarchies

Only two geldings were present on the study areas for more than a year. One of these, a very large animal, was dominant over all mares in the population, whereas the other, a 4-year-old in 1966, was only relatively higher in rank than mares of the same age. Both the geldings were larger and much heavier than mares of the same age. Montgomery (1957) also found that castrated horses tended to achieve a higher rank than mares and attributed this to their relative heaviness. In reindeer, castrates also achieved high ranks (Espmark 1964b).

Position of Foals in the Hierarchies

All foals of less than 3 to 4 months were at the bottom of the various hierarchies, although when a foal remained close to its mother it shared her status. When the mother was a dominant mare, the foal if near her was not threatened by mares subordinate to the mother, but when the same foal moved only several yards away, the subordinate mares did threaten it. No evidence was obtained however, to suggest that foals or older offspring, when near their mothers, ever threatened mares that they would not threaten when they were separated from their mothers.

During their first few days of life, foals frequently showed threat postures when other foals approached them. They laid back their ears as in the threat to bite and also turned and kicked out at the approaching animal, even when it was several weeks or months older than themselves, but they rarely threatened ponies older than this. When a mare threatened a very young foal, the foal did not react by immediately moving away; instead it looked up at the mare and snapped. Foals older than a week, usually avoided the bites and kicks of mares by quickly moving out of their way, although they also usually snapped. No evidence was obtained from this study that snapping inhibited mares from carrying out their threats. A mare to whom a foal snapped, usually continued to threaten the foal until it moved away or she bit or kicked it (see p. 144). Stallions however, seemed more tolerant of foals.

No stable hierarchy existed between young foals. Fillies were more aggressive than colts in that they bit and kicked out more than did colts, but either of any two foals was often observed threatening the other. A more stable relationship

was developed by the foals' first winter but changes were not infrequent after this.

As in rhesus monkeys *Macaca mulatta* (Koford 1963), there was a tendency for offspring of high ranking mothers to achieve high rank. During a hay feed an 8-month-old filly of a very aggressive dominant mare threatened ponies not only a year older than herself but also several 2 years older and one who was 3 years older. Most of these older fillies returned the threats and the young filly then moved out of their way on further encounters, but three of them avoided the young aggressive filly and remained subordinate to her. All these interactions occurred when the filly was separated from her mother; when near her during hay feeds, she gained much higher status. When a small amount of hay was provided in one pile on a grazing area where up to forty animals were present, the only stallion, the two most dominant mares who were group companions, and the daughter of one of them, the aggressive foal, were the only ponies that actually ate any of it.

Another filly foal of a dominant mare also proved dominant over a yearling when she was only 6 months old. It is not known whether this tendency for offspring of dominant mares also to achieve high rank was due to inheritance of an aggressive temperament or to the fact that other ponies in the population learnt to avoid a foal when it was near its dominant mother and then continued to avoid it when it was separated from her. There was also the possibility that foals learnt to be aggressive, having repeatedly watched their mothers bite and kick other mares. However, there were many exceptions to the tendency, such as in a small group of two adult mares and their two yearlings, where the yearling of the subordinate mare was dominant over the yearling of the dominant mare. Although the ranks of these two fillies was the reverse of that of their mothers, the following year another yearling daughter of the dominant mare was frequently seen threatening the subordinate mare's daughter who was then a 2-year-old.

Mares sometimes aided their foals, when the foals were threatened by other ponies. This was most usual when the foals were only a few days old, but on several occasions mares aided older offspring. When a 10-month-old filly threatened to kick a younger filly, the mother of the latter rushed towards them from over 10 yd away and immediately threatened the older filly. Mares whose colt foals had approached adult stallions also seemed anxious; they nickered and moved towards them.

Stability of the Hierarchy

It was not possible to place every mare in a position in a hierarchy as many groups led a fairly solitary existence, others would not approach hay and interactions between particular pairs of ponies were not observed. Of those mares, whose rank was known from work done in the winter of 1965 to 1966, few changed in rank up to 1968; and these were all young animals. Several kicking fights were recorded between mares who had previously peaceably co-existed and were thought to indicate that one of the mares on each occasion was attempting to rise in rank. One spring a 4-year-old and a 2-year-old mare were seen fighting twice in one day. Before that day no fights between them had been recorded and no further fights after that day; the older mare remained dominant.

No changes in rank were recorded within any group of mares, even within the few large groups. In one large group of five mares, no mare that was found to be subordinate in March 1966, ever subsequently threatened a dominant mare up to March 1968 (Table XVI).

The extreme stability of the hierarchies of adult ponies was no doubt largely due to the fact that a subordinate mare very rarely challenged a previously established more dominant mare. Both of the triangular relationships in Table XVII involved the mares BH40 and 37. If mare 37 had been dominant over mare 40, the order would have been linear, as other mares, who were younger but larger or more aggressive than 40, were dominant over her, whereas 37 was dominant over them. Unfortunately the old

mare 40 was removed in the autumn of 1967 so any further observations on the relationship between 40 and 37 was prevented; however, in the eighteen months of observation until then, 37 had always moved away when threatened by 40 and she had never returned a threat. This behaviour on the part of subordinate ponies, who almost always moved away from previously established dominant animals when threatened by them, or who often avoided them without even being threatened, supports the conclusions of Rowell (1966). She suggested that a hierarchy is maintained, or expressed chiefly by subordinate activities and that it is the low ranking animals that do most to perpetuate rank distinctions.

Dominance–Subordinacy Relationships with Other Animals

Cows were the only species of mammal with which ponies were observed freely interacting. Adult ponies were dominant over all cows and bullocks although many of the cows were horned and much heavier. Ponies threatened to bite and kick cows in the same manner as when threatening other ponies. Young ponies, especially foals, did however, avoid approaching cows although they frequently approached and chased calves.

Only one aggressive interaction was recorded between ponies and fallow deer; a yearling filly threatened to kick a doe who was grazing near her and the doe quickly moved out of the way. A 10-month-old pony filly also kicked out at two full-grown donkeys who were grooming near her.

Leadership

When ponies were grazing on a small area for several hours, movement was usually unorganized. Members of a group tended to drift apart so that contact had to be renewed at intervals by one or more ponies looking up at other group companions and walking purposefully towards them. Temporary separations, where contact calls had to be made by one animal before it became re-united with the rest of its group, were frequent.

When ponies left the open day-time grazing areas in the evenings for the valleys or woods, when they left the valleys and woods in the early mornings and when they moved to the shades or drinking places, they sometimes moved as much as a mile without grazing. These movements were more integrated and were initiated by any member of a group, even by an immature animal. This animal began to walk purposefully away from the rest of its group, whereupon the other ponies usually soon began to walk after it. Occasionally one or more ponies did not see their group companions move away but they usually looked up after several minutes and then trotted or galloped after them. Walking was the normal gait when ponies were moving long distances but especially when they neared their day-time grazing areas, when they moved down into valleys and when they were moving to shades they often trotted, cantered or galloped, even bucking and kicking out with their hind legs as they did so.

Usually a group of ponies walked in single file, both when on the narrow tracks that they had made over the heaths and around the edges of valleys and also when on tracks wide enough to accommodate several side by side. Young foals however, did often walk alongside their mothers. The order of the file in a group of ponies was not always constant but some patterns did emerge. When the ponies were walking some distance, the pony leading initially, if a young animal, soon stopped until a more dominant animal had overtaken it. In small family groups, the mother invariably led with her offspring following behind her in their order of age. Foals were alongside or immediately behind their mothers, followed by yearlings and then 2- to 3-year-old or older offspring. A similar order was observed in steppe zebras (Klingel 1964a, 1965). In groups of two adult females and their offspring, this pattern was still usually shown. The dominant of the two mares led with her offspring behind her and then the subordinate mare followed with her offspring. This was illustrated by the usual order of movement observed in a group

in 1967 where the dominant mare, BH23, had a yearling, 23A, and a 2-year-old daughter, 24, and the subordinate mare, 25, had a foal 25B, and also a 2-year-old daughter, 26 (Tables XII and XIII, Group C). BH23 invariably led, followed by 23A and then 24. The subordinate mare, 25, walked behind this family, followed closely by her foal and finally by 26, bringing up the rear.

Even in larger groups, the order followed by adults was commonly that of their order of dominance, with immature animals immediately behind their mothers and when the leading mare paused, the whole group often also paused, until she recontinued. However, this ideal order was often upset, particularly when the distance moved was short and when the group was moving quickly.

When ponies were alarmed members of individual groups collected together in a bunch. Young animals quickly rushed to their mothers, who also moved towards their young, often nickering as they did so, and subordinate mares moved towards more dominant mares of their group. The group members then stood close together with their heads held high and ears forward, looking at the source of danger; if this was near, they soon all galloped away alongside one another with no obvious leader. Such behaviour was only usually observed when riders on horseback quickly approached ponies; in the autumn the ponies are rounded up by men on horses and it was particularly after the annual round-ups that they showed signs of alarm when they saw approaching horses.

In groups with which a stallion was associated, the stallion rarely led, but more often brought up the rear of his group. Because any stallion was not usually allowed to remain in the same area for more than 2 to 3 years, he cannot at first have known the area as well as did the mare group with which he became associated. Moreover, the mare group was a stable unit before his arrival, with the dominant mare as its leader. After he had joined the group, the dominant mare still led and he followed the group. In one case, a stallion that had been closely associated with a mare group for 2 years, was occasionally seen leading it in his second winter with it but another such stallion was never seen leading his group. From the small number of cases where stallions were associated with mare groups and because of the unstable nature of these relationships due to man, it was not possible to conclude whether the stallion as the dominant member of his group was the leader but it was thought that this was not so and that the dominant mare was in fact the leader.

In many old studies of semi-wild horses, zebras and asses, the male is stated to be the leader (Lydekker 1912; Alverdes 1927) but it was not always made clear whether the male actually led his group from in front or whether he drove it from behind. Driving of mares by a stallion cannot be equated with leadership. Driving in the New Forest ponies (see p. 177) occurred mainly at the start of the breeding season when stallions were establishing their harems, after rival stallions had fought, and when mares were in oestrus. Stallions then drove one or more mares but the mares often determined the direction of movement. During the winters and during normal grazing movements, driving did not occur and leadership was usually taken by mares. Klingel (1964a) observed that in steppe zebras, a mare usually led her group with the other mares following in their order of dominance and that the stallion of the group either brought up the rear or walked parallel to the mares, and only occasionally led. The stallion's position at the rear of his group would presumably be the best position for its defence if the group was moving away from the source of danger.

Social Facilitation

Social facilitation was described by Thorpe (1963) as contagious behaviour where the performance of a more or less instinctive pattern of behaviour by one animal acts as a releaser for the same behaviour in another or in others, and so initiates the same line of action in the whole

group. This is distinct from imitation, a type of social learning, which involves a new pattern of behaviour.

Social facilitation was a common phenomenon both between members of one group of ponies and even between members of different groups. The pattern of behaviour involved included resting, grazing, walking and other types of movement, rolling, eliminative behaviour and sucking.

Resting

It was often observed that all or most members of a group rested in close proximity and at the same time, and that all members of one or more groups often rested at times when no other visible ponies in that population were resting. From observations throughout the hours of daylight when the length and time of rest periods of a number of ponies were recorded, co-ordination of resting within a group and also between groups that were close together in space was evident (see p. 101).

Social facilitation of resting between groups perhaps partly accounted for some of the observed peaks of resting activity, which could not be explained by the appearance or presence of the sun or by other weather conditions.

Grazing

Social facilitation of grazing occurred as described above where one member of a group began to graze after a resting bout and other members soon then did likewise.

Movement

There was co-ordination of purposeful movements such as up from or down to valleys, to shades or to drinking places as described on p. 130 rather than of the general grazing drifting movements. One pony usually initiated movement and other group members then followed.

Rolling

Social facilitation was most evident when one pony rolled; one or more animals often stared at the rolling pony and then also rolled. On most occasions only two animals were involved but on three occasions four ponies, which had been grazing together, rolled in quick succession during observation periods of over 6 hr. No other ponies were observed rolling during these periods. Once one pony rolled and then immediately four others rolled; all but one of these five animals belonged to the same group.

When a group of ponies encountered a sandy track or a pile of sawdust, all the members sometimes rolled, but the rolling by several animals as described above was not attributable to some particular characteristic of the ground. Occasionally when a pony rolled, another moved to the same spot where the first had rolled and then did likewise but it was more usual to see a pony roll and then one or more others look up and roll where they were already standing. This was thought to be due to social facilitation.

Elimination

Ponies frequently reacted to urine or faeces of another pony by sniffing it and then either urinating or defaecating on it (see p. 106), but it was also observed that when one pony urinated or defaecated, one or more others who looked up as it did so, then also did likewise. Sometimes the ponies involved were close together, often walking in single file, and there was the possibility that it was the smell of the urine or faeces of the first pony that caused the others to react by urinating or defaecating, and that this behaviour was the same as that seen when a pony sniffed at a pile of faeces or at urine on the ground. However, the ponies involved were often many yards apart and it was unlikely that they could smell the urine or faeces. Moreover, when mares sniffed faeces on the ground they then urinated more commonly than they defaecated (Table VI) whereas when mares saw another mare defaecate, they invariably defaecated. Similarly when mares sniffed urine, although they usually reacted by showing flehmen posture and urinating, they did sometimes defaecated (Table VII), whereas when they looked up at a mare urinating, they were never observed to defaecate; nor did

they show flehmen posture, which suggested that it was not the smell of urine that they reacted to, but the actual sight of the mare urinating. Thus the behaviour of ponies who looked up at other ponies defaecating or urinating provided another example of social facilitation.

Sucking

On numerous occasions when one of two foals that had been playing, grazing or grooming together, moved to its mother to suck, the other foal stared at its companion sucking and then moved directly to its own mother and also sucked. On one occasion two filly foals had been grooming and resting together for nearly an hour, when one sucked from her mother, who was nearby. The second filly stared at the mother and foal and then instead of moving to her own mother, who was out of sight, 'sucked' the nipples of the first filly for over 2 min (see p. 157).

Home Ranges, and Diurnal and Seasonal Movements within Them

Introduction

Aggressive interactions only occurred when one pony came within the individual distance (Burkhardt 1944) of another and no evidence of territorial behaviour was observed between groups. Each group had a home range, the restricted area over which it normally travelled in pursuit of its routine activities. Burt (1943) originally defined home range as the area normally traversed by an individual in its day to day activities of food gathering, mating and caring for its young. It is limited and constant for a given period of time and does not include areas covered on occasional long forays of a possibly exploratory nature.

The home ranges of the pony groups were recorded by plotting the positions of all known ponies on sketch maps whenever any part of the study area was visited. This method was used throughout the 3 years of study and it showed any changes in the home range of a group as well as any seasonal shift within the home range. Diurnal movement within the home range was also recorded by plotting the positions of certain groups at regular time intervals during a number of days at different times of the year.

Although it was sometimes difficult to draw boundaries for the home ranges of certain groups because of scattered outlying points, many of these points resulted from disturbance of ponies such as by buckhounds and by round-ups. Several mares in oestrus also occasionally moved outside their normal range when no stallion was present (see p. 116). The completed maps of home ranges include all points except those resulting from obvious disturbances and abnormal ones such as made by a few oestrus mares. Boundaries and areas have been used to describe the home ranges of ponies, as for blacktailed deer (*Odocoileus hemionus colombianus*) (Dasmann & Taber 1956) and coatis (*Nasua narica*) (Kaufmann 1962) because they give a truer picture of the ranges than would the use of an activity radius. The use of this was first suggested by Hayne (1949) and developed by Dice & Clark (1953) and Calhoun & Casby (1958). It is used to express the home ranges of small mammals with indefinite boundaries and is based on the distance of recapture from a recapture centre. The use of boundaries and areas and of an activity radius to describe home ranges was discussed by Kaufmann (1962) and Jewell (1966) has discussed methods of recording home ranges.

Size of Home Ranges

Home ranges of different groups often coincided with or considerably overlapped each other and varied in size from about 200 to 2500 acres (82 to 1020 ha). The presence of a restricted number of favoured grazing areas resulted in separate populations of groups, each population centred around a reseeded area or lawn during the day. Only a few others moved regularly between two populations. In the larger study area there were six populations, two centred on reseeded areas, two on streamside lawns, one

on a large woodland clearing, Balmer Lawn, and the sixth on a grass heath. Some groups ranged widely over heaths or along roadside verges for much of the time. In the second study area there was one population centred on the reseeded area. The groups of any one population tended to have very similar home ranges.

The size of the home ranges could not be correlated with the size of groups. Topography and the nature of the habitat were the most important factors in determining home range size. Within any home range there were four requirements: a grazing area, shelter, water and a shade. When these four requirements were present close together, the home range was small. Ponies of the Balmer Lawn population all had small ranges of about 200 to 400 acres (82 to 164 ha). The large woodland clearing provided their main grazing area; the surrounding woods provided shelter and food, especially in the winter; a patch of concrete by a building south of the clearing provided shade, and water could be obtained from a pond or ditches on the clearing or from a river close to it. This small area almost wholly supported over forty adult ponies (Fig. 13). In the woodland to the north of it, there were a number of small clearings to provide grazing and one group there ranged over 800 acres (330 ha); it moved distances of over 2 miles, but its home range was not as large as it might have been because of the Forestry Commission enclosures which limited its movement in certain directions. In other parts of the study areas there were large tracts of heathland between the main grazing area and the valleys or woods; and home ranges of ponies using these were subsequently larger than those where shelter and grazing were side by side. The

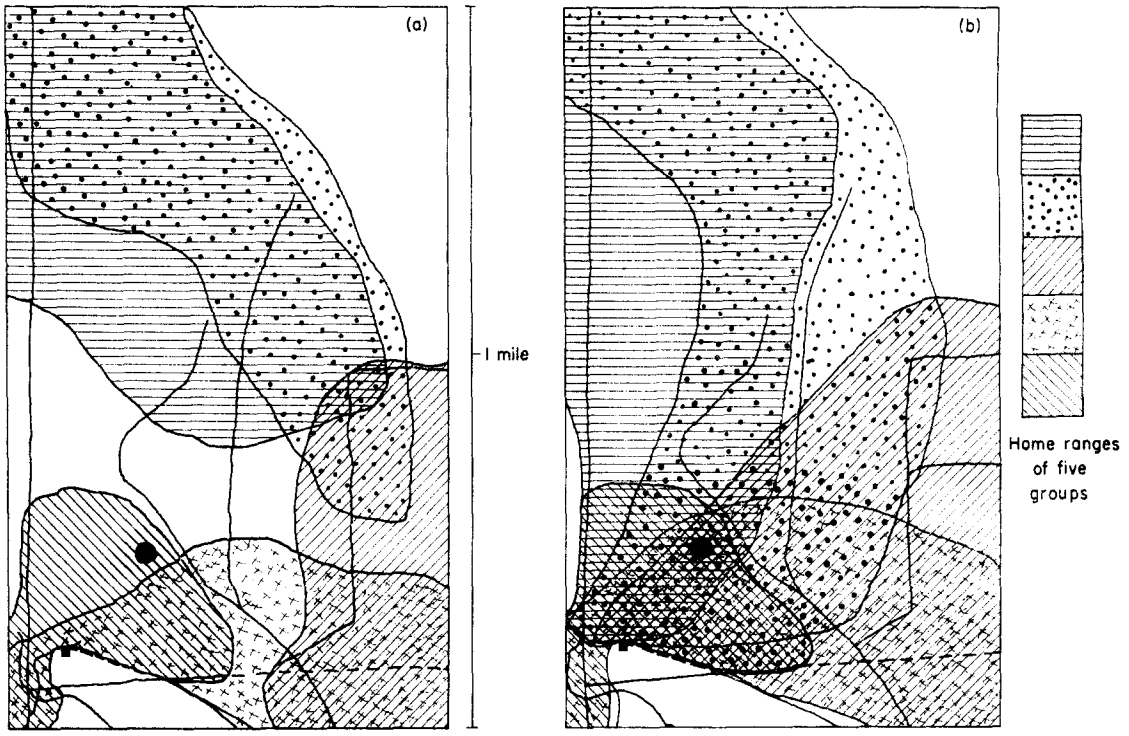


Fig. 13. Parts of the home ranges of five groups in winter 1965 to 1966 (a) and in summer 1966 (b) to show the greater degree of overlap of the ranges in the summer. The circular black patch is a pond, and the small black square is a building by which ponies 'shaded'.

largest recorded home ranges were in the second study area where the grazing was found on a reseeded area on a hill top, where the ponies also shaded. Shelter and water in the summer were found in the valleys and woods, some distance over the heaths. In the summer over ninety ponies concentrated on the reseeded area but less than half this number regularly grazed it in the winter daytime. The small amount of food to be obtained there in the winter resulted in many groups moving up to 2 miles away and grazing in other valleys or woods and along roadsides. The home ranges of these groups were therefore large, 1600 to 2500 acres (655 to 1020 ha).

The effect of population density on home ranges was not determined.

As previously mentioned the home ranges of different groups freely overlapped and there were no monopolised zones, areas within the home ranges exclusive to one group but not defended by it, as described in Soay sheep by Jewell (1966). However, there were generally one or more core areas joined by tracks within any home range. These core areas were ill-defined areas that were used more frequently by a group than were other parts of its range (Fig. 14) but the core areas of different groups also overlapped.

Although the home ranges of different groups in one population were often almost identical, with each group using the same grazing area and the same valleys and woods at night, there was a tendency for each group to use a slightly different part of the grazing area (Fig. 15). This was more evident during the winter than in the summer.



Fig. 14. The range used by a mare and her daughter in two successive winters (1965 to 1966 and 1966 to 1967), showing a 'core area' where ponies were most frequently to be seen. Each dot represents the position of the mare on different days between dawn and dusk. Cross-hatching represents woodland.

Seasonal and Diurnal Movements within the Home Ranges

Seasonal changes. Some groups, especially those with small home ranges such as the groups of the Balmer Lawn population, used the whole of their home ranges in the winter and summer; although they spent different proportions of time in different parts of it during these two seasons. More commonly the summer and winter parts of the home ranges overlapped but were not identical (Fig. 13). Certain areas of the home ranges were often only used for 2 to 3 months from July to September when ponies were using the shades; other areas were only used in late autumn when grazing was becoming limited. Several groups ranged widely during October and one group at the end of September in both 1966 and 1967 moved through woodland to outlying parts of its home range, that were not frequented at other times of the year. However, in general the winter ranges were smaller than the summer ranges, when shading movements often greatly increased a group's range.

Stallions, which were closely associated with mare groups, used the same winter ranges as

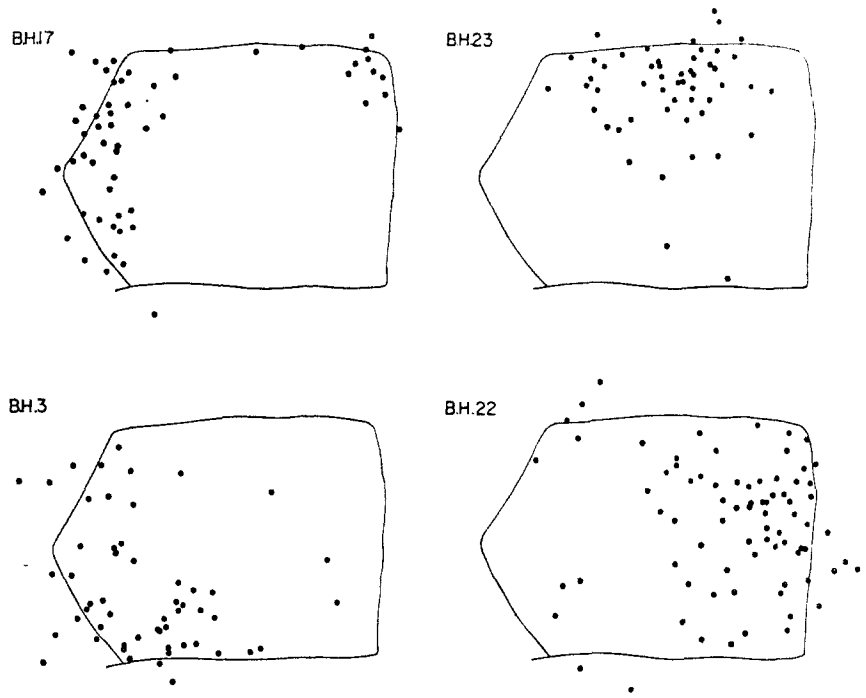


Fig. 15. Positions of representative ponies of four groups on reseeded area (Backley) in winter 1966 to 1967, showing that although there was much overlap, different groups tended to use different parts of a grazing area.

their mare groups but in the summer they ranged more widely. They then spent much time with mares belonging to other groups.

Diurnal changes. Groups that used a similar range throughout the year, centred on a particular grazing area in the morning and left in the afternoon or evening. The time of the morning return and evening departure varied not only with the time of the year, weather conditions and nature of the grazing area but also with the different groups using the same area.

In the winter the morning return began at or soon after dawn and continued throughout the morning; the evening departure similarly continued for several hours. It was latest from the streamside lawns and woodland clearings, which provided the bulk of the food for animals ranging over them, and much earlier from the reseeded areas, which only provided a small proportion of the winter food for ponies using them. The departure from these reseeded areas began in early afternoon and was usually completed well before dusk, whereas ponies were often still on the lawns and clearings after dusk. In the spring the departure from all these areas gradually became later.

The time of the morning return in the summer was consistently later on the reseeded areas than on the clearings and lawns. The earliest time recorded for a group on the former in mid-summer was 05.45 hours, several hours after dawn. Moreover, the time of the evening departure was earlier from the reseeded areas than that from the clearings and lawns. Most groups had left the reseeded areas by 19.30 hours in May and often as early as 15.00 hours in July and August, when they had to move down to the valleys to find water. Ponies were frequently still grazing on the clearings and lawns after 21.30 hours but these animals were able to drink during the day from nearby ponds, streams and ditches.

Therefore throughout the year ponies spent

less time on the reseeded areas than others did on the lawns and clearings, but not all groups grazed in the open during the day and in woods or valleys at nights. Some showed this behaviour only in the summer, whereas others grazed over heaths, in woods and along roadsides throughout the day and night in all seasons.

Weather conditions had a great effect on the times of arrival and departure from the open grazing areas. Very strong winds or heavy rain prevented or delayed ponies from moving out into the open, or if they were already there, caused them to quickly leave. This was observed at all times of the year.

The same group was often the first group to arrive on a grazing area in the morning for a period of time, similarly another group consistently was the last to leave in the evening. On 10 days of observation during the winter 1967 to 1968 there were significant differences in the times of arrival in the morning of one group and those of three other groups in the same population ($0{\cdot}02 < P < 0{\cdot}05$, and $0{\cdot}01 < P < 0{\cdot}02$); similarly there were significant differences between the times of the evening departure of one group from those of three others in the same population ($P = 0{\cdot}02$, $<0{\cdot}01$ and $<0{\cdot}01$). The group that was usually the first to arrive was not the last to leave. However, there was much variation in the times of arrival and departure in the same group at any season.

Many groups followed a very constant pattern of diurnal movement and could be found at the same part of their home range at the same time each day for several days, weeks or months. Thus although groups using the same grazing areas tended to occupy different parts of them, each group usually also used a different part at different times of the day (Fig. 16). A group of two mares, BL17 and 19, spent the nights in scrub and woodland south of a large clearing. After dawn they soon crossed a track onto the clearing and grazed on that during the day, gradually drifting north as far as a clump of oak trees and then slowly south again during the afternoon. This pattern was maintained for

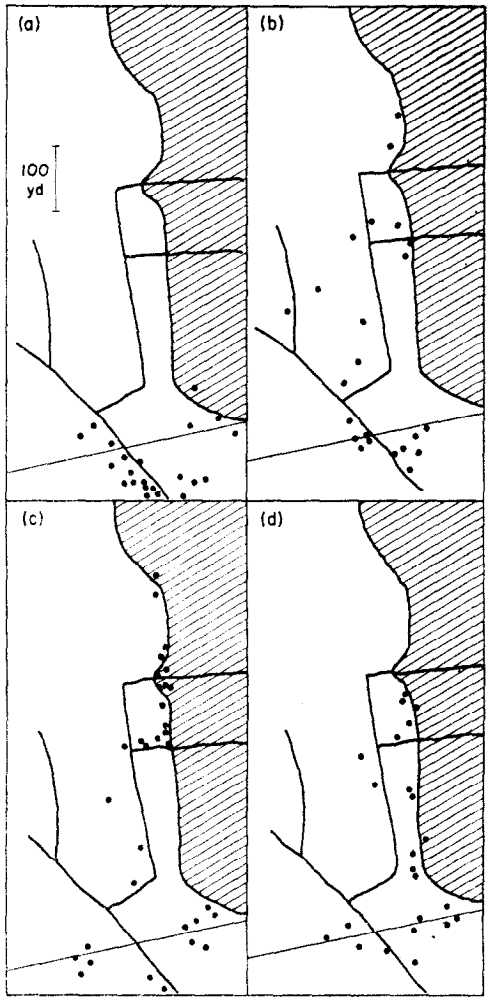


Fig. 16. Positions of pony (BL17) in winter daytime 1966 to 1967. (a) 05.30 to 08.30; (b) 08.30 to 11.30, (c) 11.30 to 14.30, and (d) 14.30 to 17.30. The constant pattern of diurnal movement which was maintained for nearly 3 months in the winter by this pony who tended to use a somewhat different part of her home range at a different time each day. Positions of ponies were checked mainly during the middle part of these time periods.

nearly 3 months in the winter of 1966 to 1967 until the end of February when the group began to spend much time in woodland to the north west of the clearing. Wind and rain affected the time that the two ponies spent grazing on the clearing.

Other patterns of movement were more short-lived, lasting only for a few days at a time. In the population on the second study area, any

group which had a choice of three valleys, often moved down into the same valley in the evenings for several days or even weeks, and then one evening suddenly moved down into a different valley.

In the summer different groups of any one population usually followed a more co-ordinated pattern of movement than the independent group movements shown in the winter. Those groups centred on the large woodland clearing, Balmer Lawn, moved onto the lawn soon after dawn from different directions from the surrounding woods. Most groups then concentrated in the east-centre of the lawn where they grazed until flies drove them quickly along well defined tracks to the pond and then to the communal shade at about 09.00 to 10.00 hours. Movements were made out from the shade at intervals during the day to drink from a nearby river or from another smaller pond near the shade, and also to graze. These excursions were usually brief and ponies soon returned to the patch of concrete where they shaded. In the late afternoon most ponies finally left the shade and moved back onto the lawn, usually all to the south and south east part of it, where they grazed until dusk or several hours after dusk. Then the various groups moved off in different directions towards the woods and scrub at the edge. This pattern was only seen on hot, calm days; on other days the ponies reverted to their more independent winter pattern (Fig. 17).

Stability of Home Ranges

Home ranges were remarkably stable during the 3 years of study and only two complete shifts in range were recorded. One of these involved a mare who had been turned out onto a strange area in the late summer of 1965. Her home range recorded during the winter of 1965 to 1966 differed from that recorded for the rest of the study period. In the second case a mare left her group companion with whom she had been associated for over 2 years and moved in the autumn of 1967 to an area adjoining her original home range. She was not seen again during 1967 or 1968 in this old range and there was no obvious reason to explain her move. All other disappearances of mares from their usual areas were found to be due to their removal or interference by man.

Slight annual shifts in home ranges were more common. Some of these may have been due to lack of observations so that a group was either seen in a new area one year where it had not been seen the previous year, or that it was not seen in an area one year where it had been seen the previous year. However, not all the shifts were due to lack of observations. One permanent extension of a home range was brought about by a stallion. Between autumn 1965 and spring 1967 a young mare was always seen with three other mares in a range centred on a reseeded area. There was another reseeded area adjoining the first but separated from it by a stream and boggy wood or carr. The few groups that moved between the two areas either crossed by a bridge or further downstream where the carr ended, but only four groups did regularly graze on either side of the stream and the two populations on the two reseeded areas were otherwise separate. The young mare never crossed the stream until Spring 1967 when a stallion, that had been freshly turned out, chased her relentlessly until finally she crossed the bridge. During the next week she had not returned to her original group and range, and she soon established a close relationship with another young mare on the new area. She remained associated with this mare for the rest of the study period and although she occasionally grazed on her original home range, she spent most of the time on the second reseeded area and the heaths and bogs surrounding that.

Another mare also began to use the grazing areas on both sides of the carr during the study period. Until she foaled in 1966 she was part of a small family group, but she left that after her foal was born and increasingly used the other of the two reseeded ares. During 1967 and 1968 she was very rarely seen on the original reseeded area. However, this was not a complete shift in

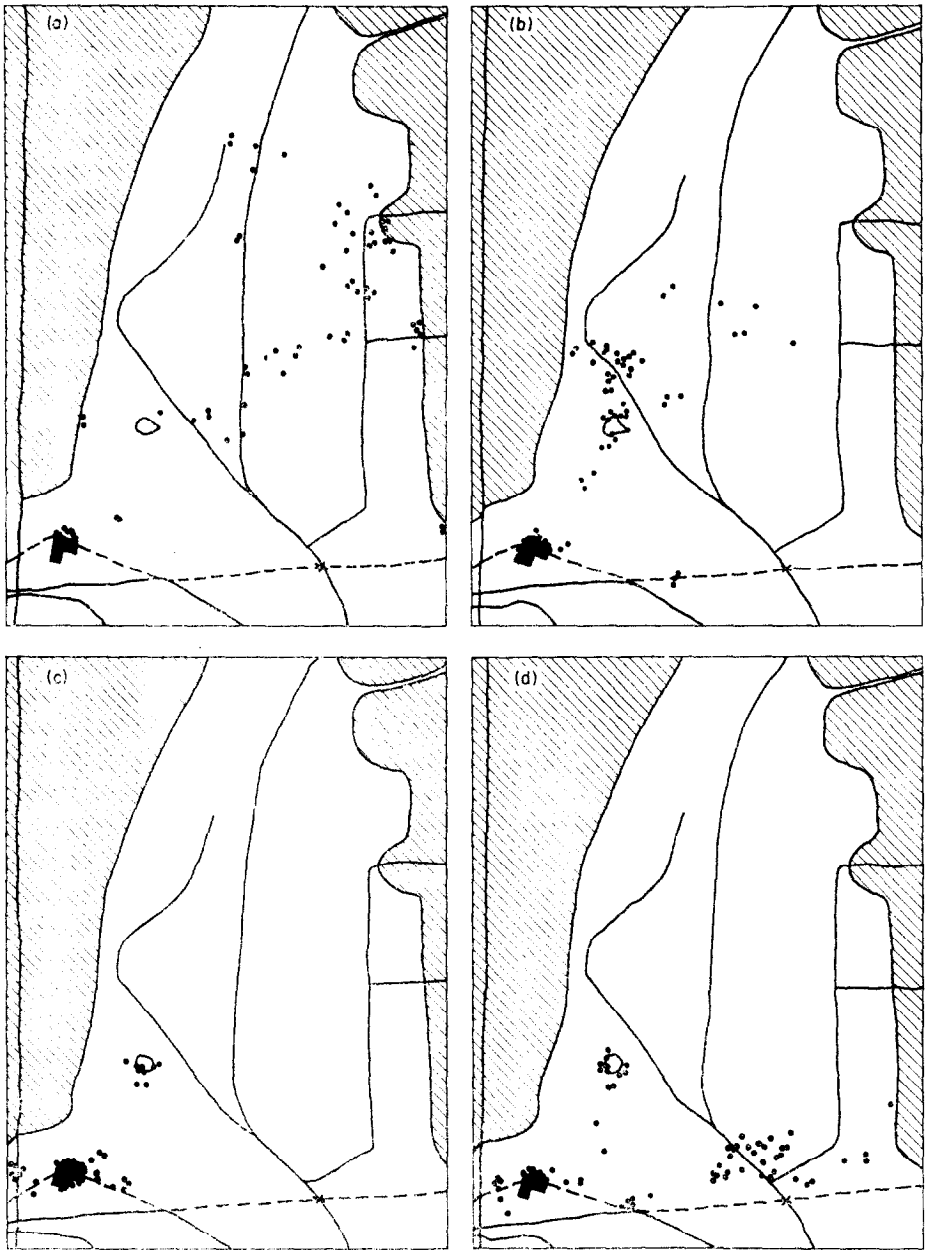


Fig. 17. Co-ordinated pattern of movement made by members of many different groups in the summer (23 June 1967) on Balmer Lawn. (a) 08.00, (b) 10.30, (c) 13.00, and (d) 15.30. Each dot represents a pony. Areas with diagonal lines are woodland. The circular patch in the south-west is the pond. The shaded block in the same area is the large building by which the ponies shaded.

home range because she still grazed further downstream on a 'lawn' where she had originally also grazed with her mother and sibling and occasionally during 1967 and 1968 she was seen with these two mares.

Several other real extensions of home range occurred with the new and old ranges overlapping considerably. Other annual changes in home ranges did not represent real changes in their extent but only preferences for different parts of it in different years. The home range of one mare included a reseeded area, a roadside and the heaths and bogs between the two. During 1966 the mare was almost invariably to be found grazing on the reseeded area during the day, and only occasionally on the roadside, but in 1967 she spent most days with her foal grazing along the roadside and correspondingly few on the reseeded area.

Homing Ability

There were numerous instances of the homing ability of the ponies during the study period. A 3-year-old mare was removed from her group companion and kept with a strange mare in a field during one winter. In the spring these two mares were released and a few days later were both seen in the original home range of the strange mare, nearly 5 miles from the field. However, within 2 weeks the young mare had returned to her original group companion and home range, which surrounded the field.

Other cases of homing occurred after stallions were taken from their home ranges and moved to new areas, where they could not serve their own offspring. Such stallions sometimes re-appeared in their old ranges, as did one stallion in the first study area. He was removed in two successive years but each time quickly found his way back. Another yearling colt was taken 4 miles from his mother and her range; he was castrated and then released and 5 days later was seen back with his mother. Other cases have been related by various owners, whom there were no reasons to doubt, of animals returning to their old home ranges within 2 to 3 days from spots of release over 12 miles away. However, not all ponies returned to their original ranges and Grzimek (1943) found no evidence for homing in horses, but he used thoroughbreds as his experimental animals, whose home ranges were only a stable and field.

Although many New Forest ponies showed great homing ability, no evidence was gained from this study how the homing was brought about. Some ponies may have found their old ranges by trial and error but the short time that they often took, seemed to rule this out and to indicate that they took a more direct route. In some cases, such as that of the 3-year-old mare mentioned above, the animal could simply have retraced its steps, using both visual and olfactory stimuli. This could explain the instances of homing where ponies were chased by men on horseback to new areas, but many ponies were removed in horseboxes and driven by road to their release points. Many were also taken to smallholdings and farms and kept in fields for up to 5 months during the winter months. After their release in the spring, some quickly returned to their old home ranges but visual and olfactory stimuli can then have been of little importance.

V. COMMUNICATION BETWEEN PONIES

Vocalizations

No tape-recordings or detailed analyses were made but four very different vocalizations were recognized in the field: (a) the snort, (b) the squeal, (c) the nicker, and (d) the whinny. Dr M. Kiley (personal communication) recorded these same four vocalizations in a short study of Camargue horses. From the Spring of 1967 all vocalizations made by the ponies were recorded by noting the type, the circumstances and the class of pony making the call.

The Snort

Snorts were made only by stallions either when they were fighting or when they approached mares in oestrus. They trotted or galloped quickly and excitedly towards such mares, with their ears forward and tails swishing, and snorted as they did so.

The Squeal

This was a high pitched cry of short duration, most commonly made by mares when stallions approached and sniffed them. These mares were usually in oestrus but non-oestrous mares, when approached by stallions, also squealed. At the same time, the mares usually raised a foreleg and pawed the air.

Squeals were also made by mares which were fighting, sometimes by dominant, aggressive mares when they were kicking out at other animals and less frequently by stallions when fighting. A mare, who was lying down, also sometimes squealed when her foal repeatedly pawed at her until she stood and allowed it to suck.

The Nicker

Both the nicker and whinny were used in a much wider range of circumstances than were the snort and squeal. They were chiefly used as various types of contact calls but the nicker functioned as an alarm call and was also directed towards approaching humans by those ponies accustomed to being hand fed, as in domestic horses (Hafez et al. 1962). It was a low-pitched guttural noise and 518 calls of this type were noted, in the following circumstances:

(1) By mares to their first day foals whenever the foals moved away from them. A foal showed little response to the nicker on its first day, but its mother usually approached her foal when she nickered. Thirty-four such calls were recorded.

(2) By mares to older foals when they were out of sight for some time or grazing some distance away. The foals usually responded by looking up and by often nickering and then continued grazing. The mothers sometimes nickered again and then also continued grazing. Similarly foals often nickered first, their mothers replied and then the foals made a second nicker. This also occurred when one group companion was apart from another; and when a foal or group companion was separated for a long time from its mother or the rest of its group. However, when a pony was 'lost', it whinnied rather than nickered, or the vocalization made was intermediate between a whinny and nicker.

Two hundred and five nickers were made by ponies who were apart from but within sight of group members or mothers, or temporarily separated from them; thus 40 per cent of all recorded nickers were made in these circumstances. Eighty-one of the 205 nickers received replies (39 per cent) and in twenty-four cases, the first pony made a repeat nicker.

(3) The nicker was also used in reply to a whinny (forty-five cases).

(4) Ponies, when approaching group companions, or foals, when approaching their mothers after a period of separation frequently nickered, and the group companions also sometimes nickered when they saw the approaching animal. Foals usually sucked after a period of separation from their mother so the nicker was a frequent preliminary to a sucking bout. This occurred in ninety-two cases, and in sixty-four

other cases the nicker was recorded when a pony approached its mother, its foal or another group companion.

(5) Any adult or immature pony occasionally nickered when they saw a strange animal or object. This occurred when a mare saw a newborn foal other than its own especially early in the breeding season, or a mare actually foaling and when a young foal saw a calf or human for the first time (thirteen cases).

(6) When stallions were showing interest in or copulating with mares, the young foals of these mares sometimes nickered and showed signs of alarm. Young foals also sometimes nickered when other mares approached their mothers. Sixteen such calls were recorded by foals in these circumstances.

(7) When mares saw approaching horses with riders, or when mares saw humans or dogs approaching their young foals, they frequently nickered. This seemed to act as an alarm signal as group members quickly bunched together and the foals rushed towards their mothers when they heard the call, even if they had previously been lying down. Nickers, used as alarm calls, were heard twenty-nine times.

(8) Certain ponies always moved towards approaching humans or stationary cars because they were often given bread or other foodstuffs by tourists. As they moved towards them, they often nickered (twenty cases).

The Whinny

This was a louder and higher-pitched call than the nicker, and it carried over longer distances. It was almost exclusively used either when a pony was separated from the rest of its group, or a foal from its mother, for some time, or as a reply to the whinnies of a lost pony. Two hundred and thirty-seven lost ponies whinnied, usually several times, and in 129 cases, replies were heard to these calls. Of the 129 reply calls, eighty-four were also whinnies (65 per cent) and forty-five were nickers (35 per cent). Although replies were not heard to the whinnies of the other 108 lost ponies, some may possibly have been inaudible to me. However, some ponies of a group were often under observation when one of their number was missing and although they sometimes looked up when they heard its whinnies, they still did not reply and often they merely continued grazing without even looking up. In 33 per cent of all cases where no reply was heard, one or more ponies did look up when they heard a whinny from a group companion. Generally only members of the same group as the lost pony ever replied to its calls but very young foals often reacted by whinnying or nickering to the calls of any pony, and young foals often replied to a group companion's call whereas other group members did not. This was less evident in foals older than 1 to 2 months. Frequently when a mare had been apart from her foal, who was older than 1 to 2 months, she looked up and called to it and the foal usually immediately replied, even when it could not see its mother, for instance when in the shade with numerous other ponies between them. Other foals of the same age did not reply to the mother's calls. This all suggested that adult ponies and even month old foals could recognize the calls of individual animals; sonograms also suggest that calls are highly individual (M. Kiley, personal communication).

The only other circumstances in which whinnies were heard were when stallions saw approaching groups of mares and when stallions approached these mares. However, the vocalization used on such occasions was often intermediate between an excited whinny and a snort.

Klingel (1967) recorded six different vocalizations in steppe zebras: two expressions of pain, one expression of 'well-being', two warning sounds and a contact call. The latter probably corresponds with the whinny as it serves to keep the zebra group members together and is individually different. (For a more detailed account of acoustic communication in horses see Odberg 1969 and Kiley 1969.)

Postures and Facial Expressions

Facial expressions of equids have been described

in detail by Antonius (1937, 1939), Schneider (1930, 1931, 1932a, b), Zeeb (1959), Schloeth (1956) and Trumler (1958, 1959b). Six expressions are recognized in equids but they are not all seen in true horses.

Yawning Expression

When yawning, ponies held their ears forward and their mouths were wide open with the upper and lower teeth exposed (Trumler 1959b). It occurred in the following situations: (a) during, immediately before or after a resting bout; (b) in oestrous mares, when standing quietly while stallions sniffed them; (c) in foals after sucking and (d) in stallions after copulating.

Greeting Expression

This was described by Antonius (1939) and Trumler (1959b) as being similar to the threat posture but that the ears were directed forwards, denoting friendliness. Zeeb (1959) denied that the greeting expression could be distinguished as a special expression in true horses, but Dobroruka (1961) claimed its existence in the Przewalski's horse and a similar expression was also seen in the New Forest ponies.

When two group companions, or particularly when a stallion and mare met, they sometimes both extended their heads so that they touched each other's muzzle and lips. Their ears were directed forward but unlike the greeting expressions described by Trumler, the corners of their mouths were rarely drawn back. Such expressions were only maintained for a few seconds and often developed into threat postures, when ponies then laid back their ears. The greeting expression of the ponies was also often a preliminary to mutual grooming, when it seemed to act as an invitation posture, but the corners of the mouth were still then only occasionally drawn back.

Flehmen Posture

Schneider (1930, 1931, 1932a, b) described flehmen posture in a wide variety of ungulates. It occurred when the upper lip of an animal was curled back to expose the teeth and Schneider thought that this posture acted as 'a careful tasting of a smell'.

In the ponies it was recorded in several situations but always as a reaction to some smell: (a) by stallions after sniffing the genital region of a mare in oestrus; (b) by stallions, mares or foals of both sexes after sniffing urine; (c) by mares, and other ponies, after sniffing freshly delivered placentae; and (d) by any pony at the sight of another showing the flehmen posture. Stallions frequently showed this posture after sniffing oestrous mares, when no other ponies were in sight; foals and mares also frequently showed the posture when no other pony was looking at them and even when other ponies could see the posture, they still showed no reaction to it. Zeeb (1959) also, although not denying that the posture could act as a signal to a mare or even a rival stallion, did not record any pony ever reacting to it. However, Dobroruka (1961) claimed that the flehmen posture acted as a definite visual signal in Przewalski's horses, as he observed that a mare, when she had seen a stallion show the flehmen posture after smelling urine, also sometimes showed the posture without smelling the urine herself, but the significance of this social facilitation of the posture remains unclear.

A similar facial expression has been observed in higher primates such as the pig-tailed monkey, *Macaca nemestrina*, by Van Hoof (1962). A male, after smelling the genital region of a female on heat, curled back his upper lip, with his lower lip pressed tightly against it and then raised his head so that his face was directed upwards, as in ungulates showing the flehmen posture. This posture acted as a definite social signal in *M. nemestrina*, where it was not only used as a reaction to olfactory stimuli, but also in a number of situations in which no obvious preceding act of smelling occurred. The eyes were often directed towards a female on heat, who reacted to the posture by presenting, but the expression has also been observed in many cases with no obvious sexual context and between

animals of both the same and different sexes and between animals of roughly all ages.

Threat Expression

This was first described by Antonius (1937) and is characterized by the backwardly directed ears. It is absent in asses, half-asses and Grevy's zebra (*Equus grevyi*). In other species of zebra, the corners of the mouth are drawn up during the posture, but this is not seen in true horses (Zeeb 1959a). The expression in New Forest ponies varied according to the intensity of the threat. In low intensity threats the ears were only slightly laid back but in high intensity threats the ears were right back and sometimes the mouth was slightly open with a suggestion of drawing back of its corners. This occurred immediately before the aggressor lunged out and attempted to bite a subordinate pony (see p. 121).

The threat posture was also observed when a stallion was driving mares (see p. 177). During this 'driving', which occurs in a wide variety of artiodactyls (Walther 1958) the stallion held his head and neck stretched out almost touching the ground and swayed his head and neck from side to side; his ears were directed backwards. This driving acts as a modified threat posture display directed to females in oestrus, which serves to intimidate those not yet ready for copulation (Zeeb 1958).

Rossigkeitsgesicht

The facial expression of mares in oestrus, indicating their readiness to copulate, occurs only in zebras, asses and half asses (Antonius 1937). It shows similarities to both the threat posture and the greeting expression and is an exaggerated opening of the mouth.

Snapping

Although Antonius and Trumler had both previously recorded grinding of the teeth and chewing movements in equids, Zeeb (1959) first fully described snapping or 'Unterlegenheitsgebärde'. The expression is characterized by the usually sidewards or slightly backward position of the ears with the ear openings on the underside, the vertical movement of the jaws, the teeth mostly covered by the lips, the corners of the mouth drawn back and the neck stretched out horizontally. During the vertical jaw movements, the lips never meet.

This expression was very common in young ponies when they were threatened and sometimes occurred prior to mutual grooming; it also occurred in a variety of other contexts, in most of which it seemed to be released by fear.

(1) Foals and yearlings often approached adult mares or stallions and snapped without provocation, with their heads almost touching the heads of the older animals. This was particularly common in young foals. Mares usually soon threatened the foals who then quickly moved away, but stallions were more tolerant. They either ignored the foals, delivered a mild threat or even sometimes responded by play-fighting with colt foals (see p. 174). Stallions also allowed fillies to sniff them and even touch their sheaths.

(2) Young foals, when temporarily separated from their mothers, often galloped from one mare to another and snapped as they approached each one. They even snapped when they approached their own mothers until they recognized them.

(3) Young foals also often snapped when their mothers made a sudden movement and this response was almost the first behavioural pattern to be seen in a foal a few minutes after parturition, when its mother turned her head to touch it. This corresponded with the 'champing' movements observed in thoroughbred foals soon after parturition (Rossdale 1967).

(4) When a stallion approached a mare and showed an interest in her by sniffing her genital region and nibbling her hind legs and rump, and when he was copulating with a mare, the mare's young foal often trotted around the pair, nickering and snapping. This also occurred when a young foal's mother was grooming with another mare.

(5) Young mares frequently snapped during their first oestrous periods when stallions sniffed, licked or nibbled them and during copulation.

Klingel (1969) observed that young steppe zebra mares showed the 'Rossigkeitsgesicht' expression during copulation and when stallions approached them, but he then described an expression more similar to snapping than to Rossigkeitsgesicht. He recorded that the ears of the young mares were held downwards and backwards, that the corners of the mouth were drawn up and that the mares chewed with slightly exposed teeth and open lips. This description seems very similar to that of the expression seen in the young pony mares, which also appeared identical to that expression seen in foals and other immature animals such as when they were threatened.

(6) Snapping was not only directed to other ponies but was occasionally a response of foals and even yearlings to approaching cows.

The total number of snapping responses recorded in all circumstances between spring 1966 and the end of the study period was almost twice as large for filly foals as for filly yearlings (Table XX). However, there were almost twice as many filly foals in the study areas during this period as there were filly yearlings so the frequency of the response was similar in foals and yearlings. Although the frequency of snapping was similar in these fillies, the yearlings showed the response much more commonly to stallions than did foals (Table XX). There was no significant difference in the total number of snapping responses in colt and filly foals (139:150), although colt foals were observed snapping to stallions on thirty-six occasions and filly foals on only twenty-five. This was not significant either, however. It was not possible to compare the number of snapping responses in colt and filly yearlings because of the very small number of colts of this age still on the Forest.

Snapping was infrequent in ponies older than about 18 months but it was recorded in 2- and 3-year-old colts and fillies directed to both mares and stallions. The response to a mare invariably occurred when the young animal was threatened by her, or occasionally prior to mutual grooming. In one case a 3-year-old mare snapped at her own mother after receiving an unexpected high intensity threat from her. This occurred after the birth of the older mare's new foal. When colts snapped at stallions it was often after they had voluntarily approached the older males, and most of the snapping responses of fillies directed to stallions occurred during their oestrous periods.

Zeeb (1959) recorded snapping or 'Unter legenheitsgebärde' as an expression of submission but although it was most commonly observed when young ponies were threatened, in those situations where a submissive expression

Table XX. Variation in Frequency of 'Snapping' with Age, Shown by the Total Number of Such Expressions Observed in Female Ponies up to 4 Years of Age

By	To				
	Foal	1 year	Adult male	Adult female	Total
Females					
Foal	5	25	92	25	147
1 year		1	42	38	81
2 years			7	6	13
3 years			5	6	11
4 years					0
Total	5	26	146	75	252

would be expected, it had no noticeable effect in preventing the older animals from carrying out their threats. Foals and yearlings were frequently bitten or kicked by mares even after they had made the snapping response. Moreover, the response was directed to humans and cows.

Zeeb also believed that snapping had originated from social, or mutual, grooming, and that the jaw movements seen in the snapping expression are intention movements of grooming. Observations of the New Forest ponies support this as on a number of occasions when a young pony approached an older one, it snapped. This acted as an invitation to the older animal to groom, as the older animal then sometimes cooperated by grooming with the younger one instead of threatening it. The snapping movements then gradually passed into grooming movements. Similarly at the end of some grooming bouts when the older animal broke off by threatening its partner, the grooming movements of the jaws of this subordinate pony passed into snapping movements.

It is of interest that an analagous posture to snapping has been described in coatis (Kaufmann 1962). The young coatis often approached adult females, that were already mutually grooming, and made inhibited biting movements with their heads jerked towards the females. The females then responded by grooming the young coatis. This inhibited biting was also recorded amongst subadults and adults especially in the breeding season.

Snapping in ponies also appeared similar to the facial expression, lip-smacking, seen in various primates. Van Hoof (1962) recorded that as a rule the expression facilitates non-hostile approach between individuals and that this may be achieved by an appeasing or reassuring effect, according to the motivation of both individuals.

Other Postures

Postures, other than these five facial expressions, that functioned as a means of visual communication were few. The most obvious was that of a mare in oestrus who stood with her hind legs apart and with her tail raised as when she was urinating. When stallions saw mares showing either the oestrous or urinating posture, they often rushed excitedly to the mares and sniffed their genital regions.

The only other posture was that shown particularly by young foals when they were alarmed. Common noises such as the alarm screech of a jay (*Garrulus glandarius*), had little noticeable effect on the ponies but any unusual noise or sudden movement such as that made by a deer quickly running from cover, the sudden sight of a human or of paper blowing by, caused signs of alarm; the sight of ridden horses and running dogs also generally caused alarm but the degree of alarm varied between ponies, at different times of the year and in different places. Ridden horses excited more alarm in the months following the annual autumn round-ups than in other months, and the wilder ponies were more alarmed by my presence when they were grazing in woodland or thick vegetation in the valleys than when they were on short grass in the open. When foals were alarmed, their tails were raised and held vertically, as they moved quickly to their mothers. This made the paler underside of the tails very conspicuous, as in fallow deer, which also hold their tails upright when disturbed (personal observation) and in which it acts as a danger signal, and as in caribou (*Rangifer arcticus*) (De Vos 1960). However, older ponies ignored these tail signals as they had usually seen the source of danger before the foals had, or foals raised their tails at events which caused no concern in adults. Thus when a cow approached a young foal the foal rushed quickly to its mother with its tail held vertically. Although no reaction was observed in adults at the sight of this posture, other young foals often immediately then also rushed to their mothers when they saw it. When older ponies were alarmed they slightly raised their tails as they galloped away from the source of danger but

the length of their tails made a vertical position impossible.

Olfactory Communication

The phenomena of smelling and marking of faeces by stallions with their own faeces or urine, and the marking of urine with urine have been described on pp. 105–107. Both mares and foals were observed marking faeces and urine of other ponies.

VI. MOTHER–YOUNG RELATIONSHIPS

Introduction—Time of Foaling

Of 294 births between 1966 and 1968, 96 per cent occurred in April, May or June; nine (3 per cent) occurred in the months of March and July and only four (1·3 per cent) in August and September (Fig. 18). None were recorded at

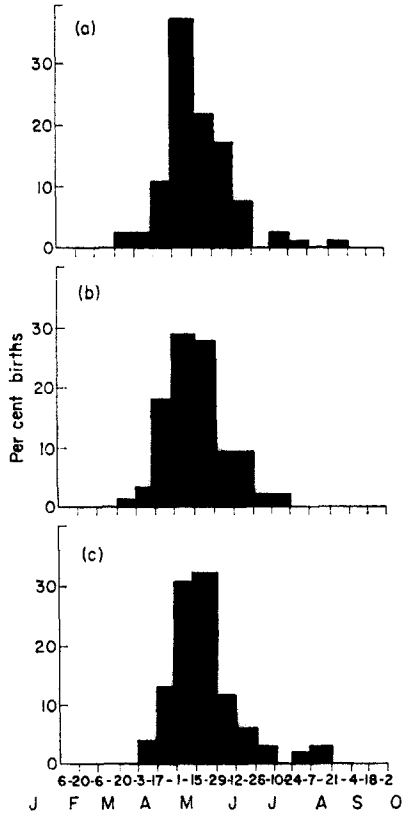


Fig. 18. Distribution of all known births in the two study areas in: (a) 1966, (b) 1967, and (c) 1968; showing a peak of foaling in May each year. Dates are given in intervals of 2 weeks and each histogram is based on eighty-nine to 108 births.

other times of the year in the study areas, although occasional foals have been born in the Forest during the winter.

Hammond (1960) believed that horses bred in the spring and summer because the hours of daylight were then increasing and suggested that ultraviolet light was the causal agent. Seasonal breeding is partly controlled by the stimulus of light (Burkhardt 1947) for when mares were subjected to increasing light, the ovaries began to function earlier. However, there must also be an inherent rhythm for when mares were kept in continuous dark, ovarian activity was delayed but not inhibited. Nutritional factors must also be of some importance in determining the breeding season, as well-fed and stabled mares such as Welsh ponies under domestication, tend to breed throughout the year (Cole & Cupps 1959).

The length of gestation in horses is on average 336 days (Hammond 1960) and Rossdale (1967) gave a similar average for thoroughbreds of 340·7 days with a range of 315 to 365 days. The shortest known gestation period in the ponies was 339 days (see p. 175). Thus a mare foaled about 11½ months after the date of conception. The actual day of parturition is also influenced by the sex of the fetus as male foals are carried longer than females (Wackernagel 1965; Flade & Frederich 1963) and by the time of year that the mare is served. Mares served early in the year have pregnancies up to 10 days longer than those served from May onwards, (Hammond 1960). Older mares also tend to have on average shorter gestation periods than younger mares (Tutt 1944).

The time of day of birth of thirty foals was either known accurately or was calculated for those where observation began before the mother had delivered the placenta, before the foal was dry or before it could stand. No night births were recorded as observation in the dark proved difficult, but of the known daylight births, 70 per cent occurred between dawn and 09.00 hours; 17 per cent between 19.00 and 12.00; 10 per cent between 12.00 and 15.00 and only 3 per cent between 15.00 and dusk. This showed that there was a marked tendency for parturition to take place during the few hours in the early morning.

Observations of steppe or Grant's zebras in zoos suggested that most births occurred in the

early morning (Wackernagel 1965), and G. Mottershead (personal communication) also believed that births of both Grant's and Grevy's zebras, took place in the early morning as foals were still wet when keepers arrived. In domestic cows there seems to be little variation in the frequency of parturition during the 24 hr of the day (Richter 1933) although in wildebeests *Connochaetes taurinus* the majority of births occurred in the morning between sunrise and noon and there was no indication of any calves born at night (Estes 1964). In sheep, births are most common during the hours of daylight (Lindahl 1964), although Hersher, Richmond & Moore (1963) concluded that births are distributed randomly throughout the 24 hr.

The early morning would seem to be an advantageous time for a birth to occur as it would allow the maximum amount of daylight for the newborn animal to be steady on its feet before dark and for the mother and young to familiarize themselves with each other. However, of 501 thoroughbred horse births, 86 per cent occurred between 19.00 and 07.00 hours with the maximal incidence between 22.00 and 23.00 hours, although the distribution about this maximum was markedly skewed towards dawn (Rossdale & Short 1967). Very similar results have been obtained in Poland by Zwolinski & Suidinski (1965) and in Sweden by Bane (1961), all working on thoroughbreds. These were kept in stables where there was human disturbance during the day and so it is possible that mares foaled at night when disturbance was least; when mares are disturbed at night, more then foal during the day, but Zwolinski & Suidinski (1965) also observed that as nights became shorter in the autumn, the frequency of foaling per hour of darkness increased, which suggested that light itself was the controlling factor. However, the behaviour of the free ranging semi-wild ponies cannot be properly compared with that of the very domesticated thoroughbred.

Pre-Parturient Behaviour

Premonitory signs of parturition have been described by Tutt (1944), Bane (1961), Rossdale & Mahaffey (1958) and more fully by Rossdale (1967). They include development of the mammae, secretion of milk sometimes several days before foaling, waxing of the ends of the teats a few hours to 2 days before foaling and lengthening of the vulval lips. The only useful sign in the ponies, which indicated that parturition was imminent, was development of the mammae, shown by the enlarged udder with diverging teats. This 'bagging-up' usually occurred only 1 or 2 days before parturition but in two old mares it was observed over 3 weeks before the event. Several hours before thoroughbreds foal they begin to sweat, they roll, paw at the ground and appear generally restless as they repeatedly lie down and stand up. All these signs were observed in preparturient New Forest mares except profuse sweating.

The mares sometimes moved away from their group companions, their yearlings or other offspring to a quiet, sheltered area before foaling; in two cases mares moved to areas outside their normal home ranges; others remained in the woods or valleys in the morning instead of moving out or up onto an open grazing area but many others foaled within a few yards of their yearlings, surrounded by their group companions or with other ponies grazing nearby. Five foals were born close to busy roads, two of them with a crowd of human spectators in attendance. Little attention was paid by other ponies to parturient mares although in two cases where group companions were nearby, they repeatedly looked up at the mares and gave excited nickers. Klingel (1966) also observed that little attention was paid by other mares to a parturient mare in their own family group.

Expectant sheep tend to separate themselves from the flock as parturition becomes imminent and seek out sheltered spots. Some also show a marked interest in the young of other mothers just before parturition (Hersher et al. 1963).

Parturition

Only three births were observed and in all of

them the mare delivered her foal when fully recumbent. In one case almost as soon as the mare had arrived on a reseeded area in the morning, she lay down and the only indication that uterine contractions were taking place was that she kept raising her uppermost hind leg. The forelegs of the foal quickly appeared and delivery was completed within 25 min of the mare first lying down. In thoroughbreds the mean length of delivery time for multiparous mares was 18 min and that for primiparous mares 21 min, with a range of 5 to 47 min (Rossdale 1967). Unfortunately observations did not begin on the other two pony mares until after contractions had begun, so that their delivery times were unknown. In one case the umbilical cord was broken when the mare rose to her feet after she saw a stallion approaching; in the other two cases it was broken by movements of the foals as they were attempting to stand.

In thoroughbreds discharge of the placenta usually occurred within 2 hr of parturition and mostly within 1 hr of it. Eighty-six per cent of twenty-nine cases occurred within 2 hr and 60 per cent within 1 hr (Tutt 1944). In the ponies placental delivery was also usually completed shortly after parturition; on several occasions, when observations began soon after parturition had taken place, the placenta had already been discharged or was discharged during the observation period while the foal was still wet. The known intervals between parturition and placental delivery were 28, 95 and 122 min but in one case, observations began at 08.00 hours within about 30 min of parturition and the placenta was not discharged until after 15.30 hours, after an interval of over 7½ hr. Mares showed signs of pain when losing their placenta as they kept lying down and rolling; in each observed case, the mare was lying down when she finally lost it. One mare lay for over 40 min prior to this event and during that time her foal kept pawing at her and pushing against her whilst it tried to suck; it trotted and galloped around her and several yards to and from her and it even nibbled at grass for a total of 15 min, appearing to eat some of it.

No mares were observed eating or even licking a placenta, although several sniffed at it and then showed flehmen posture. It was noted that Grant's and Grevy's zebras in zoos also showed little interest in their placenta, merely sniffing it or at the most nibbling it for a few seconds (G. Mottershead 1966, personal communication; Wackernagel 1965). Thoroughbred horses showed a similar disinterest (Rossdale & Mahaffey 1958). Four ponies, other than the mother, also sniffed a placenta; each showed a flehmen posture and then left it.

Post-Parturient Behaviour of the Mother

Mares began to lick their foals a few minutes after their birth and then licked almost continuously for up to ½ hr. After this licking was less frequent but occurred intermittently and for short periods throughout the foal's first day. It was concentrated around the head and tail region of the foal, especially when the foal was sucking. In one case where observations were very complete and made only 10 yd from the mother and newborn foal, the mare raised her head 3 min after parturition and lay on her sternum. She licked the fetal fluids on her own hind legs for 10 min before she stood and then turned her attention to her foal, who was also by then lying on its sternum with only its hindquarters still covered by the amniotic membranes. She began to lick its head but as she turned to do so, the foal made snapping movements of his jaws. She vigorously licked the foal's head, back and rump for 25 min during which the numerous attempts by the foal to stand were frustrated.

Thoroughbreds also vigorously lick their foals' coats (Rossdale 1967) but Wackernagel (1965) and G. Mottershead (personal communication) reported that Grant's and Grevy's zebras do not lick their offspring to any extent except for a little nuzzling around the mouth. Rossdale believed that licking of the foal's head stimulated sucking movements. It also plays an essential part in the development of

respiratory effort in goat kids in which there is an increase in oxygen content of the blood associated with licking. This leads to a greater capacity for muscular movement and strength and so hastens the kid's efforts to stand (Blauvelt 1954).

As in goats and sheep (Collias 1956; Smith 1965) licking by the mare of the fetal fluids seemed very important in establishing the relationship between mother and young and also for the development of the highly discriminative behaviour of the mother. After a mare had licked her foal, she discriminated between it and other foals; the latter were threatened whenever they approached the mare whereas she touched her own foal, nuzzled it and allowed it to suck. The great attraction of a mare to the fetal fluids was shown by events which followed when a foal fell into a ditch whilst trying to stand. The mare stood anxiously by the ditch nickering to her foal, which was then rescued and carried 15 yd from her and the ditch. She looked up at the foal and nickered but still remained by the ditch near the placenta and the spot where the foal had been born. After 3 min she still had not moved to the foal but when it was carried back to her, she began to lick it again and a normal relationship was established. At that stage, less than 40 min after parturition, the fetal fluids exerted more attraction to the mare than the foal itself.

During her foal's first day, a mare's behaviour was markedly different from that shown in the rest of its first week. She remained very near her foal, only grazing a few yards from it, and kept especially close when other ponies were nearby. She threatened any subordinate pony that approached and always moved quickly between the approaching pony and her foal; she even threatened her own other offspring. When a more dominant mare approached, she nickered to her foal, moved close to it and sometimes nudged it. She then walked quickly away, keeping between it and the dominant mare, and her foal followed at her side. First day foals often trotted or galloped a few yards from their mothers, sometimes even following other mares, but their mothers immediately nickered and rushed after them and directed them away from other ponies. When a mare and foal were isolated from other ponies, the mare showed less concern and did not always rush after the foal when it moved away from her. The foal invariably only moved two to three yards before rushing back to her.

Data from both free ranging troops of rhesus monkeys (Kaufmann 1966) and from caged groups (Hinde & Spencer-Booth 1967a) indicated that the proximity of other adult females increased the restrictiveness of mothers. However, in ponies, the proximity of other adult mares caused the mothers to stay close to their foals and rush after them whenever they moved away from them only on their first to second day. After that they allowed their foals to approach other mares, but in rhesus monkeys the restrictiveness of the mothers continued for the first 6 to 8 weeks of their infants' life.

A mare's behaviour on her foal's first day was therefore directed to keeping her foal near her and away from all other ponies, until it was able to recognize her. Foals usually seemed able to recognize their mothers by their second day by smell, and their mothers then approached them infrequently compared with their first day.

Behaviour of Newborn Foals

In the case where observations of a mother and foal were made for 5 hr after parturition from a distance of about 10 yd, the foal raised its head and pushed a foreleg and its muzzle through the amniotic membranes almost immediately after its birth. The membranes ruptured and the foal then raised itself onto its sternum. Within 5 min of its delivery, it made an attempt to stand, before the mare had begun to lick it, but its hind legs slipped on the membrane still enclosing the hind part of its body. It made two further attempts and then the mare licked its head, back and rump, and legs. Although now free from the membranes, its numerous standing attempts were thwarted by the mare's vigorous licking action, but finally 33 min after parturition, it stood. The times taken for two other foals to stand were 37 min and about 45 min.

After standing the foal then moved unsteadily alongside its mother pushing its head under her belly. It nosed backwards and forwards under her belly until the mare gently nudged its hind region so pushing it further alongside her. It then found her nipples but kept losing contact with them because of its unsteadiness, and only made sucking movements with its mouth for a few seconds at a time. It sucked successfully 52 min after parturition. During the next 3 hr, it nosed backwards and forwards under the mare's belly seven times before it found the nipples but it gradually found them more directly each time, and by the end of the 3 hr, it moved immediately to the nipples before sucking. After finding the nipples, it did not usually suck at once but pushed with its head against the udder for several seconds, presumably stimulating the mare to make milk available for it.

During the foal's first sucking bouts, the mare co-operated by standing still and flexing the hind leg furthest from the foal; this made the nipples more easily accessible. After the first few bouts, she usually limited their length or more usually the number of bouts by moving away whenever her foal approached to suck. However, when the foal persisted in its attempts by following her, she soon remained still and allowed it to suck. This probably aided the development of the mother–foal relationship by encouraging the foal to follow its mother; milk then acted as a reward for this behaviour.

Several mares showed signs of pain when their foals first attempted to suck as they squealed when the foals touched their udder. One such mare was a primiparous mare and for most of her foal's first week, she repeatedly moved away from it when it tried to suck. The foal often made more than ten attempts before she allowed it to suck. Another such mare was multiparous but her foal had fallen into a ditch before it was rescued. A further hour elapsed before the mare would remain still to suckle her foal, as her udder was then very swollen and obviously painful; the foal died the following day. Rossdale (1967) also recorded that primiparous thoroughbred mares and a few multiparous mares frustrated their foal's first attempts to suck because of apparent sensitivity.

The following response of a newborn foal was evident as soon as it was able to stand as it then moved to its mother, and moved after her when she walked away. First day foals showed a strong tendency to follow any large moving object; they frequently followed siblings or mares other than their mothers; several followed me and one even trotted alongside a van which was driven slowly past it. By their second day, foals did not show this response to other ponies or objects and they only followed their own mothers, walking behind or alongside them or trotting or galloping by their sides when the mares moved quickly. One foal, which was born in woodland, showed a marked attraction to a particular tree on its first day. It remained close by the tree, even when its mother moved up to 5 yd away, and it nibbled the tree, walked around it and to and from it. However, a successful mother–foal relationship was formed, although some New Forest Commoners observed that the attachment to a fixed object, such as a tree, can occasionally upset the relationship. A foal will sometimes completely ignore its mother and try to suck from the tree; the mare will eventually desert it.

Most first day foals sucked and nibbled at grass or other vegetation. Sometimes they chewed off leaves and other pieces of vegetation but they usually then let them fall to the ground, often tossing them into the air before doing so. Although most foals rarely ate much grass on their first day, several did eat some and one was observed nibbling at grass almost continuously for 5 min. The amount of time per hour spent nibbling or grazing by foals gradually increased with their age (Fig. 5). Roughly half of the daylight hours were spent resting by young foals up to their third month, but after this, the amount of time spent resting gradually decreased until the end of their first year.

Foals were observed rubbing, rolling, nibbling, scratching, stamping and shaking, on their first day, often within a few hours of birth, and

foals of this age also yawned and showed the flehmen posture and the snapping expression.

Behaviour of Other Ponies to Newborn Foals

Great interest was sometimes shown by other ponies, particularly by yearlings, to foals born early in the season. Yearlings, both siblings and non-siblings, stared at the foals and kept approaching them and trying to sniff or touch them, but the foal's mothers threatened them and drove them away whenever they approached too closely. After 2 to 3 days the yearlings showed much less interest in the foals. This curiosity on the part of the yearlings was thought to be because the early foals were the first foals that they had ever seen, except for those born at a similar time to themselves the previous year.

On four occasions adult mares also showed great interest in a mare and her newborn foal. On three of these the foal had been born early in the season and in the fourth instance, the foal was born in September, very late in the season. However, mares were not consistent in this behaviour shown to early and late foals as many such foals were completely ignored, although group companions of a mother usually showed some interest when they first saw her new foal. They sometimes nickered to them, approached and stared at them, but soon moved away again and showed no further interest in them. On the four occasions mentioned above, a mare rejoined her group companions and other ponies on a grazing area, after the birth of her foal. The mare and foal were quickly surrounded by other adult mares, mainly of their own group but also members of other groups. These mares stared at them and became very excited. When the mother moved away, followed by her foal, the mares whinnied and nickered and followed them until all were trotting and galloping alongside them. In the general excitement on one of these occasions, the foal galloped alongside a strange mare moving away from its mother but the mother soon galloped between them and manoeuvred her foal away. They then moved into the cover of woodland and left the mares, amongst which there were young animals, which had never foaled and multiparous mares which had either foaled the previous year or had not foaled for several years. However, their excited behaviour and great interest in the mother and foal were exceptional, and it was usually only yearlings or even older foals that showed any interest in newborn foals. Other adult mares were usually aggressive to strange foals, even very young ones, and when such a foal approached a mare before its mother could prevent it, it was threatened and even kicked.

Sucking Behaviour

Introduction

After its first few days of life, when a foal approached its mother to suck, it often nickered and usually quickened its pace, laid back its ears and often tossed its head. It then moved in front of its mother pushing under her head, before it sucked. This usually occurred from whatever direction the foal had approached so that when it walked up from behind her, it walked almost completely around her before sucking, or it walked alongside her up to her head and then turned and sucked from that side.

When the mother walked on when her foal touched her nipples, the foal again moved in front of her, across her path to stop her, and then made another sucking attempt either from the same side as before or from the other side. This was repeated until the mare stood still for her foal. Sometimes when foals were frustrated in the attempts to suck, they pawed the ground. This was also seen when mares were wading deep in ponds or ditches; their foals remained on the banks nickering to their mothers or waded out to them and then pawed at the water until they were able to suck.

When a foal was sucking, it usually stood alongside its mother with its hindquarters near her head. She often sniffed the foal when it was sucking or when it was too rough and pushed violently against her udder, she bit its hind legs. The foal's reaction to this was sometimes to kick out at its mother's head and continue grazing or

to move around so that its body was at right angles to her's or even almost parallel again but now facing in a similar direction. Foals sometimes began sucking in these positions but usually moved during the bout to the normal position. Very young foals and those whose mothers did not co-operate by making their udder easily accessible, occasionally sucked from between their mother's hind legs.

Behaviour of Mares during Sucking Bouts

A mare, which was grazing when her foal approached to suck, either co-operated with her foal by stopping grazing, standing still and flexing her hind leg on the side away from the foal, or she continued grazing and even walked on when it attempted to suck. She sometimes also actively rejected it by biting it, stamping or even kicking it, although such rejection was often the result of the foal pushing too hard against her udder. It was unusual for any mare to prevent her foal from sucking for longer than 2 to 3 min, but this did occur with some primiparous mares on their foal's first day (see p. 152) and occasionally with other mares who sometimes repeatedly kicked out at their foals. Kicking was much more of a deterrent to sucking than the comparatively mild forms of rejection such as biting and stamping, and when a mare kicked out at her foal, it usually moved away to graze instead of making another sucking attempt immediately.

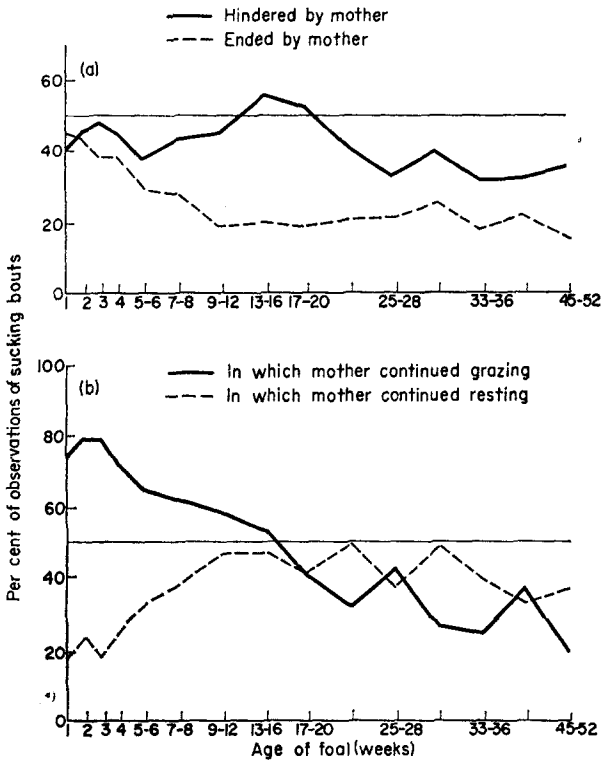


Fig. 19. Changes with foal's age: (a) in the proportion of sucking bouts that were hindered and ended by mothers, and (b) in the behaviour of mothers during sucking bouts. The number of observations of sucking bouts varied from 830 in first week foals to 132 in foals of 17 to 20 weeks to only forty-one in foals of 44 to 52 weeks.

The proportion of sucking bouts during which mares continued grazing, so making sucking more difficult for their foals, gradually decreased with the age of the foal. This decrease with age was statistically significant ($P < 0{\cdot}01$) and there were no obvious differences in this behaviour between primiparous and multiparous mares. In foals of under 1 month, the percentage of bouts in which mares continued grazing rather than stopped, was between 70 and 80 per cent; it dropped to under 50 per cent in foals of 5 months and to under 30 per cent in foals of 8 months (Fig. 19b). Those bouts in which a mother stood for her foal but in which she was already standing resting before her foal began to suck were excluded from this, but they formed between 20 and 50 per cent of all bouts (Fig. 19b). Although mares co-operated more with older foals, these old foals sucked very infrequently compared with young ones and sucking was often the only contact that mares had with their old foals some days.

Mares hindered less than 50 per cent of all bouts by moving, biting, stamping or kicking except in fourth and fifth month foals. There was no significant difference in the proportion of sucking bouts hindered with the age of the foal (Fig. 19a) ($P = 0{\cdot}2$). However, the ways in which bouts were hindered did vary with age. Thus 80 per cent of the bouts that were hindered by the mother in first week foals were hindered

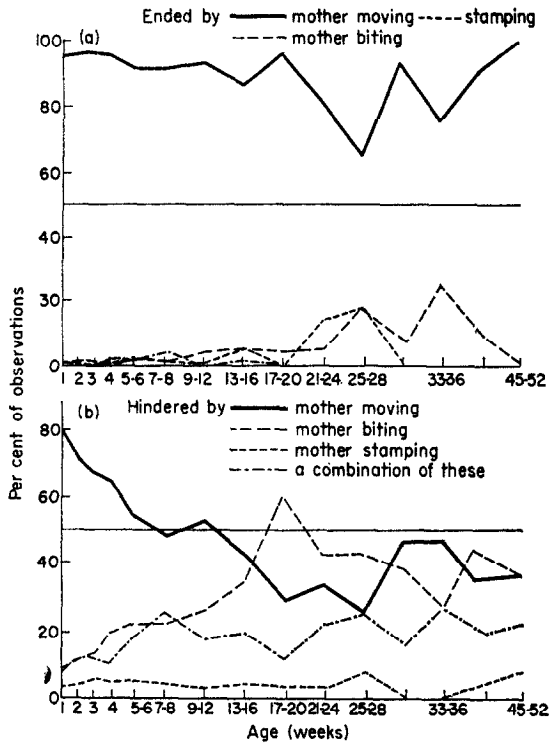


Fig. 20. Changes with foal's age in the ways in which mothers: (a) ended, and (b) hindered, sucking bouts. The different types of line have the same meaning in the two graphs, indicating the mother's behaviour which ended or hindered the suckling.

merely by her moving and only 7·5 per cent by biting the foal, 3 per cent by stamping, and the remainder by a combination of these factors. In fourth month foals, the bouts hindered by the mother moving dropped to 42 per cent whereas those hindered by her biting her foal rose to 35 per cent. After the fourth month, there was no significant difference in the proportion of bouts hindered by the mother moving or by her biting her foal, but up to the fourth month biting became more common (Fig. 20b).

Mares ended sucking bouts by the same means as they hindered them, but it was often difficult to determine whether the mother or foal ended a bout. A mare often moved or bit her foal during a bout, causing her foal to stop sucking but often after a few seconds the foal continued again and finally ended the bout of its own accord. Alternatively the foal moved away and

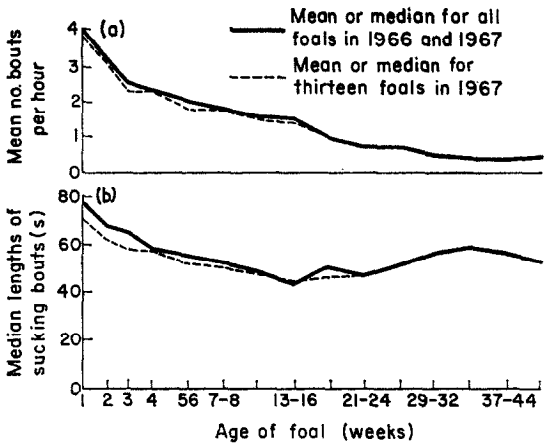


Fig. 21. Changes with age in: (a) the frequency of sucking, and (b) the length of sucking bouts. Data from all foals observed in 1966 and 1967, with the frequency and lengths of bouts of thirteen 1967 foals which were observed at more regular intervals until their sixth month.

began to graze after its mother had interrupted the bout. This usually depended on how long the foal had sucked before the mare interrupted it. Sometimes bouts were interrupted several times by the mother, but each time the foal continued sucking again. 'Bouts ended by the mother' only include those where the mother made the final move, whereas 'those hindered by the mother' include those hindered by her at the start and those hindered by temporary interruptions.

The proportion of bouts ended by the mother was highest in very young foals, 46 per cent in first week foals, but it dropped to 18 per cent in third month foals and thereafter remained below 25 per cent. The correlation of the decrease in the proportion of bouts ended by the mother and increasing age of the foal was significant ($P = < 0{\cdot}01$) (Fig. 19a). A very large proportion of these bouts ended by the mother were ended by the mother moving. Over 90 per cent were ended by this method in foals of most ages (Fig. 20a), and biting or stamping were only rarely employed. The higher readings for these methods of ending bouts in old foals was largely explained by the smaller number of bouts considered for these compared with for younger foals.

Thus 378 bouts of a total of 828 were ended by the mother in first week foals, twenty-one bouts of 103 in sixth month foals and only six of forty-one in twelfth and thirteenth month foals.

Frequency of Sucking

Data used for determining the frequency of sucking and the length of sucking bouts was obtained from all the foal record sheets completed for many 1966 foals, and a smaller number of 1967 foals at regular intervals. Unfortunately some of the twenty-four selected 1967 foals were killed at an early age and many others were removed from the Forest so that for only thirteen foals were observations completed up to their sixth month and for only six up to the end of their first year.

Sucking in first day foals was very frequent and the bouts were very variable in length, but bout length became more constant during the rest of their first week and the frequency gradually decreased. The mean frequency for first week foals was four bouts per hour (728 bouts and 186 hr of observation). Thereafter there was a steady decrease in frequency until their eighth month, so that fifth and sixth week foals sucked on average twice each hour (144 bouts), fifth month foals sucked once each hour (103 bouts) and eighth month foals only about once in every 2 hr (109 bouts) (Figs 21 and 22). The frequency then remained constant at this level of about 0·5 bouts/hr until weaning, which occurred fairly rapidly by a mare repeatedly avoiding her foal and even threatening it when it attempted to suck. Weaning usually occurred when foals were about 1 year old, a few days or weeks before their mothers gave birth to new foals. Two foals were however weaned between their tenth and eleventh months. Mares which had no new foal the following spring, allowed

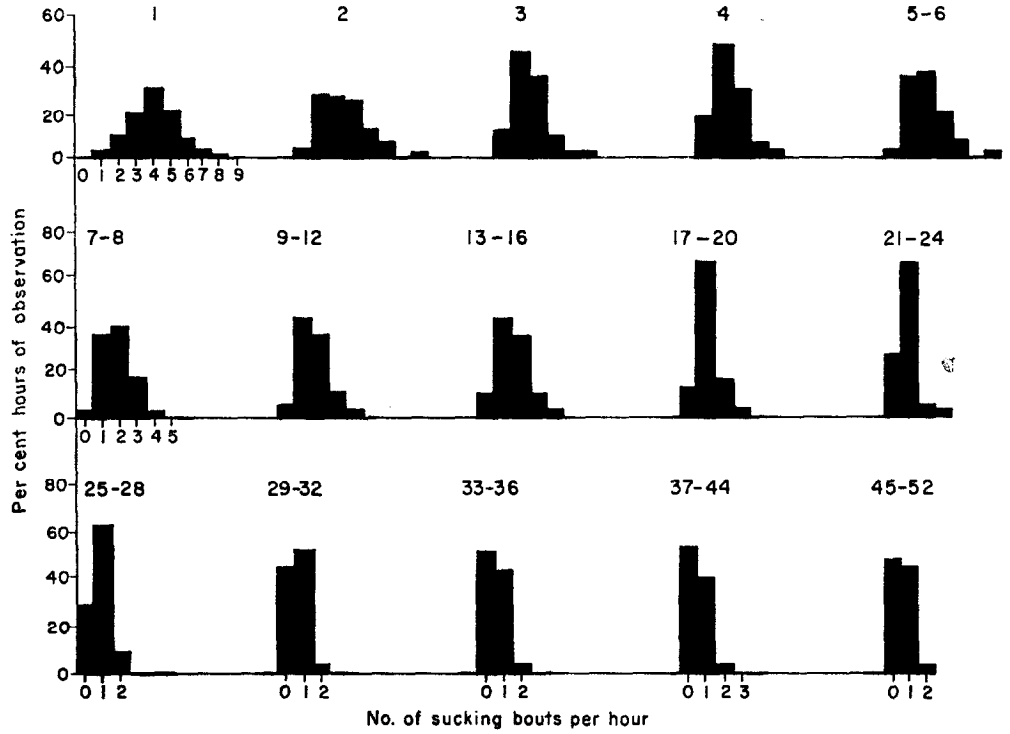


Fig. 22. Changes with age in the frequency of sucking. Data from all 1966 and 1967 foal check sheets. The figure for each age group was based on between 50 and 210 hours of observation. The figure above each histogram shows the age in weeks.

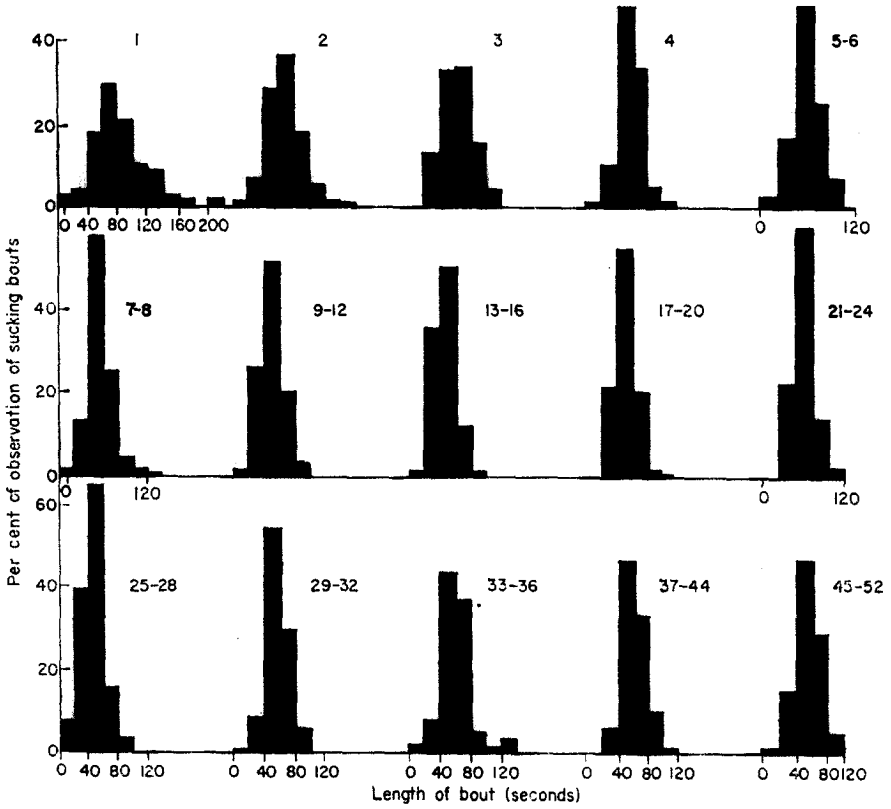


Fig. 23. Changes in the length of sucking bouts of foals during their first year of life. These histograms were each based on between fifty-nine and 728 observation sof sucking bouts. The figure above or beside each histogram shows the age in weeks of the foals concerned.

their yearlings to continue sucking until their second winter or spring and occasionally even into their third year if they still had no new foal. Three 3-year-old ponies, two fillies and one colt, were seen sucking.

Sucking commonly occurred: (a) when foals had found their mothers after a period of separation; (b) after a foal had been resting; (c) after a disturbance which resulted in the foal approaching its mother; and (d) when a mare approached her foal. The foal then usually stopped grazing, looked up and walked to meet her.

In old foals and yearlings a mother often initiated a sucking bout by approaching her offspring and standing near it. The young animal appeared to start sucking as soon as it took the nipples into its mouth as no or very little initial pushing against the udder was observed. There was milk available in the mare's udder for old foals, yearlings or older offspring as sucking movements of their mouths were obvious and in instances when they were removed from their mother, the mother's udder increased in size for several days following the separation. However, sucking in which the young animal received no milk, acted as a comforting stimulus in itself as was indicated by the following observations. A mare formed a close association with a gelding, and her foal frequently sucked the gelding's sheath; several foals sucked the nipples of older siblings, who had never had foals of their own and who cannot have had any milk; one filly foal sucked for over 2 min from the nipples of another filly foal of only 5 months.

Mares very occasionally allowed their yearlings or 2-year-olds to continue sucking even after the birth of another foal. This was observed in one yearling for almost a week after the birth of its sibling, and in one 2-year-old filly for several months. It was then removed from its mother and her young foal.

The frequency of sucking for the thirteen foals observed at regular intervals until their sixth month was very similar to but slightly lower than the frequency for all observed foals (Fig. 21).

Length of Sucking Bouts

The length of a bout was timed on a stop watch from when the foal stopped pushing violently against its mother's udder until it finally released the nipples. When bouts were interrupted such as by the mother moving, the period of interruption was subtracted from the total length of the bout to give the true bout length.

In first week foals bout length varied from only a few seconds to several minutes; the median length of 728 bouts was 78 s for all observed foals and 71 s for the selected foals (149 bouts). The smaller median was probably a truer indication of the median length of first week foals because most of the selected foals were observed for 1 hr on their first or second day, for 1 hr in the middle of their first week and for 1 hr towards the end of the week, whereas observations of 1966 foals were biased towards first day foals whose bout lengths were often very long.

After the first week the range and median length of sucking bouts both decreased until

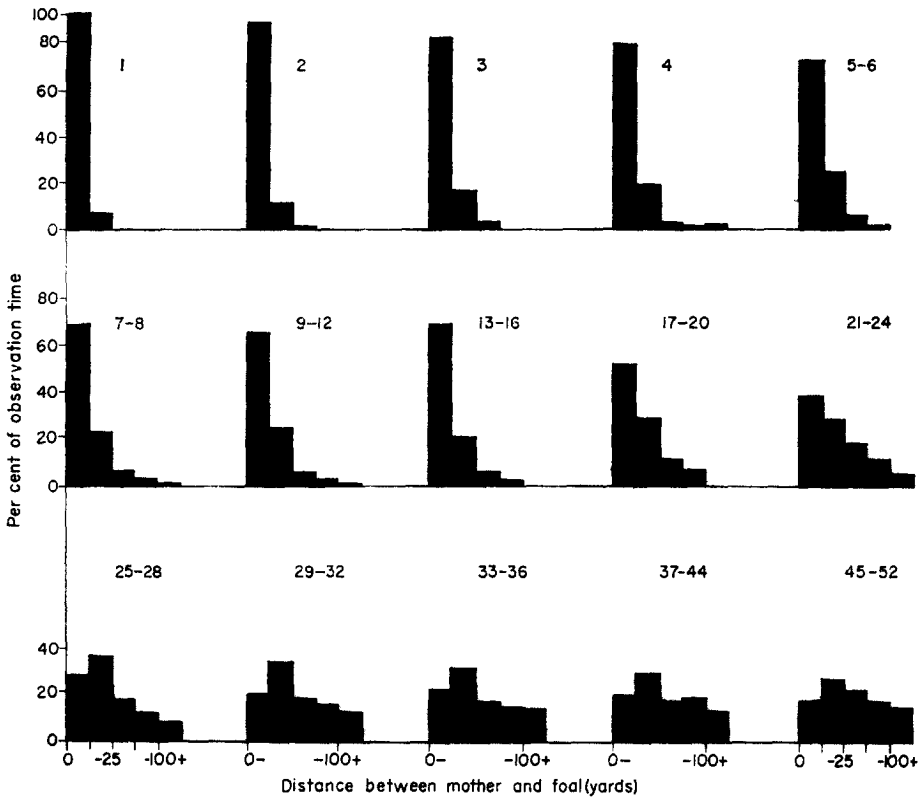


Fig. 24. Changes in the proportion of observation time that foals of different ages were within certain categories of distance of their mothers. Ages of foals are shown in weeks and each histogram is based on at least 58 hr of observation.

the fourth month (see Figs 21 and 23). The decrease in bout length was significant ($P < 0{\cdot}05$). A subjective impression was gained that foals older than about 9 months usually had longer bouts than foals of only 4 to 5 months and the median lengths of sucking bouts for all observed foals did increase again after the fourth month, but there was no significant difference in the medians between the fourth and twelfth and thirteenth months. However, when only the 1967 foals, whose observations continued up to their ninth month, were considered, there was a significant difference in median lengths with their age after the fourth month; older foals had longer sucking bouts ($P = 0{\cdot}05$).

There was no significant difference in the length of sucking bouts of colt and filly foals, nor of foals of primiparous and multiparous mares; but there was much individual variation. The filly foals of two multiparous mares (BH16 and BL10) were born within a week of each other, but one of them consistently sucked for shorter periods at all ages than the other. The differences were significant at all ages up to their ninth month except in the fifth to seventh months. Certain matched pairs of foals, but of primiparous mares (L21 and B8), also showed consistent differences in bout lengths but others showed remarkable similarities (BH25 and 41) (Table XXI).

Relationship between Foals and Mares Other than their Own Mothers

Mares only suckled their own foals and there was no evidence from this study that they ever normally accepted strange foals. No mare, other than a sibling, ever voluntarily allowed a strange foal to even touch her nipples or udder. However, on one occasion a mare was standing resting under a tree with her own foal resting by

Table XXI. Comparisons of the Median Length of Sucking Bouts of Three Pairs of Foals from Their First Week to the End of Their First Year

Age of foal (weeks)	Median length of sucking bouts					
	Foals of primiparous mares		Foals of multiparous mares			
	L21	B8	BL9	BH16	BH41	BH25
1	67·5	110	56	81	66	61·5
2	58	64·5	51	94	64	73
3	50	86	50	82	58·5	58
4	52·5	59	41·5	67	57	58
5–6	41	52	40	52	60	63
7–8	52	61	36·5	66	63·5	49
9–12	48	55	40·5	59	49·5	49
13–16	33	45	43	65	53	45
17–20	37	58	52	51·5	62	46
21–24	41	—	41	55	54·5	59
25–28	52	—	42	67	55	47
29–36	64	66	41	69	61	58

There was no significant difference between the bout lengths of foals of primparous mares and that of foals of multiparous mares but there was much individual variation in bout length. L21 and BL9 had consistently shorter bouts than B8 and BH16, respectively, throughout their first year, whereas BH14 and BH25 had bouts of similar length throughout this period

her side. Another foal, whose mother was grazing out in the open in wind and rain, approached the mare from behind and sucked successfully for over a minute. The mare then turned, looked at her own foal and then quickly turned her head to the other side and bit and chased away the strange one. All mares were aggressive to strange foals of even 1 to 2 days and they threatened them and then bit or kicked them when they approached. After their first day, foals quickly moved away when threatened but some first day foals were bitten and kicked, although this was usually avoided by the behaviour of the young foals' mothers. These mares rushed after their young foals whenever they moved towards another pony and manoeuvred themselves between their foal and the pony (see p. 166). Mares in the same groups as a foal's mother, and siblings, were more tolerant of the foal than other mares, as were most stallions.

Although no mare accepted a strange foal under normal circumstances, this was brought about experimentally. The skin of a filly foal, which was killed in a road accident when 3 months old, was draped over a younger foal, whose mother had been killed, and the dead foal's mother then accepted the strange foal as its own and suckled it. Thus as in the recognition of their lambs by sheep, olfactory stimuli must play a very important role in the recognition of a foal by its mother. The colour of the dead foal's coat draped over the other foal may also perhaps have been important in bringing about the strange foal's acceptance by the mare. It is noteworthy that this mare accepted another foal when her own was as old as 3 months.

Changes in Mother–Foal Relationship with Foal's Increasing Age

Distances between Mother and Foal

The gradually changing relationship between a mare and her foal during its first year was indicated by the changing proportions of time that the foal spent within five categories of distance from its mother with its increase of age. (a) Within 5 yd of its mother, (b) 5 to 25 yd, (c) 25 to 50 yd, (d) 50 to 100 yd, (e) over 100 yd.

The proportions of time that foals of different ages spent within these five categories were determined from the foal check sheets, but the distances were only estimated, The small distances were easily judged but the discrimination between larger distances was obviously less accurate. However, initially many estimates were then measured and it was thought that they were reasonably accurate and at least able to be used for comparisons because they were all made by the same observer.

There was a gradual decline in the amount of time that a foal spent within 5 yd of its mother from 94 per cent in its first week to 52 per cent in its fifth month and to about 20 per cent by its eighth month. After the eighth month there was no significant change up to the end of a foal's first year, except in those few foals that were weaned before this. The amount of time a foal spent within the other four categories of distance increased until its eighth month, again after which no significant change was evident (Fig. 24).

First week foals were rarely observed more than 25 yd from their mothers and never more

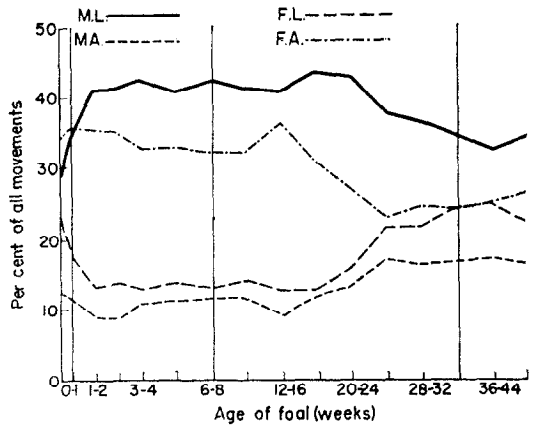


Fig. 25. Changes with foals' age in the proportion of leaves and approaches made by mothers and foals of the total number of all movements made by them both. M.L., mother leaves; M.A., mother approaches; F.L., foal leaves; F.A., foal approaches. Vertical lines indicate change in scale on time axis.

than 50 yd from them. Even up to the age of 5 months, foals spent less than 10 per cent of the observation time more than 50 yd from their mothers, and even when a year old, less than 35 per cent of the time at such a distance. Foals in their twelfth and thirteenth months however, spent over 45 per cent of the observation time within 25 yd of their mothers; this reflected the long and close relationship between a mare and her foal.

No quantitative data were obtained for foals older than 1 year, but the close relationship between a mare and her yearling, even when the mare had a new foal, was continued up to the time the young animal became sexually mature or left its mother's group. Although the yearling or older offspring was often weaned, it still rested near its mother, groomed with her and followed her.

A younger sibling often helped in prolonging the relationship between a yearling and its mother, because a close association was formed between the two siblings; the older animal then grazed near the younger one, which usually remained near its mother. However, the reverse also occurred as sometimes the close association of two siblings encouraged a greater independence of the younger one with regard to its mother. It spent much time close to its older sibling and grazed further from its mother than did a foal with no sibling. This was also apparent when there were two foals in the same group. They remained close to each other after their first 3 to 4 weeks, grazing and playing further from their mothers than foals with no such companions.

Close associations were also occasionally established between foals of two different groups. One colt foal was more frequently seen with a young filly foal and her mother than with his own mother, who was often several hundred yards away and out of sight. The great independence of this colt began in his third week and continued throughout his first year. However, no consistent significant differences were found in the distances that colts and fillies spent from their mothers.

The nature of the habitat where observations were made also influenced the data. When a mare and her foal were in woodland they remained closer together than when on open grassland. This was because when on open grassland such as a reseeded area, any group tended to remain in a small area of a few acres for much of the day. A foal was therefore unlikely to become separated from its mother compared with in woodland or on rough ground where ponies drifted on when grazing and covered larger distances. The absence of tall vegetation on the reseeded areas also enabled a foal to move far from its mother and yet still be able to see her.

The increase in the distances that foals moved from their mothers with their age was correlated with the foals' increasing ability to recognize their mothers from other ponies. A foal of a few days, when it awoke after a resting bout spent lying down, usually moved to the nearest mare irrespective of her size and colour. The nearest mare was usually its mother because she remained so close to it when it was lying down, but when the mother was grazing near other mares, her foal sometimes moved to the wrong mare. It seemed able to recognize its mistake when very close to the strange mare, presumably by differences in smell, and it then moved to the next mare until it encountered its mother or until she called or moved to it. By the time a foal was 2 to 3 weeks old, it was able to recognize its mother by her shade. Foals whose mothers were dark brown or black moved to the nearest dark brown or black mare when they awoke, even from a distance of 50 yd or more. They also recognized their mothers by sound at this age and moved directly to them when the mares nickered. Mistakes were made though, even by yearlings. They moved to mares of similar shade to their mothers from distances of 100 yd or more, but when 3 to 4 yd away, recognized their mistake presumably by both differences in smell and by more detailed visual differences. They then turned, looked around and usually walked directly to their real mothers.

Movements of Mother and Foal

Leaves and approaches. In an attempt to determine the part played by the mother in bringing about her foal's increasing independence, all 'movements' that were made by both mothers and their foals towards (approaches) and away from (leaves) each other were recorded on the foal check sheets.

An approach or leave was of any distance from less than 1 yd to over 100 yd and was recorded as a movement when a pony at least moved all four feet and when there was a pause of 30 s or more before another movement. This method of recording had some disadvantages because in normal grazing behaviour a pony slowly walked forward moving only two feet at a time. Movements were still then recorded only when there was a definite pause between further drifting movements. Mares also sometimes grazed in a circle around their foals so making neither leaves nor approaches. Such movements were excluded. Many other movements made by mares when their foals were older than about 6 months were only incidental in bearing any relation to their foals as they were often out of sight of each other; these were recorded as approaches or leaves according to whether they were towards or away from their foals, even when the foals were over 100 yd away.

The total number of approaches made by mares and their foals did not equal the total number of leaves made by them. Leaves made by mothers often followed in succession as part of normal grazing behaviour, whereas their approaches were usually more direct and purposeful. The number of leaves made by them therefore greatly exceeded the number of approaches made by them. In old foals approaches were also direct as when they moved to their mothers to suck, but young foals made numerous short approaches each time their mothers drifted away from them; their approaches exceeded their leaves. Although the total number of leaves made by both mothers and foals exceeded their total number of approaches, the ratio only varied from 1:1·1 to 1:1·4 throughout the foals' first year of life.

Many factors affected the number of movements made by mares and their foals, such as the amount of time each spent lying down. In foals this varied with their age (Fig. 6) and mares also spent more time resting in July and August when their foals were mostly between 3 and 5 months old. The availability of food and hence the nature of the habitat and the time of year also affected the number of movements made, as did play in foals. Young foals of up to about 4 weeks spent much time playing around their mothers and later with other young ponies, and this greatly increased their movements.

Proportion of movements made by mares and by their foals. The proportion of leaves made by mothers of foals up to 6 months was over 40 per cent of the total number of movements made by them and their foals, except in their foals' first week. After the foals' sixth month, the proportion of leaves made by mothers slightly decreased (Fig. 25). The proportion of leaves made by foals similarly increased after their sixth month and was also higher during their first week than during the period from their second week to sixth month.

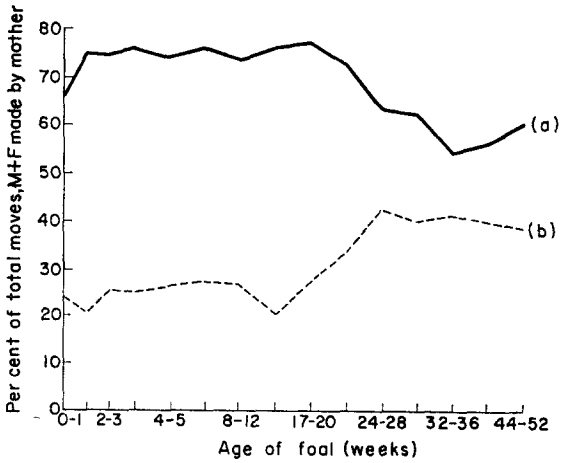


Fig. 26. Changes with foals' age in: (a) the proportion of leaves made by mothers of all leaves made by both of them and their foals; and (b) in the proportion of approaches made by mothers of all approaches made by both of them and their foals.

The proportion of approaches made by foals of the total number of movements decreased after about their fifth month, when approaches made by mothers increased slightly (Fig. 25). Thus mares made more leaves than did foals throughout the foals' first year and foals made more approaches than did their mothers throughout this period. This was more evident when leaves and approaches were analysed separately (Fig. 26). It was then shown that mares always made over 50 per cent of the total number of leaves made by them and their foals and, except in their foals' first week, they made over 70 per cent of the leaves up to their foals' sixth month. They made less than 40 per cent of the total number of approaches throughout their foals' first year and less than 30 per cent up to the time their foals were 5 months old.

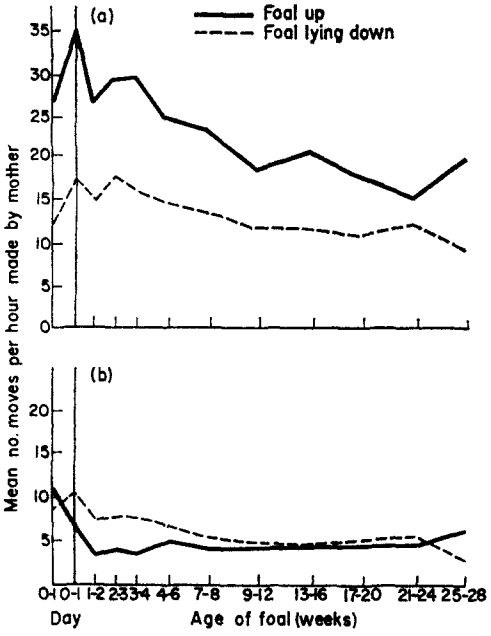


Fig. 27. Changes with foals' age in the number of leaves (a) and approaches (b) made by mothers per hour when their foals were lying down and per hour when they were standing up.

However, there was no indication from Figs 25 and 26 whether changes after the foals' fifth and sixth months were due to mares actually making fewer leaves and more approaches or due to their foals making more leaves and fewer approaches. The method used by Hinde & Spencer-Booth (1967b) to ascertain the part played by infant rhesus monkeys in bringing about their increasing independence, could not be used for the ponies. Their method involved subtracting the percentage of leaves made by infants from the percentage of approaches made by them; the difference between these gave an index of the extent to which a change in proximity between a mother and infant was due to the one or the other. They recorded an infant's approach or leave only when it moved from more than 2 ft away from its mother to less than 2 ft from her, and vice-versa, and so the total number of approaches made by mothers and infants equalled the total number of leaves made by them. As this was not so in the ponies, a different method had therefore to be used.

An indication of whether mares or their foals were responsible for the differences in the proportion of movements made by them as the foals grew older, was given by comparing their number of movements in a given unit of time for foals of various ages (Figs 27 and 28). It was then found that mares, perhaps surprisingly, made more leaves per hour in their foals' first week than during the rest of their foals' first year, and

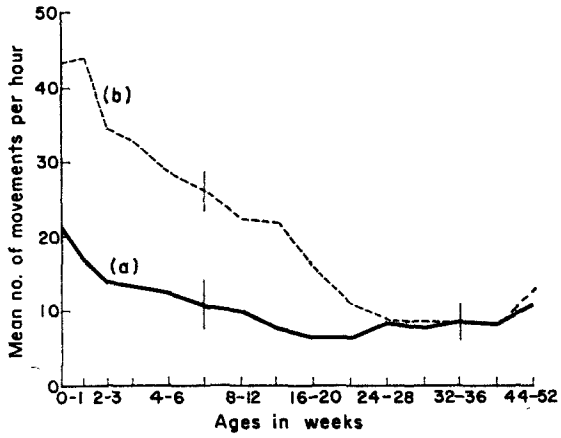


Fig. 28. Changes with foals' age in the number of leaves (a) and approaches (b) made by them per hour.

that there was a significant decrease in the number of leaves per hour made by them up to the end of their foals' first year ($P < 0{\cdot}001$). The large number of leaves recorded in their foals' first week was explained by the fact that during this period mares characteristically made very frequent and short movements alternately toward and away from their foals. There was no significant difference however, in the number of approaches per hour made by mothers from when their foals were 1 week old to 1 year old, although this number was greater in the foals' first week and especially on their first day, than during the rest of their first year (Fig. 27).

Changes in the proportion of approaches made by mothers and foals after the foals' fifth month (Fig. 26) were therefore due to the foals themselves making fewer approaches as they grew older, rather than to mares making more approaches. This is shown in Fig. 28 where the mean number of leaves and approaches per hour made by foals was plotted against their age. As foals spent so much more time lying down up to their third month than during the rest of their first year, the time that they spent lying down was excluded. Thus the number of movements per hour refers to the number of movements per hour of the foal being up.

There was little significant change in the number of leaves per hour made by foals after their third month; up to this age there was a decrease (Fig. 28). The high number of leaves per hour made by young foals was largely due to play movements, when they repeatedly galloped to and from their mothers (see p. 171). The changes in the proportion of leaves made by mothers of the total number of leaves made by them and their foals (Fig. 26) were therefore due to them making actually fewer leaves as their foals grew older and not to the foals making more leaves. It must be mentioned that in general, the length of movements increased as foals grew older.

Discussion. The decrease in the number of approaches made by foals with age was correlated with numerous factors, notably a decrease in their frequency of sucking, a decrease in the proportion of time that they spent within 25 yd of their mothers (Fig. 24), an increase in their interest in other foals, and young ponies and an increase in their ability to recognize their mothers from a distance.

The foals' increasing independence, which meant decreasing contact with their mothers, was however, probably largely brought about by their mothers' behaviour. Thus mares moved further from their foals as they grew older and they sometimes avoided them when they approached to suck. Mares also delayed their foals' sucking bouts by more active methods as the foals grew older (Fig. 20); they bit their older foals rather than simply moving away from them. Hinde & Spencer-Booth (1967b) also emphasized that in rhesus monkeys the mothers play a considerable role in bringing about their infants' gradual independence by avoiding or punishing the infants when they approach.

Hinde & Spencer-Booth (1967b) also found that young rhesus monkeys played a progressively increasing role in the maintenance of proximity with their mothers; however in the ponies the situation was very different. Until their fifteenth week, the number of leaves made by infant monkeys exceeded their number of approaches. This was the period when they were exploring their environment and during which their mothers watched over them closely and restrained them when they moved too far away. Restraining behaviour by the mothers decreased after their infants' second month, when the infants began to play an increasing role in maintaining proximity. They then approached their mothers more than they left them. In the ponies, restraining behaviour by mothers, in so far as they remained very close to their foals and prevented them from approaching other ponies, was very much in evidence during their foals' first day and to a lesser extent during the rest of their first week, but it was almost non-existent after this.

The existence of 'restraining behaviour' by mares on their foals' first day was indicated by

differences in the number of movements made by them at this time from the number made during the rest of their foals' first year or even the rest of their first week. Thus mares made more approaches per hour when their foals were up on their first day than at any other time (Fig. 27). The proportion of leaves made by mares of all movements made by them and their foals was also less on their foals' first day than during the rest of their first year, but this was largely due to the foals making more actual leaves per hour at this time (Fig. 28) and not due to the mares making fewer leaves per hour; in fact mares made more leaves per hour during their foals' first week than at any other time (Fig. 27).

Mares therefore approached their foals frequently on their first day, rushing after them whenever they moved towards other ponies, but they also frequently moved away from their foals. However the following response was well developed in foals only a few hours after their birth and these young foals moved after their mothers and were consequently guided away from strange mares. Because of this early response, first day foals still approached their mothers more than they left them, unlike young rhesus monkeys. Moreover, whereas infant monkeys played an increasing role in maintaining proximity with their mothers after their second month, young foals played a decreasing role in doing so after only their first week. Foals were still largely responsible for the maintenance of contact however, right up to the end of their first year, in that they still approached their mothers more than their mothers approached them.

The first 15 weeks of an infant rhesus monkey's life therefore in some ways corresponds with only the first to second day of a foal's life; this is explained because of the foal's precocial nature, and existence of its early following response and its ability to recognize its mother.

That mares also played important roles in maintaining contact with their foals, at least up to their sixth month, was shown by differences in their behaviour when their foals were up and when they were lying down and so unable to follow them. Mares made almost twice as many leaves per hour when their foals were up compared with when they were lying down, up to their foals' sixth month (Fig. 27). After this age analysis of movements per hour when foals were up and when they were not, became meaningless because foals then spent so little time lying down. Mares also made fewer approaches when their foals were up than when they were not; this difference however, was less marked by the foals' third month. It is also of interest that on their foals' first day, mares actually made more approaches per hour when they were up than when they were lying down (Fig. 27).

The distance that mothers moved from their foals when the latter were lying down, gradually increased with their age. When very young foals were lying down, their mothers only grazed a few yards from them, moved back towards them and then away again. This was repeated for as long as the foals were lying down. The distance was over 50 yd when foals were 1 month old but mares still then frequently approached their foals during their resting period. By the time foals were 2 to 3 months old, their mothers usually remained some distance from them and the foals then had to find their mothers when they awoke. However, the mothers still made fewer leaves per hour when their foals were lying down than when they were up.

Both mares and foals were therefore responsible for maintaining their relationship and yet still bringing about the foals' increasing independence. As Hinde & Spencer-Booth (1967b) stressed, the relationship between mothers and their young is a developing interacting pattern. The behaviour of each at every stage is determined by properties of both, which in turn depend upon the behaviour of both at preceding stages.

Protection of Foals by their Mothers

During a foal's first day its mother remained close by it and protected it from other ponies by

threatening any that approached or by manoeuvring her foal away from them. This occurred to a much lesser extent on the foal's second day, and after that the foal soon learnt to avoid other mares when they threatened it. Its mother then only occasionally came to its aid, although on several occasions when another young pony threatened a foal or yearling, the foal's or yearling's mother moved to them and threatened the other pony.

Mares also protected their very young foals from stallions, but these were rarely aggressive to foals. When one stallion attacked a foal of about one week and shook it by its neck, the foal's mother galloped up to them and tried to move between the two, even kicking out at the stallion. Other mares were also observed kicking out at stallions, who approached them and their very young foals, but this was not seen when the foals were older than 1 to 2 weeks.

Dobroruka (1961) described a method of ultimate defence of their foals by Przewalski's mares. This consisted of a mare kicking out with her hind hooves at the enemy and at the same time urinating so that the stream of urine was sprayed by the hooves and directed towards the enemy. This was not thought to be a means of defence in New Forest pony mares. It was occasionally observed in mares with young foals, when they were chased by stallions, but most commonly occurred when oestrous mares with or without foals, were being driven by stallions. Mares in oestrus urinated frequently even when no stallion was near them; when a stallion began to drive such a mare, she then sometimes also urinated as she trotted or galloped in front of him. This spurting of urine was sometimes but not always accompanied by kicking out with the hind legs; the urine rarely touched the stallion, who ignored it and continued driving the mare.

Spurting of urine by a gelding was once observed when he was being chased by a stallion. It was then thought to be associated with fear, but might perhaps have been a method of defence although it did not stop the stallion from chasing him. Spurting of urine was never observed directed to other mares.

A number of rodents and lagomorphs, in some species the male only but in others both the male and the female, enurinate or squirt urine at conspecifics (Wickler 1967). There is a distinct relationship between enurination and sexual and agonistic behaviour. Various primates also either expel urine dropwise on the substrate when running, or take urine onto the surfaces of their hands and feet and then deposit it on the substrate or on their bodies. This 'Urinwaschen' of primates such as *Saimiri* and *Cebus*, is also associated with anger, strong sexual arousal or fright (Kirschshofer 1963). Moreover, many other mammals do expel urine in fright (Wickler 1967); adult male dogs often show revived puppy-urinating in fear situations (Berg 1944) and this urinating in some species seems to act as a submissive gesture in adults (Kühme 1965). Thus, by analogy with other mammals and from observations of the circumstances in which the sudden spurting of urine occurred in ponies, it seemed very likely that their spurting of urine also occurred in fear situations or in a sexual context, and that it was not a means of defence.

Although there was generally little aid by mothers to their older foals, when these were threatened by other ponies, they did try to protect their foals when they were in danger from dogs or when they were approached by humans or ridden horses. This protection continued well after their foals' first week. When dogs chased a foal or snapped at its legs, the foal's mother quickly moved to them and kicked out at the dogs; when ridden horses approached a foal, its mother nickered to it and moved towards it. The foal usually then rushed to its mother, who led it away. Most mares were very tame, allowing humans to approach them closely. The foals of such mares soon became very tame and their mothers, except on their first day, allowed them to approach humans or humans to approach them. A few mares however, would not normally allow humans to approach within 10 to 20 yd of

them and these mares protected their foals from humans. When the 3-week-old foal of one such mare moved right up to the observer and sniffed her, its mother galloped up to it, nickered to it, quickly turned and kicked out at the observer and then led her foal away.

There was little opportunity to observe the behaviour of mares to their seriously injured or dead foals as these were usually soon removed from the Forest. Lame foals were common however, and mothers of badly lame foals usually modified their behaviour to adjust to their foal's incapacity to move quickly. When walking long distances, the mares frequently stopped and waited for their foals to catch up with them; when foals were unable to stand, their mothers usually remained near them.

Mares also usually remained near their dead foals, grazing to and from them as did mares to live foals that were lying down; the distance mares moved from their dead foals similarly depended on the foals' age. Observations were limited because road accident victims were quickly removed but two incidents were recorded. One foal died on its second day and its mother prevented me from approaching it by rushing up and threatening me whenever I approached the dead foal. The mare repeatedly grazed up to 10 yd from her foal and back towards it; she sometimes touched it and nickered to it, but then continued grazing again. This continued for 4 hr after the foal's death and perhaps for longer but unfortunately further observations were not possible. The mare's behaviour during the 4 hr was very similar to that of any mare with a 2-day-old foal, which was lying down. Another foal of about 4 months was found dead and its mother was grazing 20 to 30 yd from it. Ponies also grazed unconcernedly near dead adult group companions, ignoring them as they did live companions that were lying down. It is not known how long mares remained near dead foals; the mother of the foal that died on its second day had left the foal 2 days after its death and the mother of an 11-month-old foal that died in a bog, was grazing about half a mile from the dead foal on the day following its disappearance.

Relationship between Mares and their Older Offspring

Occasionally, mares which were due to foal in the spring, weaned their previous year's foals in the winter, but most mares weaned them only a few weeks or even days before their next foals were born. Weaning was then sometimes abrupt with a mare suddenly threatening or avoiding her yearling whenever it approached her to suck.

Immediately before parturition most mares left their yearlings or older offspring and sought a quiet, sheltered spot, but two mares with newborn foals were observed within a few yards of their yearlings. One colt grazed only 7 to 8 yd from his mother and new sibling, whilst the mother delivered the placenta.

Several hours after parturition most mares had returned to their yearlings or these had found their mothers. These mares then showed a drastic change in behaviour towards their older offspring, although the change only lasted for 1 to 2 days. It varied greatly from a mare chasing away her yearling and biting or kicking out at it when it approached within about 20 yd of her and her new foal, to only very mildly threatening her yearling when it was within a yard or two of her and the foal. One mare showed no noticeable change in behaviour towards her colt yearling; she suckled it even after the birth of her new foal and she grazed with the colt much further away from her foal than did other mares from their foals. This was however, atypical and the majority of mares kept their yearlings or 2-year-olds at about 10 yd from them during their foals' first day.

After this first day the relationship between a mare and her older offspring was much as before the birth of the new foal. Although the yearling no longer sucked, it still rested and grazed near its mother, groomed with her, rushed to her when alarmed and followed her when she made purposeful movements. However, it did not now walk immediately behind her but behind its

new sibling, who walked alongside or behind its mother. The yearling seemed mainly responsible for maintaining the close relationship with its mother.

A mare which did not foal when her previous year's foal was 1 year old, showed no observable change in behaviour towards her yearling during this animal's second spring and summer. Unfortunately, quantitative data were not obtained because foal record sheets were not continued for young ponies older than 1 year. Most yearlings of such mares were weaned in the late autumn or winter of their second year; of twenty-four yearling fillies with no younger siblings, only one was still sucking in the following spring, when she was 2 years old. This animal was unusual in that it then continued sucking after the birth of a new sibling. Two other ponies that were 2 years old in spring 1966, also continued sucking into their third year but these still had no younger siblings.

There was little immediate obvious change in the mother–young relationship when older offspring were weaned at about 18 months or later. Even after the birth of a new sibling, the 2-year-old ponies still remained with their mothers. However, during the summer, some of these young ponies spent short periods away from their mothers, and this usually occurred when the fillies were in oestrus. During the next winter, some of them again spent short periods away; these periods gradually increased in length and the fillies formed temporary associations with other groups or single ponies.

Of nineteen young ponies whose first younger siblings were born when they were 2 years old, only one left its mother permanently before its third winter. Some then left their mothers the following spring when, as 3-year-olds, some had foals of their own. Others remained with their mothers throughout their fourth year and then left. Of forty-three fillies which had never been disturbed by man, twenty-seven (63 per cent) were still with their mothers when they were 3 years old; of twenty-two fillies, only seven (32 per cent) still remained with their mothers when they were 4 years old.

Very few young mares remained with their mothers after they had foals of their own. However, even after this, the relationship between the young mares and their mothers was not entirely broken. They still sometimes rested or groomed together and one 13-year-old mare still groomed with her mother, although they were in separate groups.

In many cases particularly with the 3- to 4-year-old fillies, separation from their mothers' group seemed to begin after the birth of a new sibling. Their mothers then became temporarily aggressive towards them, and directed all their attention to their new foals. The birth of a new sibling did not however, cause any permanent separations of yearlings from their mothers.

It might be expected that a much stronger bond would develop between mares and those young which continued sucking into their second or third year, than between mares and young which were weaned before or when they were only 1 year old. Of the seven animals mentioned above that were still associated with their mothers when they were 4 years old, all but one of them were still sucking into their second year and two even into their third year. This suggested that a stronger bond was formed between late weaned animals and their mothers than between early weaned foals and their mothers. This obviously depended on whether the young ponies had new siblings when they were yearlings or not until they were 2 to 3 years old.

The death of a new sibling, or its removal at an early age, might also be expected to strengthen the bond between a mother and her older offspring, as, for example, the mother would then be able to groom more frequently with the older offspring. Commoners reported that a yearling may sometimes begin to suck again, if its young sibling is killed, but although several foals were killed, or died, in their first and second weeks, their yearling siblings did not begin to suck again. It is of interest, however, that of the seven 4-year-old fillies that formed long associations with their mothers, five had once

had younger siblings but these had been removed before their fourth month. Another of the 4-year-olds had never had a younger sibling and only one had a sibling present in the group.

Although the absence of a younger sibling therefore seemed to prolong the association between a young pony and its mother, the reverse also occurred. Thus two siblings often became very close companions and as long as the younger one remained with its mother, so did the older one. In such cases, the presence of a younger sibling prolonged the association. However, when a third sibling was born, the two older sibling companions then split away from their mothers. This occurred with a yearling colt and his 2-year-old sister, and with a 2-year-old filly and her 3-year-old sister. In these cases it seemed that the close relationship with the older sibling had prematurely weakened the bond between the younger sibling and its mother.

All these observations refer mainly to fillies because so few colts were left with their mothers after their first or second autumn. Two colts that were left with their mothers after their second year, both remained with them into their third year. One was then removed and the other left his mother's group when he was three years old.

To summarize, it is not possible to generalize about the factors leading to the weakening of the bond between a mother and her young. Separation from their mothers occurred when the young were 1 year old to when they were over 4 years old; two mares of 5 and 8 years were still in their mothers' group at the end of the study period. Weakening of the bond could sometimes be related to the birth of a new sibling or to the onset of sexual maturity in the young ponies and in many cases, to the presence or absence of other siblings or other unrelated young animals in the same group. However, all variations occurred from a mare with four offspring together in a family group, to a mare, which foaled in successive years but whose offspring left her as 2- to 3-year-olds, or even as yearlings.

VII. PLAY

Definitions of play in mammals have been fully discussed by Loizos (1966). In this present study play was characterized by various exaggerated motor activity patterns and by patterns of behaviour which were normally only observed in adults, e.g. mounting behaviour. However, both the exaggerated and more normal motor activity patterns were observed in adults; secondly they were often incomplete, being only isolated parts of a sequence of behaviour patterns shown by adults, and they were not obviously primarily directed towards their normal ends.

Play in ponies included locomotory, ingestive, aggressive, grooming and sexual patterns of behaviour. It was chiefly observed in immature ponies between the late spring and autumn, and only rarely in the winter months when food was scarce.

Play between Young Foals and their Mothers, and Solitary Play

Some of this play behaviour was perhaps strictly a form of exploratory behaviour but the movements involved were often exaggerated; it has therefore been included here as play.

From their first day, foals showed a great interest in their mothers' bodies. They nibbled at various parts of them, particularly the hind legs and the tail and mane, which they chewed and pulled. They stood at right angles to their mothers, backed up so that their rumps were pushed against their mothers' bodies and then they kept kicking out with their hind legs at the mothers' bellies. They also jumped up so that their forefeet were resting on their mothers' backs or necks; true mounting often occurred with correct orientation of foal to mother.

Mares were very tolerant of these activities and rarely showed any response to their foals' behaviour. They continued grazing and only either moved or stamped a leg when the foals were very persistent in their biting. However, by the time that foals were about 2 weeks old, their rough biting and nibbling of any part of their mothers' bodies developed into gentler grooming movements of their mothers' necks. The mares then began to respond to their foals by also nibbling their necks; true mutual grooming ensued.

Other play in very young foals took the form of a foal galloping very fast repeatedly to and from its mother, or away from her and then in irregular circles around her, for several minutes at a time. It bucked and kicked out its hind legs as it galloped, and jumped over or swerved to avoid any small obstacles in its way. Finally it rushed up to its mother, sometimes rearing up at, or mounting her, or biting and pulling at her neck and mane.

This 'galloping' play was even seen in foals 2 to 3 hr after their birth, but the distance to which they moved from their mothers on their first day was only usually 2 to 3 yd. The distance to which they galloped gradually increased so that by their fourth week they moved up to 50 yd from their mothers and sometimes further. Such play then became increasingly uncommon, when

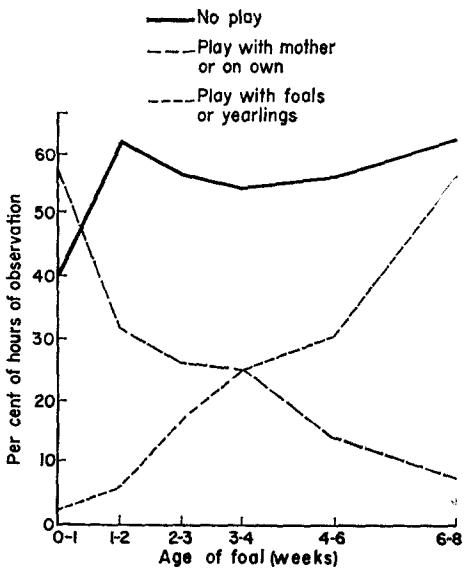


Fig. 29. Changes with age in foals' choice of play partners. This was based on a minimum of 67 hr observation of each group.

interactions with other foals became more numerous (Fig. 29). Thus the percentage of all hours of play centred around their mothers, in which foals galloped to and from or around their mothers dropped from 63·5 per cent in first week foals, to 17·6 per cent in foals in their fourth week, to 0 per cent in foals in their seventh and eighth weeks.

Although a foal's mother was almost always used as the centre for such play, one foal on its first day used a tree as such a centre. It galloped to and from a large oak tree, remained very close to this and kept chewing at the bark; it paid very little attention to its mother during this time. Occasionally attachments to large fixed objects such as trees, can impede the normal early establishment of the mother–foal relationship. Older foals often showed an interest in trees and nibbled at the bark but this was of a different nature and occurred after the mother–foal relationship had been established. These old foals used the trees as rubbing posts rather than as 'mother substitutes'.

Young foals were very inquisitive. They stared at and often approached, sniffed and nibbled strange objects and then frequently suddenly rushed back to their mothers; they picked up various objects such as twigs or pieces of paper in their mouths, tossed them into the air and pawed at them. Older foals also sometimes stared at and approached calves, especially when these were lying down. The foals sniffed the calves and often galloped after them, but no calves were observed playing with foals. They always rushed to their mothers when disturbed by foals. These types of behaviour in which no other pony was involved, have been included as play behaviour. They became much less common after a foals' first month as did play centred around mothers. The percentage of hours of observation in which foals played on their own or around their mothers decreased from 56 per cent in their first week (220 hr of observation) to 25 per cent in their fourth week (68 hr) to only 7·4 per cent in their seventh and eighth weeks (67 hr) (Fig. 29).

Play between Foals and Yearlings

Few interactions occurred between foals before their second to third week. Play in first week foals was recorded in only 2·6 per cent of 220 hr of observation and in second week foals in only 6·3 per cent of 158 hr (Fig. 29). It merely consisted of two foals staring at each other until one approached the other; after touching muzzles, one usually then laid back its ears and threatened or actually bit or kicked the other, before rushing back to its mother. The two foals often continued to stare at each other until again one approached and the sequence was repeated.

After their third week playful interactions between foals became more numerous. At this age foals moved further from their mothers for longer periods, during which they frequently approached, sniffed and nibbled other foals. If one of two foals was threatened, it did not immediately rush back to its mother but either moved a few yards away, stopped and returned to the other foal, or this other foal followed it until it turned. They then continued nibbling or grooming one another or they chased after one another, kicking out with their hind legs and bucking and rearing as they galloped. Such play involved foals of either sex and often began after one foal approached another that was lying down. The first foal repeatedly pawed at the resting foal until it stood up.

True play between foals younger than about two weeks and older foals was not observed. When an old foal approached a very young one, it was soon threatened by the young foal's mother. A 5-week-old filly repeatedly approached and stared at a young colt of less than a week, but she kept at a distance of about 10 yd from him and his mother. She then galloped in circles around him but although he showed great interest in her and several times pranced 3 to 4 yd towards her, he always quickly returned to his mother and did not join the filly.

Play between Colts

Although little difference in play behaviour was observed between colts and fillies up to the

end of their first month, young colts did mount their mothers or other ponies more frequently than did fillies. Colts in their second to fourth weeks reared up and mounted ponies forty-one times in 201 hr of observation, whereas fillies of the same age only mounted ponies seven times in 260 hr. Therefore colts mounted once in about 5 hr and fillies only once in 37 hr. The foals usually mounted their own mothers but seven of the forty-one observations of mounting by colts referred to them mounting other foals, and one of the seven observations of mounting by fillies referred to her mounting another foal.

The play of colts older than about 4 weeks became markedly different from that of fillies. Colts spent long periods play-fighting together in pairs. Each tried to bite his opponent's head and neck; they reared up and pawed at each other; each tried to bite the forelegs of the other until one of them sank to his knees, and each tried to bite the other's hind legs so that circle fights developed with each foal moving away from the other but still gripping the other's hind legs. They also galloped after each other and bit each other's rump. This chasing usually ended in another play fight. The various means of fighting described above were all observed in the real fights between adult stallions and have also been described in steppe zebras (Backhaus 1960). Most play bouts of colt foals included all these components and they often alternated with bouts of mutual grooming.

Male play differs from female play in laboratory raised rhesus monkeys by the second month (Harlow & Harlow 1962). By the second year play of male and female juvenile baboons also differs (de Vore 1963). The females groom more frequently and spend more time with newborn infants. Male juveniles play more roughly and for longer periods.

Particular pairs of colts often played together and the two partners of a pair were only rarely seen playing with other colts. Sometimes three or four animals were involved but any one play bout generally involved only two animals; more often took part in the chases however. Play fights were observed both between colts of similar age and between colt foals and yearlings.

Play most commonly occurred in the colts' first summer; it was little in evidence during the winter but was renewed in the spring when the colts were yearlings. The small number of colt foals left on the Forest for the winter months might partly explain the small number of play fights seen then, but even when two colts were left in the same area they rarely played during the winter. In the spring they showed a marked increase in play activity. Similarly, elk, *Cervus canadensis*, calves and yearlings played when pasture was abundant but play was almost absent when food was scarce (Altmann 1952).

Play between Colts and Fillies

Mutual grooming bouts between colts and fillies were common but the colts usually soon became rough. They gripped their partners' necks, bit at their heads, fore and hind legs, reared up and even mounted them. The fillies then tried to leave, first often laying back their ears and biting or kicking out at the colts. The colts sometimes followed the fillies and initiated further grooming bouts until again they became too rough and started to bite the fillies.

Although most fillies avoided colts when they became rough, some soon approached the colts again. They then initiated grooming bouts or even nibbled at the colts' heads and legs, reared up at them or chased them. This active behaviour on the part of the fillies was most evident where two foals of opposite sex were in the same group. They often then established a very close relationship. However, one colt foal and a filly foal from different groups also established such a close relationship and the colt was rarely seen away from the filly and her mother after his third week.

Each colt foal of such a pair seemed to prefer the company of his filly companion to that of other colts or fillies. Each remained very close to his companion when grazing or resting, often with his head resting on her back. When other fillies approached him, he either ignored them or

sniffed them briefly, whereas his filly companion usually threatened them. When other colts approached him, he occasionally had brief play fights with them but soon returned to his filly companion. Unfortunately, further development of these particular associations was not observed because one foal of each of the pairs was removed in its first autumn.

When a colt approached a filly foal with whom he had no close association, he soon began to nibble her or rear up at her. The biting of the filly by the colt was not however, aggressive. Although he nibbled and bit at various parts of her body, he rarely laid back his ears and the biting seemed to be more playful than that of the filly. The filly sometimes ignored the colt for a few seconds but when she did bite back this was usually an aggressive gesture. Her ears were laid back and after biting the colt or kicking him, she left.

Play between Fillies

Most interactions between fillies consisted of mutual grooming bouts or were of an aggressive nature; these were often thought to be playful, however, because of various exaggerated movements shown by the foals before or after such interactions. Thus foals often pranced or bucked as they moved towards or away from each other. Other forms of play between fillies were rare although some chases did occur when two fillies galloped about after one another. Of sixty-nine playful interactions between filly foals only 10 per cent were such chases, the remainder being grooming bouts and aggressive interactions.

The sight of young animals galloping about together often seemed to stimulate other foals or yearlings to join in with them. On three occasions two yearling fillies were galloping wildly together on very windy days and their behaviour even stimulated several adult mares to gallop with them for a few minutes.

Play between fillies was much less common than play between colts or between colts and fillies. Thus, of 423 playful interactions observed between foals in 1966 and 1967, half (50 per cent) involved a colt and a filly and 34 per cent involved two colts, compared with only 16 per cent involving two fillies. The precocious sexual nature of young colts might perhaps explain the higher incidence of play in male foals than in females. Much of the play between colts and fillies did contain elements of sexual behaviour, such as mounting and the nibbling of the hind legs and rump of the fillies.

Play between Young Animals and Adults

Play with Adult Mares

Young foals, especially in their first month, frequently nibbled, kicked out at and reared up at their mothers. Occasionally such behaviour was directed to other mares in the group, although most mares did not allow foals other than their own to approach them too closely and they threatened them if they did so. However, older siblings and young mares of 2 to 4 years were more tolerant; they allowed certain foals to rear up at them and nibble and chew their manes, tails and legs, but although they were more tolerant in that they did not bite or kick the foals, they largely ignored them as did the foals' own mothers.

No true play was recorded between foals or yearlings and adult mares, except where the wild galloping of the immature ponies stimulated mares to join in for short periods. A 3-month-old colt repeatedly nibbled the rump and hind legs of a 2-year-old mare from a different group, and also mounted her; she was very tolerant and did not kick out at him or even threaten him. The colt's behaviour closely resembled true sexual behaviour rather than play, as the young mare was in oestrus and he sniffed her genital region before nibbling or mounting her. He also had a full erection of his penis and made repeated pelvic thrusts when he mounted her (see p. 186).

Play between Young Animals and Stallions

Both colt and filly foals showed a great interest in stallions. When foals were older than 2 to 3 weeks, they often stared at stallions and approached and snapped. They then sometimes

sniffed the stallions' muzzles and even their sheaths of their penes, or nibbled their tails and legs. Most stallions, and particularly, geldings, were very tolerant of the foals' behaviour; they usually ignored the foals or gave very mild threats and only occasionally actually bit or kicked out at them.

M. Kiley (personal communication) also observed that in Camargue horses, the stallions showed a great tolerance towards foals and that foals frequently approached them and sniffed various parts of their bodies. When stallions threatened these foals, the foals snapped but when the stallions continued to threaten them, they then bent their knees and eventually lay on the ground with their legs uppermost. Thus they adopted a similar position to that used by puppies when threatened by adult dogs. After foals had adopted this position, the stallions immediately stopped threatening them and moved away. This ultimate appeasement posture was not observed in foals in the New Forest.

Although young fillies did approach stallions and even sucked their sheaths, this was more common amongst colt foals. Thus of forty-one observations in 1966 and 1967 of foals younger than 4 months that approached stallions and sniffed or nibbled them, thirty-one involved colts (76 per cent) and only ten involved fillies. Several colts of 3 to 4 months often stood resting by stallions and five young colts were seen play-fighting with stallions on one or more occasions. The mothers of two of these colts seemed anxious when they saw their colts with a stallion; they approached them, stared and kept whinnying or nickering. Klingel (1967) recorded that steppe zebra foals and stallions play running games.

The play fights that occurred between a colt foal or yearling and an adult male were similar to those between two colts. They gripped each other's mane and neck and bit at each other's fore and hind legs. The colt often reared up so that his forefeet were resting on the stallion's neck but stallions did not rear up when playing with well grown yearlings. During the play fights, stallions were not aggressive to colts and only bit them gently; they never kicked out with their hind legs as they did in fights with rival males. When a stallion ended a play fight by walking away, the colt frequently followed him and tried to initiate another such fight by rearing up and pulling at his mane.

No play fights were observed between stallions and 2-year-old males, although occasionally they nibbled at each other's heads. Stallions were more tolerant of 2-year-olds than they were of 3-year-old males, which were usually treated as rivals. Some of the fights between adult stallions late in the breeding season were however, suggestive of play fights: no obvious incident caused such fights; they were usually longer than real fights and very little pawing with the forefeet or kicking out with the hind feet occurred during them.

VIII. SEXUAL BEHAVIOUR

Oestrous Cycle

Oestrous cycles of New Forest mares corresponded closely with those described for thoroughbred horses. (Hammond 1960; Asdell 1946; Berliner 1959). A post-partum oestrus occurred soon after foaling; this foal heat usually began several days after a mare had foaled and continued for up to a week, but it sometimes did not begin for over 2 weeks after the date of foaling. After the foal heat there were successive oestrous periods of about 1 week in length and these continued throughout the spring and summer at intervals of about 3 weeks if the mare failed to conceive. The length of oestrus was however very variable. A 15-year-old mare showed signs of being in oestrus for almost 5 weeks and a 3-year-old for 2½ weeks. Hammond (1960) stated that the length of oestrus, although on average 7 days, did tend to be longer in very young and very old thoroughbred mares, and Nishikawa (1959) also stated that a long oestrus, the so-called continuous oestrus, was relatively frequent in the first oestrous cycle of young mares.

Oestrous cycles began in some mares in March and continued until September, although several mares displayed oestrous postures in October and one mare even copulated in late November. The mares first to come into oestrus were usually young virgin mares or older mares, that had failed to conceive the previous year or that had aborted during the winter. As the majority of pregnant mares did not foal until May (Fig. 19) most come into oestrus in the second half of this month.

Several young mares first came into oestrus in their second summer when they were only 14 to 15 months old, but most not until their third summer. Young thoroughbred mares are sexually mature when 16 to 17 months old on average (Nishikawa 1959). Adult stallions generally ignored these very young mares when they were in oestrus, and allowed yearlings or 2-year-old colts to attempt copulation with them. In the 3 years from 1965 to 1968 only one mare out of 107 foaled when she was 2 years old (1 per cent) and only fourteen of 104 when they were 3 years old (13 per cent). The small proportion of young mares that foaled would seem to be advantageous as these young mares were especially vulnerable to parasitic worms and they were also gaining their adult teeth; some died in the winter without having the added stress of a developing fetus.

It proved difficult to calculate the exact length of gestation periods of New Forest mares as many copulated on numerous occasions over a period of several days; possible further oestrus periods may also well have been overlooked. The shortest gestation length was about 339 days as the number of days between the births of two successive foals (the Foaling Index) was 345 and the mare was observed copulating on the sixth day after the first of the two foals was born. However, she may have copulated after this sixth day in which case the gestation length would have been shorter.

Many mares were observed copulating during their foal heats, and no further oestrous periods were then recorded for many of them. From an analysis of foaling indices, assuming the gestation length was on average 340 days, it also seemed likely that most mares conceived during their foal heats or during the oestrus following the foal heat, and that few conceived after this second oestrus. Thus, if a mare conceived at the foal heat on the ninth day after foaling, she would foal the next year about two weeks earlier than she did the previous year. Her foaling index would therefore be 350. The foaling index for mares that conceived during their second oestrus would be about 378 and that for mares conceiving during their third oestrus about 406. The foaling indices were known accurately for fifty-nine mares, which foaled in successive years. They were between 336 and 364 days in 27 per cent of these mares, between 365 and 392 in 64 per cent of the mares and over 393 in only 9 per

cent, although one mare's foaling index was 489. Very few mares therefore conceived after their second oestrus following parturition.

A pony mare in oestrus showed a typical posture, which has also been described in zebras (Trumler 1958). She stood with her tail raised and hind legs slightly apart and repeatedly protruded her clitoris through the vulval lips; this 'winking' also occurred immediately after a mare had urinated. The oestrous posture was observed when a stallion was very close to a mare and when no stallion was near her. In both cases it sometimes served as a stimulus to any stallion that saw it as he then often rushed to the mare and sniffed her. Sometimes stallions also rushed excitedly to mares that had just urinated as their urinating posture was identical to that of the oestrous posture. However, after sniffing the genital region of such a mare, a stallion soon left.

When a stallion moved towards a mare in oestrus, she often trotted or galloped in front of him. Her movements were exaggerated and she swished her tail. Sometimes she spurted urine; this spurting of urine was most commonly seen in young mares that were just coming into oestrus, although, throughout oestrus mares did urinate frequently and in small amounts when stallions were near them.

The behaviour of mares and stallions varied during different stages of oestrus. Early in the breeding season stallions trotted or galloped after mares that were coming into oestrus and drove them for long periods. Occasionally a stallion mounted a mare but she usually kicked out at him or forced him to dismount by moving forward. When mares were in full oestrus and therefore fully receptive, driving by stallions ceased and copulation occurred at irregular intervals. However, later in the season driving of mares even when they were in the early stages of oestrus was not common. Many mares were then in oestrus at the same time and stallions only showed any interest in mares when they were in full oestrus (see p. 181).

In steppe zebras, Klingel (1969) observed that the young mares commonly showed the typical oestrous posture but that older mares stood with their hind legs straddled and tails raised only when stallions actually approached and sniffed them. He concluded that the conspicuous oestrous posture of young mares promoted outbreeding as it attracted stallions from other family groups or from bachelor groups. These stallions then tried to abduct the young mares from their mothers' groups. The young mares would therefore be less likely to copulate with stallions in their mothers' groups; these stallions were their own fathers. Adult zebra mares did not need a conspicuous oestrus as they copulated with the family stallion resident in their own group and he could detect those mares in oestrus by olfactory means. No such difference in the display of the oestrous posture was observed between young and old New Forest pony mares; both displayed when some distance from a stallion and when close to him.

A further difference in the oestrous behaviour of zebra and of pony mares was the absence of the facial expression, 'Rossigkeitsgesicht' in the latter. Young inexperienced pony mares did however, snap when stallions approached and sniffed them.

Harems and Interactions between Stallions

Harem Formation

The first movements by stallions towards forming their 'harems' of mare groups began as early as February. Typically a stallion galloped up to one or more groups and with his head lowered and ears laid back, he drove the mares before him, swaying and shaking his head as he trotted or galloped. His head sometimes almost touched the ground. Occasionally he rushed out in front of the mares to prevent any of them from breaking away or to move them in a direction away from a rival stallion.

Driving was especially noted when two stallions encountered one another in the spring. After a fight each stallion galloped to some mares and drove them together into a tight bunch. Stable 'harems' were not properly established however, until the end of April. Many stallions,

which were kept on farms and smallholdings during the winter were then turned out onto the Forest; until then any stallion was usually the only male present in any area and relatively little driving occurred, except of individual mares in oestrus.

Driving of females by a male has been recorded in reindeer by Espmark (1964a), in caribou by Lent (1965), in moose (*Alces alces shirasi*) by Altmann (1959) and in many other artiodactyls by Walther (1958). Walther referred to it as 'Paarungsmarch' and considered it to be a modified threat posture directed to females.

Interactions between Stallions

In May there was much fighting between stallions and much movement of stallions from one area to another. The outcome of interactions depended largely on the age and strength of the animals involved. When one was much younger than the other, the older male soon drove him away, galloping after him and biting or kicking him whenever he approached. No real fights ensued but the presence of the young male did cause the old one to collect all the mare groups in the area into a tight bunch and to keep rounding up and driving back any mares that tried to leave. This continued for as long as the young stallion remained in the vicinity but he usually disappeared after 2 to 3 days. Sometimes an old stallion tolerated a young one sharing the same grazing area throughout the breeding season and even allowed him a harem of one or two mare groups. A 5-year-old stallion was present on an area with over fifty mares. He chased away a 3-year-old male whenever the latter approached any of the mares, except for one group of an old mare, her yearling and a 3-year-old mare. The younger stallion remained with this group throughout the season, whereas the older stallion copulated with mares in all the other groups.

When two stallions were of similar age or strength numerous fights occurred at first, followed by long bouts of driving of the mares. After about a week if the two still remained in the same area, they divided up the mare groups in that area and established their harems. Where numerous mare groups shared the same grazing area, as on a reseeded area, the two stallions each had a fairly constant daytime harem of some of the groups, but other groups formed no regular part of either harem. These groups were not jealously guarded by one male against the other as were the groups in the harems.

These observations were illustrated by the events which occurred in the second study area between 1965 and 1968. No stallions were present on this area in the winter of 1965 but at the end of April 1966 two adult stallions were released onto it. They both remained there throughout the breeding season, grazing on the 36 acres of reseeded area most days with up to ninety mares.

Many fights occurred between these two stallions during early May but fights became less frequent when each stallion had established a harem. During the nights each stallion usually remained with one particular mare group, with which he became very much more closely associated than with other groups in his harem. During the days he grazed and rested close to his own particular group but also near the other groups making up his harem. He seemed able to recognize the mares from all these groups by sight and defended all of them from the rival male. Certain mare groups belonged to neither harem and moved freely from one to the other.

Although the harems were not always kept strictly separate, they tended to occupy different parts of the reseeded area. Thus each time a mare belonging to one stallion moved towards the other, the first stallion galloped after her. He then often had a brief ritualized fight with the other, who trotted or galloped forward to meet him. The two stallions sniffed each other and sometimes pawed at each other but soon broke off to sniff a nearby pile of faeces upon which they both defaecated. They then galloped back to their own mares, the first driving back the straying mare as he did so. After such incidents

each stallion drove all his mares into a tight bunch and mares which didn't belong to either harem were sometimes rounded up too.

No real geographically defined territories existed despite the tendency for the two stallions and their harems to spend most of the time on opposite parts of the reseeded area. The part that each occupied frequently changed. Moreover, when one stallion did not move up from the valleys with his own mare group until late in the morning, the other stallion grazed over the whole area with mare groups of both harems. The two stallions and all their mares also used the same shade, which was a group of trees near the centre of the reseeded are. In the shade mares of the two harems were often intermingled and the stallions themselves only stood a few yards apart, each standing by his own mare group or by a mare in oestrus.

The so-called harems were not real harems as they did not consist of the same mares at all times. In the daytime each harem was usually fairly constant and made up of the same mares but some mares were often absent when they remained in the valleys and woods. In the evenings the mare groups of each harem moved off the reseeded area in different directions and at different times. Stallions often tried to delay their evening departure by rounding up and driving back any group that tried to walk off, but eventually this became impossible as so many groups were involved. Thus, at nights the harems were non-existent although each stallion did usually remain with his own group. Sometimes when a mare was in oestrus, a stallion spent the night with her or she followed him down into the valleys in the evening together with his own group.

In the autumn of 1966 one of the stallions was removed but the other remained with his mare group throughout the winter. In the spring of 1967 a younger stallion was released onto the area; after numerous initial fights with the resident male, he became associated with a mare group. This group formed the nucleus of his harem which was much smaller than that of the older resident.

In the autumn of 1967 the young stallion was removed but the other was again left out for the winter. He was challenged the following spring only by a 3-year-old stallion. Whenever this young male approached, the old male drove all the mares present into a bunch and prevented them from moving near the young one. At first the young one was seen with one or two mare groups but the old resident eventually drove these groups to his own large number and chased away the young stallion each time he moved within about 200 yd of them. No fights were observed as each time the old stallion galloped towards the young one, the latter galloped away from him. After 4 days the young male disappeared from the area and the old resident remained on his own throughout the season. The absence of any rivals did not necessitate prolonged bouts of driving and subsequent harem formation. However, on the few occasions that a neighbouring stallion wandered onto the area with a number of strange mares from his normal range, driving by the old resident of the mares on the reseeded area did occur.

Yearling and 2-year-old colts were tolerated by stallions within their harems and were not treated as rivals. They were allowed to attempt to copulate with young mares but when they approached adult mares in oestrus, the stallions drove them a short distance away. Some fights were observed between young colts and adult males but these were thought to be forms of play (p. 174).

Comparison of the Harems of New Forest Ponies with those of Other Equids

The harems of New Forest ponies seem to be atypical of wild equids whose social organization has been studied. They are presumably only the result of the artificially small number of stallions on the Forest.

In steppe zebras (Klingel 1964) and Sable

Island horses (Bruemmer 1967) the harem of each stallion is a single mare group and each mare group has a stallion attached to it, so forming a family group. Surplus stallions remain together in bachelor groups. That such an organization would probably exist in the New Forest ponies, if the sex ratio was more equal, is suggested by the fact that each stallion does usually become more closely associated with one mare group than with any others in his harem. He more jealously defends the mares in this one group than the mares in other groups. Morever, he remains with this group even during the winter.

Few detailed studies have been made of other wild equids but Alverdes (1927) recorded that in Asiatic wild horses, presumably Przewalski's horses, the stallions have harems of five to fifteen mares, and that South American semi-wild horse stallions also have harems of twelve to eighteen mares. Similarly, the jack Asiatic wild ass (*Equus hemionus*) collects a harem of three to fifty jennies, and the jack Nubian ass (*Equus asinus*) also collects together ten to fifteen jennies, which he defends against other jacks (McNight 1958).

Although these harems of females are larger than most of the mare groups belonging to each steppe zebra stallion or each Sable Island horse stallion, they are stable units, which remain with their males throughout the year. They therefore correspond closely with the zebra and Sable Island mare groups. In contrast, the harems of New Forest pony stallions are not stable and they are only in evidence during part of the year. It is therefore only each stallion's own particular mare group, which corresponds with the harem of other wild equids.

Pre-Copulatory Behaviour

Behaviour of Stallions and Mares Prior to Copulation

Typically, immediately before copulation occurred, a stallion stood behind a fully receptive mare, sniffed her genital region and showed the flehmen posture. Sometimes he snorted or whinnied excitedly whilst sniffing her. He began to nibble and lick her rump, hind legs and sometimes her forelegs, and showed erection of his penis. He then mounted her and after several pelvic thrusts usually achieved intromission. Occasionally a stallion mounted a mare when he had no erection and then, after dismounting, he either left or began to nibble and lick the mare again before making further copulatory attempts.

Stallions copulated in thirty-eight (62 per cent) of sixty-one observations in which they mounted fully receptive mares. Of these thirty-eight successful attempts the stallions achieved intromission twenty-one times (55 per cent) after mounting the mares only once. In the other successful attempts they mounted the mares a second or even a third time before achieving intromission; this occurred when a stallion was either not fully erect during his first attempt or when he was fully erect but failed to penetrate the mare's vulva on his first attempt after several pelvic thrusts. Of the twenty-three unsuccessful attempts the stallions mounted the mares once only in nineteen of them (83 per cent) and then moved away; they mounted twice in the remaining four attempts but then also moved away from the mares.

The behaviour of an adult mare in full oestrus was usually very passive after a stallion had begun to show some interest in her. She stood quietly with her hind legs straddled and her tail raised. Occasionally she squealed or pawed the air with her forefeet, and often turned her head and touched the stallion's muzzle during his intensive sniffing and nibbling of her. She also often urinated in small amounts, after which the stallion sniffed the urine on the ground or her genital region and again showed the flehmen posture.

Young, inexperienced mares in oestrus also stood with their hind legs apart and tail raised when a stallion approached and began to sniff them, but they showed signs of fear. They frequently moved away from the male and snapped or even kicked out at him. Mutual

grooming was observed on several occasions between stallions and young mares prior to copulation; one bout between a stallion and a 3-year-old mare continued for almost 30 min. Mutual grooming has also been observed between oestrous females and males of certain primates (Sparks 1967); in these it is thought to act as an appeasement gesture whereby the male overcomes the female's fear. It also seems probable that in ponies, mutual grooming initiated by stallions served to overcome the young mares' fear. It was not observed between stallions and old mares as a part of the pre-copulatory behaviour. After a stallion had mounted a young mare, she often continued to snap or sometimes walked forward. The stallion then moved with her on his hind legs until either she moved faster and caused him to dismount, or she stopped and he achieved intromission. Adult mares, who were mounted when they were not in full oestrus, also sometimes moved forward and caused the male to dismount.

True pre-copulatory behaviour was very brief, usually lasting for less than 1 min; as mentioned above, it was longer with a few young mares as it then involved bouts of mutual grooming. In other cases however, it was almost non-existent; a stallion then stopped grazing, looked up at a mare and rushed towards her, whinnying excitedly. On reaching her, he touched her nose upon which she squealed, sometimes pawed the air and then turned and presented to him. He immediately mounted with no preliminary sniffing or nibbling.

The small amount of pre-copulatory behaviour in ponies is in contrast to the situation in Grevy's zebras. These zebra stallions require 20 to 25 minutes of foreplay with a mare before they will copulate (Zeeb & Kleinschmidt 1963).

Behaviour of Other Ponies towards Stallions and Oestrous Mares

Dominant mares, which were either in oestrus or particularly those which were not in oestrus but were members of a stallion's own mare group, often tried to prevent other mares from approaching the stallion. These dominant mares remained close to him and threatened the more subordinate oestrous mares that came near him. This jealous behaviour may have been a factor contributing to the low proportion of young mares, which produced foals; such behaviour has also been recorded in zebras by Zeeb (1958).

Offspring of a mare in oestrus, either yearlings, 2- or 3-year-old fillies or even foals, occasionally prevented their own mother from copulation, by rushing around her and trying to move between her and the stallion. However, most foals stood a few yards from their mothers and interested stallions, nickered and snapped, but did not interfere.

Changes in the Behaviour of Stallions during the Breeding Season

Although the actual behaviour of a stallion towards a fully receptive mare immediately prior to copulation was similar throughout the breeding season, stallions did seem to play varying roles in bringing about copulation throughout this time. They were more active in seeking out mares very early in the spring but, later on when they had rival stallions and when several mares were often in oestrus at the same time, they played a much less active role. Mares were then chiefly responsible for bringing about copulation.

When a mare was in oestrus in early spring a stallion often remained close by her for several days. During the early part of oestrus he frequently drove her for short distances and occasionally tried to mount her, but she usually avoided his attempts. After several days she became receptive and then allowed him to sniff, nibble and mount her. Such temporary associations between a stallion and a mare were mainly due to the stallion, who kept near the mare even when she was not fully receptive. However, she did also frequently approach him during the latter part of their association.

These associations soon broke up when a rival male appeared on the area. The resident stallion became occupied with alternately fighting or chasing away the rival, and driving and

rounding up a number of mare groups. Even when the harems were established, the temporary associations between a single stallion and a single mare were not in evidence as several mares were often then in oestrus at the same time. Later in the season a stallion only remained with a fully oestrous mare for a few minutes before copulating; they then separated and the stallion soon approached or, more commonly, was approached by a different mare.

Temporary associations where a male tends a single female for several hours or days, have also been described in caribou (Lent 1965) and in moose (Altmann 1959). However, bulls of these species have no harems; they merely remain with successive cows for short periods throughout the rutting season. The bull moose tends a cow for 7 to 12 days during which he displays to her, drives her intermittently and copulates several times. He then leaves her and tends another cow. This is repeated throughout the months of August to November. In reindeer (Espmark 1964a), the bulls do have harems but when a cow is on heat, a bull will follow her, drive her intermittently for several hours and eventually copulate. Such an association only occurs when no other cows are in oestrus and therefore closely parallels the situation in ponies. The temporary consortships formed between a fully oestrous female and a male baboon also bear similarities to the temporary associations of an oestrous mare with a stallion. Consort pairs are common in primates but in the langur (*Presbytis entellus*) at least, the pairs do not stay together at night (Jay 1963).

Mares played a much more active part than stallions in bringing about copulation, except in the early spring when a few stallions were responsible for the temporary associations. When a mare was in full oestrus, she repeatedly approached a stallion, stood close by him or in front of him and displayed oestrous posture. The stallion showed little interest for much of the time and kept moving away. She followed him and grazed near him with her tail raised until eventually he sniffed her and copulated. After copulation had occurred the mare often remained in the vicinity of the stallion, soliciting at intervals.

Some mares even left their group companions or moved with their companions out of their normal home range when in oestrus. They sought out stallions and only returned after copulation had occurred. This active behaviour in seeking out a stallion was often necessary because of the small number of stallions on the Forest. After about the middle of May each stallion remained with the same mare groups in the same areas; mares in outlying areas where there were no stallions, would not have copulated unless they had searched for stallions. Ewes also will actively seek rams when they are in oestrus (Lindsay 1966); they then approach the rams and solicit by pushing against them. Similarly, reindeer cows at the peak of oestrus keep in the immediate vicinity of a bull (Espmark 1964a) and moose cows in oestrus also actively search for bulls by calling (Altmann 1959). Moreover, Washburn & De Vore (1962) recorded that oestrus in baboons may disrupt all other social relations and that the female may even leave her preference group and child to seek a male.

Quantitative data were not obtained for the number of copulatory attempts initiated by stallions and by mares, because of the difficulty in deciding which did in fact initiate them. Mares always determined the approximate time that copulation occurred according to their state of oestrus. When a mare was receptive, a stallion determined the precise time that copulation occurred by finally moving to her or by finally reacting to her oestrous posture. However, the mare really initiated copulation by previously approaching the stallion and presenting to him numerous times. Thus in late April 1967 seven mares tried to initiate copulation; they remained close to a stallion throughout a day and solicited repeatedly by standing directly in front of him and presenting to him. He ignored them for most of the time and even threatened them but during the day he copulated with three of them.

After the start of the breeding season stallions

generally only attempted to copulate with receptive mares, but, as already mentioned, in March and April they sometimes tried to copulate with mares that showed no signs of being in oestrus. The attempts with unreceptive mares were most commonly made by those stallions which had been taken off the Forest the previous autumn and hay-fed during the winter. When they were turned out in the spring, they drove mares for long periods and often singled out mares, which they then drove relentlessly. They bit them and tried to mount them. Eventually the mares either managed to break away from the stallions, fell, or a rival stallion or another pony took the stallions' interest. Such stallions even singled out yearling or 2- or 3-year-old fillies in which resident stallions rarely showed any interest. On one occasion a stallion chased a 3-year-old until she crossed a bridge and moved to an area where she had never previously been seen. The young mare remained on this area away from her original group companions but soon formed a close association with another young mare. These two mares were occasionally seen on the original area after several weeks had elapsed.

The copulatory attempts with unreceptive mares and the incessant driving of them by these stallions were thought to be due to the stallions having been so well fed during the winter and to their not having seen any mares since the previous autumn. The normally very low intensity stimulus of an anoestrous mare was an adequate stimulus to these stallions, whereas, later in the breeding season, only a mare at the peak of oestrus would act as such a stimulus and release copulatory behaviour. However, some of the mounting of young mares seemed to be of an aggressive nature; the stallions showed no erection of the penis when they mounted the mares, and they often bit them roughly.

Attacks were also made by these stallions on young mares and foals; three stallions were probably responsible for the death of six foals and the injury of several others. Two of the stallions were observed badly mauling a foal and a yearling filly, and five other foals disappeared from the same area within 2 weeks. Injuries on the body of one found dead suggested that a stallion had been responsible. On the occasion where a stallion was observed mauling a foal, he had only been out on the Forest for 5 days. He singled out the foal's mother and drove her almost continuously for over 30 min. The foal galloped alongside its mother, who was not in full oestrus, until eventually it became separated from her. The stallion immediately rushed to it, gripped its neck with his teeth and shook it. The mare then galloped to them and manoeuvred herself between them.

Hinde (1966) stated that when an animal is thwarted and cannot therefore complete a sequence of behaviour, its behaviour may then take a number of forms. It may respond to a normally inadequate stimulus or it may even show aggressive behaviour. The aggressive mountings by stallions of some mares or their attacks on foals may have been examples of such frustration-induced aggression; the attacks resulted from the stallions' high level drive to copulate being thwarted by the mares moving away and refusing their mounting attempts.

The aggressive mounting of mares by stallions in frustration situations seems to parallel the aggressive mounting of females, whether in oestrus or not, by males of certain species of Old World monkeys (Wickler 1967). This occurs, for example, when a monkey is shown food but the food is then withheld from it. Wickler believed that the frequent occurrence of such 'rage-induced copulation' (Wut-Kopulationen) supported his view that there is a strong relationship between sex and aggression; this relationship is also suggested by other extra-sexual social implications of the male copulatory posture, such as dominance mounting which has been observed in various primates by many observers and in wolves by Schenkel (1948).

Records of attacks on young animals by the males of many species of ungulates and other mammals are not uncommon, particularly in the rutting or breeding season. Thus there are records of an attack on a foal by a male Indian

wild ass (*Equus hemionus khur*) by David (1966), of attacks by reindeer bulls on calves or even cows by Espmark (1964a), and of attacks by males of several species of ungulates in captivity of young animals, even of other species, by Walther (1965). However, various observers have interpreted the attacks by the males as due to some cause other than induced by frustration.

Walther suggested that some attacks occur because the males, when in captivity, do not have the opportunity to satisfy their fight instincts against males of the same species. Frustration, especially in the breeding season, leads to substitute opponents being sought out and attacked. Thus zebra stallions in captivity have been known to attack their own foals and even small antelope calves or Watusi cattle bulls, cows or calves, when no rival stallions were present. These attacks are therefore also induced by frustration and the males then responded to inadequate stimuli.

However, other attacks have been interpreted as redirected activities. The attacks on calves by reindeer bulls occur when the calves approach them when they are marking and threatening approaching rival bulls (Espmark 1964a). There is then a conflict situation involving a tendency for the bull to flee and a tendency for it to attack the rival. The tendency for it to attack is redirected onto a calf or even a cow. Similarly, there must be a conflict situation when a stallion is attempting to copulate with a mare, and this must involve the tendency for him to behave sexually towards her and the tendency to attack her because she is close to him. He then redirects his aggression onto a nearby object, which may be a foal. If the attacks on foals were examples of redirected aggression, they would occur throughout the breeding season because a conflict situation must exist whenever a stallion approaches a mare in oestrus. As the attacks only occurred in the few weeks after fresh stallions had been turned out onto the Forest in the spring, it was concluded that they were frustration-induced rather than redirected activities.

However, the distinction between frustration-induced aggression and redirected aggression is not always clear. Two or more incompatible tendencies may be the source of frustration (Brown & Farber 1951) in which case frustration-induced aggression could merely be a form of redirected aggression. Wickler (1967) also believed that the 'rage-induced copulation' in certain Old World monkeys gave the impression of a redirected activity.

Although the attacks on foals and aggressive mountings of other young animals were not a true part of pre-copulatory behaviour, they have been included here because they illustrate differences in the behaviour of stallions left on the Forest throughout the winter from that of stallions turned out in late April. Moreover, they illustrate the difference in the behaviour of the freshly turned out stallions, which attempt to copulate with anoestrous mares, from that of the same stallions later in the season, when they will usually only attempt to copulate with mares at the peak of oestrus.

Copulation

Length and Frequency of Copulation

When an oestrous mare remained still for an adult stallion he usually achieved intromission after one or two mounting attempts; ejaculation then occurred after several pelvic thrusts. The stallion remained mounted for a few seconds after this with his head resting on the mare's back, and then he withdrew his penis and immediately dismounted.

The time taken from intromission to withdrawal and dismounting was very short in adult stallions of 3 years or more. The range in length of forty observations was 12 to 26 s, with a median of 15 s. It was not always possible to accurately determine the time taken from intromission to ejaculation but in the few cases where it was possible it varied from 9 to 15 s. The length of the resting period, i.e. the time from ejaculation to withdrawal varied from 5 to 10 s.

Young and inexperienced males had much difficulty in achieving copulation because of

their failure to penetrate the mare's vulva and sometimes because of their failure to ejaculate after they had achieved intromission. Failure of intromission was due to various causes: (a) the small size of the colt made copulation physically impossible; (b) mounting was disorientated, although this was very rare except in young foals; or (c) pelvic thrusts were disorientated. This was the most usual cause of failure.

Old and experienced mares moreover, did not tolerate young colts and they bit or kicked out at them when they approached to sniff them. Adult stallions also generally chased away colts from adult oestrous mares but allowed them to attempt to copulate with young females of up to 4 years, whom they themselves usually ignored.

During the receptive period, a mare copulated several times, usually within 2 to 3 days but occasionally over a much longer period of several weeks. Sometimes she copulated twice with an interval of only 5 min; she also sometimes copulated with two different stallions on the same day. The behaviour of the stallions of defending certain mare groups from rival males largely prevented this, but mares from those groups which formed no part of any harem, were able to copulate with two or more different stallions. Even a mare from a closely guarded group occasionally did so; on one such occasion a mare at the peak of oestrus was resting in a shade and a stallion to whose harem she did not belong was standing close by. He approached her, sniffed her, quickly mounted and achieved intromission. The other stallion then looked up and saw the pair in copulo, whereupon he immediately galloped towards them. As he did so, the intruding stallion saw him and quickly dismounted and galloped away.

When more than one mare in a harem was in oestrus at the same time, the stallion often copulated with several different mares on the same day. On 27 April 1967 seven mares, which were in oestrus were grazing and resting on the reseeded area in the second study area. They remained near the stallion for much of this time and between 10.20 and 16.15 hr he copulated six times, twice with three different mares. He also made four other unsuccessful copulatory attempts, two of them with two mares with which he did not copulate during that observation period. The remaining two mares were both 3-year-olds; although they solicited throughout the period, constantly approaching him and presenting in front of him, they were ignored or threatened by him.

Herbert (1968) found that when rhesus monkey males had a choice of several oestrous females, they usually had a favourite and did not mate with the non-favourite. Conversely in sheep and goats when several males attempt to mate with one female, the female may show a preference for one of them. In the New Forest ponies, females rarely had the choice of several adult males with which to mate but when they did, they usually mated with both stallions.

Copulation occurred at any time of the day. Of eighty-six observations of copulation, 33 per cent occurred between 06.30 and 10.30; 45 per cent between 10.30 and 14.30, and 22 per cent between 14.30 and 18.30 hr. Although more were observed around mid-day this partly reflected the time when more hours were spent in the field. Thus, of 1808 hours of observations between April and August in 1966 and 1967, 31 per cent were between 06.30 and 10.30, 40 per cent between 10.30 and 14.30, and 29 per cent between 14.30 and 18.30 hr. From a consideration of the number of hours of observation throughout the day, it therefore seemed that the frequency of copulation was similar in each of the three time periods.

Although no evidence was obtained, it seemed likely that copulation also occurred during the night. Ponies were active during the summer nights, grazing in the valleys and woods. Moreover, mares in oestrus often left the daytime grazing areas in the evenings with stallions, and the following morning they arrived on the grazing area together.

There was no evidence from this study that copulatory behaviour was at a greater level at dusk and dawn than at other times of the day.

This is in contrast to the behaviour of various cervids such as wapiti (Altmann 1952) and reindeer (Espmark 1964a), whose rutting activity is at its greatest level at these times. However, in reindeer at least, the animals are inactive and resting during the night, whereas ponies were not.

Behaviour of Other Ponies during Copulation

As mentioned above, dominant mares and the offspring of mares in oestrus sometimes tried to prevent other mares or their mothers from approaching a stallion. When a stallion managed to copulate with a mare, other mares in oestrus, group companions of the copulating mare and members of the stallion's own mare group sometimes stood close by the pair. They also sometimes rushed excitedly around the pair, even approaching and touching them. Foals, yearlings or older offspring of the mare often stood quietly near the pair and stared at them, or they rushed to and from or around them, nickering and snapping. A mare sometimes trotted or galloped up to a stallion, who was copulating with her daughter and tried to separate them. Dominant mares, especially those in the stallion's own group, even bit or kicked out at a young mare when she was copulating.

However, despite such incidents, most acts of copulation took place where no other mares showed any interest, although they were grazing or resting only a few yards away. They often did not even look up at the pair; yearling fillies and young colts however, showed more interest. These did not always approach the pair, but stared intently at them.

Post-Copulatory Behaviour

After a stallion had dismounted, he stood quietly for several seconds and then he often either sniffed the ground on which some ejaculate or the mare's urine had fallen, or he sniffed her genital region. After showing the flehmen posture, he then usually moved away from her. Sometimes he did not sniff either the ground or the mare's genital region but yawned and then began to graze or briefly drove other mares.

The mare after several seconds also sometimes made the first move away from the stallion, occasionally first touching his muzzle. The stallion moved from the mare after copulation in nine (26 per cent) of thirty-five observations whereas the mare moved from the stallion in twenty-one cases (60 per cent) whilst the stallion stood resting; both simultaneously moved from each other in five of the observations (14 per cent). The mare soon began to graze after she had moved a few yards from the stallion. On two occasions a mare followed a stallion when he moved from her, but usually the pair separated with very little or no contact soon after copulation. No post-copulatory bouts of mutual grooming were observed, as occur in zebras (Zeeb 1958).

Development of Sexual Behaviour in Immature Ponies

Mounting Behaviour

Mounting by foals of their mothers, their siblings or occasionally of another mare or a gelding in their mothers' groups was commonly observed in their first few weeks of life. Both colt and filly foals showed mounting behaviour, but it was much less common in fillies (p. 172). Moreover, mounting by fillies was not observed after their fourth week.

When a foal mounted its mother during its first week, it reared up from any side of her, so that its forefeet rested on her neck or on the side of her back. However, mounting with correct orientation of a foal's body to its mother's did occur during its first week, and after this time, incorrect mounting was unusual. It was also rare in yearling or 2-year-old colts.

Sexual Behaviour of Colts

Nibbling by colt foals of their mothers' hind legs, as the nibbling by stallions of the hind legs of mares in oestrus, was common, but it had no sexual context in foals and was thought to be play behaviour.

Full erections of the penis were observed in a colt younger than 1 month; they were common in 2- to 3-month-old colts when the colts were resting, when they were play-fighting together and also when they were grooming with filly foals. Colts also sometimes mounted filly foals and nibbled them, but mounting was not observed with an erect penis and the colts made no pelvic thrusts.

When their mothers were in oestrus, colts snifled their urine or their genital region and showed the flehmen posture, but, with one exception, no colt took any further interest in a mare in oestrus. The exception was a 3-month-old colt, who repeatedly approached a 2-year-old filly in oestrus and who showed a pattern of behaviour identical to that shown by adult stallions towards mares in oestrus. After approaching the filly, he sniffed her genital region, showed the flehmen posture and sniffed and nibbled her hind legs and rump. She stood still with her tail raised and hind legs slightly apart, and he then mounted her with an erect penis and made numerous pelvic thrusts. However, his small size prevented intromission and after a few seconds he dismounted. This pattern was repeated many times over a period of an hour.

The age at which a colt was first seen to achieve copulation varied from 15 months to almost 3 years. Before the 15-month-old colt copulated successfully, he made numerous attempts to do so. After his success, he soon copulated with two other young mares; none of these mares foaled the following year. Even after his first success, copulation still took much longer and more mounting attempts than that by adult stallions. Sometimes the colt achieved intromission but he then dismounted without ejaculating.

No other yearling colt was observed copulating but many showed great interest in mares in oestrus, sniffing, nibbling and even mounting them. Adult mares rarely allowed such colts to mount them but young inexperienced mares were more tolerant. One colt made his first copulatory attempts when 2 years old.The previous breeding season he remained with his mother and was never observed mounting a mare, but in the spring of his third year he remained away from his mother for 4 days with a 2-year-old filly, who was in oestrus. During this time he remained close by her, grazing near her some of the time but often whinnying excitedly and trotting to her, sniffing, nibbling and mounting her. Within one ½-hr period he made over ten mounts and numerous pelvic thrusts with a fully erect penis. He did not achieve intromission because the thrusts were disorientated. The young mare stood quietly for many of his attempts but after he had been mounted a few seconds, she often walked forward or even kicked out at him.

Another colt was not observed making any copulatory attempts until he was almost 3 years old. Until then he was closely associated with his mother's group. There were also two adult stallions on the area with only about fifty mares, and these stallions probably prevented him from mounting any mares as on several occasions when he did approach oestrous mares, the old stallions chased him away. When the two old stallions were removed, the young male then copulated successfully. However, it is possible that earlier attempts were made by him but were not observed.

Sperms may occur in the testes of thoroughbred colts at about 13 months but both testes are not of mature weight until about 27 months, and 20 months is the earliest recorded age of a colt that had testes of mature weight (Nishikawa 1959). However, most colts can produce some sperms at 16 to 20 months (Cole & Cupps 1959). New Forest pony colts were such an age in the autumn of their second year, when very few mares were still showing oestrous cycles. Thus most colts would be expected to copulate for the first time in the spring of their third year.

The behaviour of adult, experienced mares and of stallions prevented these young colts from copulating with adult mares, but the colts were able to mount young mares of up to 4 years. In steppe zebras however, the rivalry between stallions ensured that only fully adult males

could establish family groups and therefore copulate; the youngest family stallions were 5 to 6 years old (Klingel 1965). Younger males remained in the bachelor groups until they were old enough to compete for mares. They therefore did not have the opportunity that the young pony colts did, to attempt copulation with young mares. King (1965) found that Grant's and Grevy's zebra colts reached puberty at 2½ and 4 years respectively, but that they were also living in the bachelor groups at this time. They did not consort with mares until a year or two later.

Sexual Behaviour of Fillies

Thoroughbred fillies have their first oestrus when 16 to 17 months old (Nishikawa 1959) and steppe zebra fillies between 12 to 15 months (Klingel 1965). New Forest pony fillies also showed signs of oestrus before they were 2 years old. Thus one filly came into oestrus when 14½ months and two other fillies had their first oestrus in their second summer (p. 175).

Although the fillies showed signs of oestrus as yearlings, very few conceived at this age. Thus of 107 mares only one foaled when she was 2 years old. This may be because the fillies were not fertile even though they showed the oestrous posture. Klingel (1965) believed that steppe zebra fillies were not fertile until they were 2½ years old, although they had their first oestrus as yearlings and King (1965) also found that conception in Grevy's zebras did not generally occur until at least two or three oestrous cycles had elapsed.

The pony stallions also usually ignored very young mares and although colts sometimes copulated with them, these colts were probably infertile themselves. Only one of the three fillies mentioned above, that showed signs of oestrus as yearlings, was mounted by a stallion. That occurred after the filly had repeatedly presented to him in the manner of an adult mare. He at first ignored her but eventually mounted her. However, he did not copulate and quickly dismounted and left her.

Unlike colt foals, filly foals showed no precocious sexual behaviour. When they reached puberty and showed their first oestrus, they then solicited in front of males, presenting to them and urinated frequently, in much the same manner as an older mare; prior to this, no behaviour was observed that could be classed as sexual, except the mounting that occasionally occurred in very young foals.

Masturbation

Both adult mares and stallions showed signs of eroticism when they were rubbing against external objects such as trees, stumps and posts. This was evident in mares particularly when they were rubbing the base of their tails, their buttocks and their vulval regions. Such a mare stretched up her head, swayed it from side to side and quivered her lips, as she was rubbing. This usually continued for several minutes. Two very tame ponies frequently approached me and after I had rubbed and scratched their necks and around their ears, they turned around and invited rubbing of the tail region. They remained for as long as the rubbing continued, and if it ceased, they sometimes stepped backwards as if to invite further grooming.

Stallions when rubbing swayed their heads and quivered their lips as did mares, and they showed erection of the penis. This also occurred when stallions were mutually grooming even with anoestrous mares. There therefore seemed to be a definite association between grooming and erection. Darling (1937) similarly associated rubbing with erection of the penis in red deer (*Cervus elaphus*) and referred to rubbing as masturbatory. Deniston (1956) however, who observed erection of the penis in moose when the bulls were rubbing their antlers against trees, believed that the two phenomena were separate and unrelated responses.

When stallions were standing resting, they often showed erection of their penis and repeatedly swung the penis forward against the belly. Wickler (1967) made very similar observations in certain primates. He found that the males of wild *Cercopithecus aethions* sat when resting with their thighs spread and penis

erected. The penis was then repeatedly but irregularly struck against the stomach with a jerky action. Male baboons (*Papio anubis*, *P. ursinus* and *P. hamadryas*) also sit around with their thighs spread and penis hanging extended but not erect, whilst the rest of the group eats. Although these males have been referred to as sentinels or guards, there is no evidence for this. Wickler concluded that these males serve to warn conspecifics of the presence of a group and that 'they have retained the basis of the response which served for urine-marking of territory in the lower primates, but actual urine discharge is lost and replaced by optical conspicuousness of the genitals'.

The possibility of such a function in the ponies cannot be ruled out because the stallions did generally only show erection of the penis and jerky movements of it, during the breeding season, when they were standing resting near their harems; such movements were also very conspicuous. However, urine discharge for the 'marking' of faeces of mares is not lost in ponies. The erection and movements of the penis in a resting stallion may have been only a form of masturbation, with no significance for other stallions or mares.

IX. DISCUSSION

The first two parts of this paper briefly describe the New Forest ponies and the study areas, and contain some data concerning the ecology of these areas and the population dynamics of the ponies. The third part concerns the general maintenance behaviour of these animals, much of which has previously been similarly described for horses and other equids. Part IV on social behaviour comprises the bulk of the paper and describes in detail the group organization of the ponies and the relationships between them both within and between groups.

There is a great scarcity in the literature of detailed reports of the social behaviour of wild or feral equids with the exception of Klingel's work on steppe, Grevy's and mountain zebras. This study on New Forest ponies was much frustrated by man's interference with the ponies, which resulted in the breaking up of groups, the removal of most foals at about 4 months of age and the removal of most adult males from the Forest. However, despite this interference, the resulting observations of social behaviour can be usefully compared with those observations of Klingel on zebras. Studies are at present being made on feral horses in the western United States, on Camargue horses and on Welsh ponies in the Gower peninsula and it will be of much interest in the future to compare the social organization of these equids with that of the New Forest ponies and zebras (see 'Postscript', p. 192).

Many comparisons have been made throughout this paper with primates, as well as with other ungulates, mainly because there is such a large volume of detailed literature available on the social behaviour of primates and because ponies and primates have much in common with regard to their group organization.

Wickler (1967) in describing primate groups, described a group as a relatively stable association of a limited number of conspecifics which stay together without the existence of a common extra goal. The animals differentiate between group members and foreign conspecifics; a rather stable rank order within the group further shows that group members recognize each other individually. An exchange of members between groups does not normally occur. The small stable associations involving two or more adult mares clearly fit into this definition of a group. The only members of a pony group that normally leave the group, are immature animals; these might drift from one group to another staying for a few days or months before moving on. Sometimes two groups were very closely associated in the summer and could be confused for a single unit, but they generally separated from each other in the evenings. The smallest stable units are therefore here called groups and the few close, but nonetheless temporary, associations between two groups are called super-groups. There seems to be a maximum size for a group of about seven animals and most groups only contained two or three adults.

The New Forest pony groups compared very closely with groups of steppe and mountain zebras (Klingel 1964, 1965, 1966, 1967, 1968, 1969), the maximum size for any of these groups being only seven or eight animals. A single stallion, the family stallion, was attached to each zebra group, with surplus stallions forming bachelor groups; only a small proportion of pony groups included a stallion but this is explained by the small number of stallions in the whole Forest. The age that immature animals left the groups was similar in the ponies and in the zebras.

A large number of pony mares did not form an association with other mares; they were solitary or associated only with one or more of their own offspring. This is unlike zebras and might be due to the artificial situation in the New Forest; owners of mares often remove them, particularly in the late autumn, and so disrupt relationships. Imanishi (1950) also found that in the seventy or so semi-wild horses in Toimisaki, one-third lived solitary, whereas the rest were associated into small groups of four to eight animals.

Allogrooming in the ponies, unlike primates, was almost always mutual, and within the group mutual grooming was common. Allogrooming is thought to be important for the cohesion of the group as it works against enforcement of the hierarchy, serving to reduce avoidance or fear responses in subordinates and so allowing dominant animals to maintain social contact with those of lower status (Sparks 1967).

Mutual grooming was rare between ponies from different groups and when it did occur was more usual between young animals from the different groups. When adult mares were involved, it is thought that either some family relationship was indicated or that the mares had once belonged to the same group. It is emphasized that the restriction by any adult mare in her choice of grooming partners to one or two members within her group was not because she did not approach many others closely. Close contact, particularly in the summer in the shade, occurred between all the ponies of any one population and a population might number up to one hundred individuals. Moreover, even within a large group one mare did not necessarily groom with every other mare in the group and it is of some interest that in a group of only two mares and some of their offspring, the two mares were never observed grooming together in the 3 years of study. They only groomed with their offspring and yet the two mares formed a very stable unit. Similarly, in monkey troops allogrooming relationships are not random. Each monkey narrows its choice of grooming partners within the troop (Sparks 1967). In red avadavats (*Amandara amandara*) too, each bird narrows its choice of clumping partners within the flock and only individuals which form a clumping group will tend to allopreen each other (Sparks 1962).

During a grooming bout between ponies, the subordinate animal very rarely broke off to scratch or nibble itself, whereas the dominant animal frequently did so. Moreover, the subordinate rarely ended a bout. It is of interest that Reynolds (1961) observed in rhesus monkeys that when a subordinate groomed a dominant monkey, she groomed busily with constant adjustment to the groomee's change of position, whereas when a dominant groomed a subordinate, she groomed with less vigour and often broke off to scratch herself (Sparks 1967).

A clearly defined stable hierarchy was found to exist not only within a group but also between all groups of a population, i.e. those that came regularly into contact. Dominance, which has been defined as the priority of access to a desired object (Chance 1956; De Vore 1965) was largely determined by age in the ponies, with size and hence presumably weight and strength, important amongst older animals. This is in agreement with most other studies of dominance in ungulates where the closely related factors size, weight and strength usually determine rank, and these factors are obviously to some extent correlated with age. The size of antlers or horns, however, is also important in determining rank in such ungulates as deer and cows.

The rank order of any one group of mares was a simple linear stable one. This is unlike the rank orders in baboon troops (De Vore 1963) and langur groups (Jay 1963), where dominance patterns among females are unstable and fluctuate with changes in their reproductive cycle. The rank orders of males in these primates are however stable. In the New Forest ponies, as far as can be determined from the small number of stallions co-existing, the stallions also show a stable linear hierarchy.

Any interactions involved only two individuals, although mares did aid their foals when these were threatened and foals, when close to their mothers, tended to assume their mothers' rank. There was no real dominance of one group over another although a very aggressive mare in one group could make her group effectively dominant over another group in food situations. Imanishi (1950) after making brief studies of semi-wild horses in Toimisaki, concluded that a large group was dominant over a smaller one, but this was not apparent in the ponies.

There was some evidence from this study that

the offspring of a high ranking mother tended to achieve high rank themselves. Koford (1963) also found this tendency among rhesus monkeys and Sade (1966) and Marsden (1968) have confirmed that rank of the mother does influence that of her offspring. De Vore (1963) believed that infants of dominant female baboons were probably affected by their mothers' dominant behaviour, and Imanishi (1960) found that infants of dominant female Japanese monkeys grow up in the troop's centre and apparently learn from adults round them a domineering attitude towards peripheral troop members, and are therefore more likely to adopt roles of dominance and leadership in later life. It seems likely that foals of dominant mares are similarly affected by their mothers' attitude to more subordinate mares.

Leadership of a group by the dominant mare of the group was the rule in the ponies and the rest of the mares followed in their order of dominance with their offspring following them in their order of youth. Such a single file movement has only been previously described in steppe and mountain zebras (Klingel 1964, 1965, 1966, 1967, 1968, 1969). In the semi-wild horses of Toimisaki, Imanishi (1950) found that the male was not necessarily the leader of a group of mares. Observations on leadership in other ungulates are scarce or anecdotal. Scott (1945) recorded that although goats showed little tendency to follow one leader, sheep did consistently follow a single leader, the oldest ewe. Other flock members follow this ewe in single file but in no set order. Grubb & Jewell (1966) however, found that Soay sheep had no definite flock leader but that older animals usually initiated movement. In primates, males usually lead the groups; thus in langurs (Jay 1963) several males lead the troop but no regular pattern is apparent in the rest of the troop. From the evidence available for zebras and New Forest ponies, it seems that the position of the males in these equids is usually at the rear of the group or at the group's side. It is possible for a stallion to 'lead' his group by directing its movement from behind. His position would protect the youngest and most subordinate animals from attack from the rear and when the group was fleeing from danger, his position would again be the best for the group's defence.

The mother–foal relationship has been largely discussed in the appropriate section and mention will only be made here of the actual development of the bond which compares closely with that described for sheep and goats.

The importance of the first $\frac{1}{2}$ hr after parturition, when a mare licks her foal and quickly learns to discriminate between it and other foals, has been mentioned in Part IV. This closely parallels the known situation in sheep and goats. In the latter only 5 to 10 min of contact are necessary for a mother to establish the identity of her own kid (Hersher, Richmond & Moore 1963). The birth membranes and fluids are obviously of prime importance in the formation of the mother–young relationship in ponies as they are in sheep and goats. Collias (1956) found that ewes could be attracted even by rags soaked in birth fluids, but they would still accept lambs when these had been washed with strong detergents. The birth membranes are therefore important but not essential for the formation of the bond.

After the mother–foal bond has been established, sight seemed to be important for the recognition of the foal by the mare at a distance; when they were in direct contact, smell was probably important as the mare usually nuzzled her young foal when they approached one another. Similarly, in sheep, sight is the most important sense for the recognition of the lamb by the ewe at a distance and some other more precise sensory cue such as smell, is important when lamb and ewe are in direct contact (Lindsay & Fletcher 1968). Auditory cues to recognition are probably not very important in sheep as ewes will respond to bleats of any young. However, ponies did seem to respond to their own foals' calls and not to calls of other foals. When foals were about a month old, they also seemed to

distinguish their own mother's calls from those of other mares. Foals of this age could also recognize their mothers by sight as well as by sound, but lambs could not do so until 4 to 5 months of age (Hersher, Richmond & Moore 1963).

Postscript

Since this paper was written, several behavioural studies have been started on various equids, including feral horses in the north and west of the United States of America, Connemara ponies, wild asses and Cape Mountain zebras.

X. SUMMARY

Between October 1965 and October 1968 nearly 4000 hr were spent observing the semi-wild ponies in the New Forest. Observations were made mainly in daylight in two study areas totalling over 5 square miles and containing about 300 mares, their offspring and up to twelve stallions.

Qualitative and some quantitative data were obtained on aspects of maintenance behaviour including grazing, resting, elimination and grooming, but the social organization of the ponies received more attention.

Ponies were associated into small groups of which about 60 per cent were family groups consisting of one adult mare and a number of her offspring; about 28 per cent consisted of two mares and their offspring and only 12 per cent of more (up to six) mares. Associations between adult unrelated mares were very stable but immature ponies changed groups. Any stallion generally became associated with one mare group. The organization of the ponies has been compared with that of other wild equids.

Dominance–subordinance relationships between mares in a group and of different groups which regularly came into contact were also stable; hierarchies were chiefly linear but some were complicated by triangular relationships. The rank of a mare was largely dependent on her age and size. Stallions were dominant over most mares in competition for food, but mares in oestrus or with young foals did threaten stallions.

Mutual grooming occurred most commonly between members of a group or between related ponies in different groups.

Each group occupied a small, stable home range but home ranges of different groups freely overlapped. Diurnal and seasonal patterns of movements were recorded within the ranges.

Auditory, visual and olfactory means of communication between ponies have been described.

A detailed study of the changing mother–young relationship was made by means of check sheets on which the behaviour of mares and their foals was recorded every ½-min for 1 hr, at intervals throughout the foals' first year. Data were analysed to compare the behaviour of colts and fillies and that of foals of primiparous mares and of multiparous mares, and to assess differences in behaviour with time of day, but chiefly to demonstrate changes in the behaviour of mare and foals with the foals' age.

Play was observed between immature ponies and between immature and adult ponies. Colts played more frequently than did fillies.

Various aspects of sexual behaviour, particularly the active behaviour of mares in oestrus in seeking out stallions, and changes in stallions' behaviour during the breeding season, are described.

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party, noting on the document filed, or on the transmitting letter, that a copy has been so furnished.

* * * * *

PART 966—RULES OF PRACTICE IN PROCEEDINGS RELATIVE TO ADMINISTRATIVE OFFSETS INITIATED AGAINST FORMER EMPLOYEES OF THE POSTAL SERVICE

■ 4. The authority citation for 39 CFR part 966 continues to read as follows:

Authority: 31 U.S.C. 3716; 39 U.S.C. 204, 401, 2601.

■ 5. In § 966.4, revise paragraph (c), and add a sentence at the beginning of paragraph (d) to read as follows:

§ 966.4 Petition for a hearing and supplement to petition.

* * * * *

(c) Within thirty (30) calendar days after the date of receipt of the Accounting Service Center's decision upon reconsideration, after the expiration of sixty (60) calendar days after a request for reconsideration where a reconsideration determination is not made, or following an administrative offset taken without prior notice and opportunity for reconsideration pursuant to paragraph (b)(1) of this section, the former employee must file a written petition electronically at *https://uspsjoe.justware.com/justiceweb*, or by mail at Recorder, Judicial Officer Department, United States Postal Service, 2101 Wilson Blvd., Suite 600, Arlington, VA 22201–3078.

(d) A sample petition is available through the Judicial Officer Electronic Filing Web site (*https://uspsjoe.justware.com/justiceweb*).* * *

* * * * *

■ 6. Revise paragraph (a) of § 966.6 to read as follows:

§ 966.6 Filing, docketing and serving documents; computation of time; representation of parties.

(a) *Filing.* After a petition is filed, all documents required under this part must be filed using the electronic filing system unless the Hearing Official permits otherwise. Documents submitted using the electronic filing system are considered filed as of the date/time (Eastern Time) reflected in the system. Documents mailed to the Recorder are considered filed on the date mailed as evidenced by a United States Postal Service postmark. Filings by any other means are considered filed upon receipt by the Recorder of a complete copy of the filing during normal business hours (Normal Recorder office business hours are between 8:45 a.m. and 4:45 p.m., Eastern Time). If both parties are participating via the electronic filing system, separate service upon the opposing party is not required. Otherwise, documents shall be served personally or by mail on the opposing party, noting on the document filed, or on the transmitting letter, that a copy has been so furnished.

* * * * *

Stanley F. Mires,

Attorney, Federal Compliance.

[FR Doc. 2015–16141 Filed 6–30–15; 8:45 am]

BILLING CODE 7710–12–P

DEPARTMENT OF THE INTERIOR

Fish and Wildlife Service

50 CFR Part 17

[4500030115]

Endangered and Threatened Wildlife and Plants; 90-Day Findings on 31 Petitions

AGENCY: Fish and Wildlife Service, Interior.

ACTION: Notice of petition findings and initiation of status reviews.

SUMMARY: We, the U.S. Fish and Wildlife Service (Service), announce 90-day findings on various petitions to list 30 species and one petition that describes itself as a petition to reclassify one species under the Endangered Species Act of 1973, as amended (Act). Based on our review, we find that eight petitions do not present substantial scientific or commercial information indicating that the petitioned actions may be warranted, we find that one petition does not present substantial information that the petitioned entity may be a listable entity under the Act, and we find that one petition does not present substantial information that the petitioned entity may be a listable entity under the Act and does not present substantial scientific or commercial information indicating that the petitioned action may be warranted, and we are not initiating status reviews in response to these petitions. We refer to these as "not-substantial petition findings." Based on our review, we find that 21 petitions present substantial scientific or commercial information indicating that the petitioned actions may be warranted. Therefore, with the publication of this document, we are initiating a review of the status of each of these species to determine if the petitioned actions are warranted. To ensure that these status reviews are comprehensive, we are requesting scientific and commercial data and other information regarding these species. Based on the status reviews, we will issue 12-month findings on the petitions, which will address whether the petitioned action is warranted, as provided in section 4(b)(3)(B) of the Act.

DATES: To allow us adequate time to conduct the status reviews, we request that we receive information on or before August 31, 2015. Information submitted electronically using the Federal eRulemaking Portal (see **ADDRESSES**, below) must be received by 11:59 p.m. Eastern Time on the closing date.

ADDRESSES: Not-substantial petition findings: The not-substantial petition findings announced in this document are available on *http://www.regulations.gov* under the appropriate docket number (see Table 1, below). Supporting information in preparing these findings is available for public inspection, by appointment, during normal business hours by contacting the appropriate person, as specified under **FOR FURTHER INFORMATION CONTACT**.

TABLE 1—NOT-SUBSTANTIAL PETITION FINDINGS

Species	Docket No.	Docket link
Blue Ridge gray-cheeked salamander.	FWS–R4–ES–2015–0042 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0042*
Caddo Mountain salamander	FWS–R4–ES–2015–0043 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0043*
California giant salamander	FWS–R8–ES–2015–0044 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R8-ES-2015-0044*
Colorado checkered whiptail	FWS–R6–ES–2015–0048 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R6-ES-2015-0048*
Distinct population segment of North American wild horse.	FWS–R8–ES–2015–0049 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R8-ES-2015-0049*

TABLE 1—NOT-SUBSTANTIAL PETITION FINDINGS—Continued

Species	Docket No.	Docket link
Gray wolf, excluding Mexican wolf, in the conterminous U.S.	FWS–HQ–ES–2015–0072	*http://www.regulations.gov/#!docketDetail;D=FWS-HQ-ES-2015-0072*
Olympic torrent salamander	FWS–R1–ES–2015–0056 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R1-ES-2015-0056*
Pigeon Mountain salamander.	FWS–R4–ES–2015–0058 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0058*
Weller's salamander	FWS–R4–ES–2015–0065 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0065*
Wingtail crayfish	FWS–R4–ES–2015–0067 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0067*

Status reviews: You may submit information on species for which a status review is being initiated (see Table 2, below) by one of the following methods:

(1) *Electronically:* Go to the Federal eRulemaking Portal: *http://www.regulations.gov*. In the Search box, enter the appropriate docket number (see Table 2, below). Then click the Search button. You may submit information by clicking on "Comment Now!" If your information will fit in the provided comment box, please use this feature of *http://www.regulations.gov*, as it is most compatible with our information review procedures. If you attach your information as a separate document, our preferred file format is Microsoft Word. If you attach multiple comments (such as form letters), our preferred format is a spreadsheet in Microsoft Excel.

(2) *By hard copy:* Submit by U.S. mail or hand-delivery to: Public Comments Processing, Attn: [Insert appropriate docket number; see table below]; U.S. Fish and Wildlife Service, MS: BPHC, 5275 Leesburg Pike; Falls Church, VA 22041–3803.

We request that you send information only by the methods described above. We will post all information received on *http://www.regulations.gov*. This generally means that we will post any personal information you provide us (see the Request for Information section, below, for more details).

TABLE 2—SUBSTANTIAL PETITION FINDINGS

Species	Docket number	Docket link
Alligator snapping turtle	FWS–R4–ES–2015–0038 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0038*
Apalachicola kingsnake	FWS–R4–ES–2015–0039 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0039*
Arizona toad	FWS–R2–ES–2015–0040 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R2-ES-2015-0040*
Blanding's turtle	FWS–R3–ES–2015–0041 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R3-ES-2015-0041*
Cascade Caverns salamander.	FWS–R2–ES–2015–0045 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R2-ES-2015-0045*
Cascades frog	FWS–R1–ES–2015–0046 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R1-ES-2015-0046*
Cedar Key mole skink	FWS–R4–ES–2015–0047 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0047*
Foothill yellow-legged frog	FWS–R8–ES–2015–0050 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R8-ES-2015-0050*
Gopher frog	FWS–R4–ES–2015–0051 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0051*
Green salamander	FWS–R4–ES–2015–0052 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0052*
Illinois chorus frog	FWS–R3–ES–2015–0053 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R3-ES-2015-0053*
Kern Canyon slender salamander.	FWS–R8–ES–2015–0054 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R8-ES-2015-0054*
Key ringneck snake	FWS–R4–ES–2015–0055 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0055*
Oregon slender salamander	FWS–R1–ES–2015–0057 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R1-ES-2015-0057*
Relictual slender salamander	FWS–R8–ES–2015–0059 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R8-ES-2015-0059*
Rim Rock crowned snake	FWS–R4–ES–2015–0060 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0060*
Rio Grande cooter	FWS–R2–ES–2015–0061 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R2-ES-2015-0061*
Silvery phacelia	FWS–R1–ES–2015–0062 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R1-ES-2015-0062*
Southern hog-nosed snake	FWS–R4–ES–2015–0063 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R4-ES-2015-0063*
Spotted turtle	FWS–R5–ES–2015–0064 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R5-ES-2015-0064*
Western spadefoot toad	FWS–R8–ES–2015–0066 ..	*http://www.regulations.gov/#!docketDetail;D=FWS-R8-ES-2015-0066*

FOR FURTHER INFORMATION CONTACT:

Species	Contact information
Alligator snapping turtle.	Andreas Moshogianis; (404) 679–7119
Apalachicola kingsnake.	Andreas Moshogianis; (404) 679–7119
Arizona toad	Michelle Shaughnessy; (505) 248–6920
Blanding's turtle	Laura Ragan; (612) 713–5350
Blue Ridge gray-cheeked salamander.	Susan Cameron; (828) 258–3939, ext. 224
Caddo Mountain salamander.	Andreas Moshogianis; (404) 679–7119
California giant salamander.	Dan Russell; (916) 414–6647
Cascade Caverns salamander.	Michelle Shaughnessy; (505) 248–6920
Cascades frog	Paul Henson; (503) 231–6179
Cedar Key mole skink.	Andreas Moshogianis; (404) 679–7119
Colorado checkered whiptail.	Leslie Ellwood; (303) 236–4747
Distinct population segment of North American wild horse.	Doug Krofta; (703) 358–2527
Foothill yellow-legged frog.	Dan Russell; (916) 414–6647
Gopher frog	Andreas Moshogianis; (404) 679–7119
Gray wolf, excluding Mexican wolf, in the conterminous U.S.	Don Morgan; (703) 358–2444

Species	Contact information
Green salamander	Andreas Moshogianis; (404) 679–7119
Illinois chorus frog	Laura Ragan; (612) 713–5350
Kern Canyon slender salamander.	Dan Russell; (916) 414–6647
Key ringneck snake.	Andreas Moshogianis; (404) 679–7119
Olympic torrent salamander.	Eric Rickerson; (360) 753–9440
Oregon slender salamander.	Paul Henson; (503) 231–6179
Pigeon Mountain salamander.	Andreas Moshogianis; (404) 679–7119
Relictual slender salamander.	Dan Russell; (916) 414–6647
Rim Rock crowned snake.	Andreas Moshogianis; (404) 679–7119
Rio Grande cooter	Michelle Shaughnessy; (505) 248–6920
Silvery phacelia	Paul Henson; (503) 231–6179
Southern hog-nosed snake.	Andreas Moshogianis; (404) 679–7119
Spotted turtle	Wende Mahaney; (207) 866–3344
Weller's salamander.	Susan Cameron; (828) 258–3939, ext. 224
Western spadefoot toad.	Dan Russell; (916) 414–6647
Wingtail crayfish ...	Patty Kelly; (850) 769–0552, x 228

If you use a telecommunications device for the deaf (TDD), please call the Federal Information Relay Service (FIRS) at 800–877–8339.

SUPPLEMENTARY INFORMATION:

Request for Information

When we make a finding that a petition presents substantial information indicating that listing, reclassification, or delisting a species may be warranted, we are required to promptly review the status of the species (status review). For the status review to be complete and based on the best available scientific and commercial information, we request information on alligator snapping turtle, Apalachicola kingsnake, Arizona toad, Blanding's turtle, Cascade Caverns salamander, Cascades frog, Cedar Key mole skink, foothill yellow-legged frog, gopher frog, green salamander, Illinois chorus frog, Kern Canyon slender salamander, Key ringneck snake, Oregon slender salamander, relictual slender salamander, Rim Rock crowned snake, Rio Grande cooter, silvery phacelia, southern hog-nosed snake, spotted turtle, and western spadefoot toad from governmental agencies, Native American Tribes, the scientific community, industry, and any other interested parties. We seek information on:

(1) The species' biology, range, and population trends, including:

(a) Habitat requirements;

(b) Genetics and taxonomy;

(c) Historical and current range, including distribution patterns;

(d) Historical and current population levels, and current and projected trends; and

(e) Past and ongoing conservation measures for the species, its habitat, or both.

(2) The factors that are the basis for making a listing, reclassification, or delisting determination for a species under section 4(a)(1) of the Act (16 U.S.C. 1531 *et seq.*), which are:

(a) The present or threatened destruction, modification, or curtailment of its habitat or range (Factor A);

(b) Overutilization for commercial, recreational, scientific, or educational purposes (Factor B);

(c) Disease or predation (Factor C);

(d) The inadequacy of existing regulatory mechanisms (Factor D); or

(e) Other natural or manmade factors affecting its continued existence (Factor E).

(3) The potential effects of climate change on the species and its habitat.

(4) If, after the status review, we determine that listing is warranted, we will propose critical habitat (see definition in section 3(5)(A) of the Act) under section 4 of the Act for those species that fall within the jurisdiction of the United States, to the maximum extent prudent and determinable at the time we propose to list the species. Therefore, we also specifically request data and information for the 21 species for which we are conducting status reviews on:

(a) What may constitute ''physical or biological features essential to the conservation of the species,'' within the geographical range occupied by the species;

(b) Where these features are currently found;

(c) Whether any of these features may require special management considerations or protection;

(d) Specific areas outside the geographical area occupied by the species that are ''essential for the conservation of the species''; and

(e) What, if any, critical habitat you think we should propose for designation if the species is proposed for listing, and why such habitat meets the requirements of section 4 of the Act.

Please include sufficient information with your submission (such as scientific journal articles or other publications) to allow us to verify any scientific or commercial information you include.

Submissions merely stating support for or opposition to the actions under consideration without providing supporting information or analysis, although noted, will not be considered in making a determination. Section 4(b)(1)(A) of the Act directs that determinations as to whether any species is an endangered or threatened species must be made ''solely on the basis of the best scientific and commercial data available.''

You may submit your information concerning these status reviews by one of the methods listed in the **ADDRESSES** section. If you submit information via *http://www.regulations.gov*, your entire submission—including any personal identifying information—will be posted on the Web site. If you submit a hardcopy that includes personal identifying information, you may request at the top of your document that we withhold this personal identifying information from public review. However, we cannot guarantee that we will be able to do so. We will post all hardcopy submissions on *http://www.regulations.gov*.

Information and supporting documentation that we received and used in preparing this finding will be available for you to review at *http://www.regulations.gov*, or you may make an appointment during normal business hours at the appropriate lead U.S. Fish and Wildlife Service Field Office (see **FOR FURTHER INFORMATION CONTACT**).

Background

Section 4(b)(3)(A) of the Act requires that we make a finding on whether a petition to list, delist, or reclassify a species presents substantial scientific or commercial information indicating that the petitioned action may be warranted. To the maximum extent practicable, we are to make this finding within 90 days of our receipt of the petition and publish our notice of the finding promptly in the **Federal Register**.

Our standard for substantial scientific or commercial information within the Code of Federal Regulations (CFR) with regard to a 90-day petition finding is ''that amount of information that would lead a reasonable person to believe that the measure proposed in the petition may be warranted'' (50 CFR 424.14(b)). If we find that substantial scientific or commercial information was presented, we are required to promptly commence a review of the status of the species, which we will subsequently summarize in our 12-month finding.

Section 4 of the Act (16 U.S.C. 1533) and its implementing regulations at 50 CFR 424 set forth the procedures for adding a species to, or removing a species from, the Federal Lists of Endangered and Threatened Wildlife and Plants. A species may be determined to be an endangered or threatened species due to one or more of the five factors described in section 4(a)(1) of the Act (see (2) under Request For Information, above).

In considering what factors might constitute threats, we must look beyond the exposure of the species to a factor to evaluate whether the species may respond to the factor in a way that causes actual impacts to the species. If there is exposure to a factor and the species responds negatively, the factor may be a threat, and, during the subsequent status review, we attempt to determine how significant a threat it is. The threat is significant if it drives, or contributes to, the risk of extinction of the species such that the species may warrant listing as an ''endangered species'' or a ''threatened species,'' as those terms are defined in the Act. However, the identification of factors that could affect a species negatively may not be sufficient for us to find that the information in the petition and our files is substantial. The information must include evidence sufficient to suggest that these factors may be operative threats that act on the species to the point that the species may meet the definition of an ''endangered species'' or ''threatened species'' under the Act.

Evaluation of a Petition To List the Alligator Snapping Turtle as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0038 under the Supporting Documents section.

Species and Range

Alligator snapping turtle (*Macrochelys temminckii;* previously *Macroclemys temminckii*); Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Mississippi, Missouri, Oklahoma, Tennessee, and Texas.

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from The Center for Biological Diversity, requesting that 53 species of reptiles and amphibians, including the alligator snapping turtle, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the alligator snapping turtle (*Macrochelys temminckii;* previously *Macroclemys temminckii*) based on Factors A, B, C and D. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the alligator snapping turtle, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Apalachicola Kingsake as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0039 under the Supporting Documents section.

Species and Range

Apalachicola kingsnake (*Lampropeltis getula meansi*); Florida

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from The Center for Biological Diversity, requesting that 53 species of reptiles and amphibians, including the Apalachicola kingsnake, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Apalachicola kingsnake (*Lampropeltis getula meansi*) based on Factor A. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Apalachicola kingsnake, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Arizona Toad as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R2–ES–2015–0040 under the Supporting Documents section.

Species and Range

Arizona toad (*Anaxyrus microscaphus*); Arizona, California, Nevada, New Mexico, and Utah

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the Arizona toad, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Arizona toad (*Anaxyrus microscaphus*) based on Factor E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Arizona toad, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Blanding's Turtle as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R3–ES–2015–0041 under the Supporting Documents section.

Species and Range

Blanding's turtle (*Emydoidea blandingii*); Illinois, Iowa, Indiana, New Hampshire, New York, Maine, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, Ohio, Pennsylvania, South Dakota, and Wisconsin, United States; Ontario, Quebec, and Nova Scotia, Canada.

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the Blanding's turtle, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Blanding's turtle (*Emydoidea blandingii*) based on Factors A, B, C, D, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Blanding's turtle, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Blue Ridge Gray-Cheeked Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0042 under the Supporting Documents section.

Species and Range

Blue Ridge gray-cheeked salamander (*Plethodon amplus*); North Carolina

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the Blue Ridge gray-cheeked salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial information indicating that listing the species may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0042 under the "Supporting Documents" section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the Blue Ridge gray-cheeked salamander salamander or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Caddo Mountain Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0043 under the Supporting Documents section.

Species and Range

Caddo Mountain salamander (*Plethodon caddoensis*); Arkansas

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the Caddo Mountain salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial scientific or commercial information indicating that the petitioned action may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0043 under the "Supporting Documents" section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the Caddo Mountain salamander or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the California Giant Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R8–ES–2015–0044 under the Supporting Documents section.

Species and Range

California giant salamander (*Dicamptodon ensatus*); California

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the California giant salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial scientific or commercial information indicating that the petitioned action may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R8–ES–2015–0044 under the "Supporting Documents" section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the California giant salamander or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Cascade Caverns Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R2–ES–2015–0045 under the Supporting Documents section.

Species and Range

Cascade Caverns salamander (*Eurycea latitans*); Texas

Petition History

On July 11, 2012, we received a petition dated July 11, 2012 from the Center for Biological Diversity, requesting that 53 species of reptiles and amphibians, including the Cascade Caverns salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Cascade Caverns salamander (*Eurycea latitans*) based on Factor A. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Cascade Caverns salamander, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Cascades Frog as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R1–ES–2015–0046 under the Supporting Documents section.

Species and Range

Cascades frog (*Rana cascadae*); California, Oregon, and Washington

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the Cascades frog, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Cascades frog (*Rana cascadae*) based on Factors A, C, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Cascades frog, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Cedar Key Mole Skink as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0047 under the Supporting Documents section.

Species and Range

Cedar Key mole skink (*Plestiodon egregius insularis*); Florida

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the Cedar Key mole skink, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Cedar Key mole skink (*Plestiodon egregius insularis*) based on Factors A, B, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Cedar Key mole skink, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Colorado Checkered Whiptail as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R6–ES–2015–0048 under the Supporting Documents section.

Species and Range

Colorado checkered whiptail (*Aspidoscelis neotesselata*); Colorado

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the Colorado checkered whiptail, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial scientific or commercial information indicating that the petitioned action may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R6–ES–2015–0048 under the ''Supporting Documents'' section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the Colorado checkered whiptail or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Distinct Population Segment of North American Wild Horse as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R8–ES–2015–0049 under the Supporting Documents section.

Species and Range

North American wild horse (population of the species *Equus caballus*); U.S. Federal public lands

Petition History

On June 17, 2014, we received a petition, dated June 10, 2014, from Friends of Animals and The Cloud Foundation, requesting that the distinct population segment (DPS) of North American wild horses on all U.S. federal public lands be listed as an endangered or threatened species under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner(s), as required by 50 CFR 424.14(a). In an October 3, 2014, letter to the petitioner, we responded that we reviewed the information presented in the petition and did not find that the petition warranted an emergency listing. This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial information indicating the petitioned entity may qualify as a DPS and, therefore, a listable entity under section 3(16) of the Act. The petition does not present substantial information supporting the characterization of North American wild horses on all U.S. Federal public lands as a DPS, because the discreteness criteria were not met. Therefore, this population is not a valid listable entity under section 3(16) of the Act, and we are not initiating a status review in response to the petition. Our justification for this finding can be found as an appendix at http://*www.regulations.gov* under Docket No. FWS–R8–ES–2015–0049 under the "Supporting Documents" section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the North American wild horse or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Foothill Yellow-Legged Frog as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R8–ES–2015–0050 under the Supporting Documents section.

Species and Range

Foothill yellow-legged frog (*Rana boylii*); Oregon and California

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the foothill yellow-legged frog, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the foothill yellow-legged frog (*Rana boylii*) based on Factors A and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the foothill yellow-legged frog, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Gopher Frog as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0051 under the Supporting Documents section.

Species and Range

Gopher frog (*Lithobates capito*); Alabama, Florida, Tennessee, Georgia, South Carolina, and North Carolina

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the gopher frog, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the gopher frog (*Lithobates capito*) based on Factors A, C, D, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the gopher frog, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To Reclassify the Gray Wolf, Excluding Mexican Wolf, in the Conterminous U.S. as a Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–HQ–ES–2015–0072 under the Supporting Documents section.

Species and Range

Gray wolf, excluding the Mexican wolf (population of the species *Canis lupus*); conterminous United States.

Petition History

On January 27, 2015, we received a petition dated January 27, 2015, from the Humane Society of the United States (HSUS) and twenty-two undersigned petitioners (The Center for Biological Diversity, The Fund for Animals, Born Free USA, Friends of Animals and Their Environment, Help Our Wolves Live, The Detroit Zoological Society, Midwest Environmental Advocates, Predator Defense, National Wolfwatcher Coalition, Northwoods Alliance, Wisconsin Federated Humane Societies, Minnesota Humane Society, Howling for Wolves, Detroit Audubon Society, Sault Sainte Marie Tribe of Chippewa Indians, Wildlife Public Trust and Coexistence, Minnesota Voters for Animal Protection, Friends of the Wisconsin Wolf, Wolves of Douglas County Wisconsin, Justice for Wolves, and Wildwoods (Minnesota)), requesting that the gray wolf, excluding the Mexican wolf subspecies, be reclassified as threatened throughout the conterminous United States (U.S.) under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). On March 10, 2015, we received electronic copies of the published references cited in the January, 27, 2015 petition from HSUS. In a March 27, 2015, letter to HSUS, we responded that we reviewed the information presented in the petition and did not find that the petition warranted an emergency listing. This finding addresses the petition.

Finding

Based on our review of the petition, we find the petition does not provide substantial scientific or commercial information indicating the petitioned entity may qualify as a DPS and, therefore, a listable entity under section 3(16) of the Act. Although any further evaluation of the petition was unnecessary because this is a sound basis for a not-substantial finding, due to the level of controversy surrounding the legal status of gray wolf under the Act and the high interest in this petition specifically we further evaluated the petition by analyzing the five listing factors under section 4(a)(1). Based on our review of the petition, sources cited in the petition, and our files we find the petition does not provide substantial scientific or commercial information indicating that gray wolves, excluding Mexican wolves, in the coterminous U.S. may be likely to become an endangered species within the foreseeable future (a threatened species) due to any one of the five listing factors. We come to the same conclusion when we consider whether collective information presented in the petition represents substantial information. The petitioner's information with respect to unoccupied suitable habitat is based on a misinterpretation of the Act. Moreover, despite making allegations with respect to disease, and small population size, the petitioners provided no information to support their claim. Inadequate existing regulatory mechanisms are not an independent source of threat, but relate to amelioration of threats under the other factors. Therefore, the petition only provides information with respect to possible overutilization from recreational hunting and trapping, and the information is not substantial. Thus the petition provides no information to combine with the information regarding possible overutilization from recreational hunting and trapping. In any case, even if the petition had presented information with respect to other sources of mortality, the existing state plans regulating take of wolves only allow take above certain population thresholds, such that if the other causes of mortality increased above certain levels, hunting and trapping would be reduced to prevent the population from dipping below those thresholds. So those plans have a built-in response to possible concerns relating to cumulative impacts. Accordingly, we are not initiating a status review in response to this petition.

Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–HQ–ES–2015–0072 under the "Supporting Documents" section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the gray wolf or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Green Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0052 under the Supporting Documents section.

Species and Range

Green salamander (*Aneides aeneus*); Alabama, Georgia, Indiana, Maryland, Mississippi, Ohio, Pennsylvania, North Carolina, and South Carolina.

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the green salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the green salamander (*Aneides aeneus*) based on Factors A, B, C, D, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the green salamander, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Illinois Chorus Frog as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R3–ES–2015–0053 under the Supporting Documents section.

Species and Range

Illinois chorus frog (*Pseudacris illinoensis* or *Pseudacris streckeri illinoensis*); Illinois, Missouri, and Arkansas

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the Illinois chorus frog, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Illinois chorus frog (*Pseudacris illinoensis* or *Pseudacris streckeri illinoensis*) based on Factors A and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Illinois chorus frog, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Kern Canyon Slender Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R8–ES–2015–0054 under the Supporting Documents section.

Species and Range

Kern Canyon slender salamander (*Batrachoseps simatus*); California

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the Kern Canyon slender salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such

and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Kern Canyon slender salamander (*Batrachoseps simatus*) based on Factors A, D, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Kern Canyon slender salamander, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Key Ringneck Snake as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0055 under the Supporting Documents section.

Species and Range

Key ringneck snake (*Diadophis punctatus acricus*); Florida

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the Key ringneck snake, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Key ringneck snake (*Diadophis punctatus acricus)* based on Factors A and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Key ringneck snake, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Olympic Torrent Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R1–ES–2015–0056 under the Supporting Documents section.

Species and Range

Olympic torrent salamander (*Rhyacotriton olympicus*); Washington

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the Olympic torrent salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial scientific or commercial information indicating that the petitioned action may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R1–ES–2015–0056 under the ''Supporting Documents'' section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the Olympic torrent salamander or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Oregon Slender Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R1–ES–2015–0057 under the Supporting Documents section.

Species and Range

Oregon slender salamander (*Batrachoseps wrighti;* previously *B. wrightorum*); Oregon

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the Oregon slender salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Oregon slender salamander (*Batrachoseps wrighti*) based on Factors A and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Oregon slender salamander, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Pigeon Mountain Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0058 under the Supporting Documents section.

Species and Range

Pigeon Mountain salamander (*Plethodon petraeus*); Georgia

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the Pigeon Mountain salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial scientific or commercial information indicating that the petitioned action may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0058 under the ''Supporting Documents'' section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the Pigeon Mountain salamander or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Relictual Slender Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R8–ES–2015–0059 under the Supporting Documents section.

Species and Range

Relictual slender salamander (*Batrachoseps relictus*); California

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the relictual slender salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the relictual slender salamander (*Batrachoseps relictus*) based on Factors A, D, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the relictual slender salamander, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Rim Rock Crowned Snake as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0060 under the Supporting Documents section.

Species and Range

Rim Rock crowned snake (*Tantilla oolitica*); Florida

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from The Center for Biological Diversity, requesting that 53 species of reptiles and amphibians, including the Rim Rock crowned snake, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Rim Rock crowned snake (*Tantilla oolitica*) based on Factors A and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Rim Rock crowned snake, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Rio Grande Cooter as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R2–ES–2015–0061 under the Supporting Documents section.

Species and Range

Rio Grande cooter or Western River cooter (*Pseudemys gorzugi*); Texas and New Mexico, United States; Coahuila, Neuvo Leon, and Tamaulipas, Mexico

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from The Center for Biological Diversity, requesting that 53 species of reptiles and amphibians, including the Rio Grande cooter, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the Rio Grande cooter (*Pseudemys gorzugi)* based on Factors A, B, and D. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the Rio Grande cooter, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List Silvery Phacelia as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R1–ES–2015–0062 under the Supporting Documents section.

Species and Range

Silvery phacelia (*Phacelia argentea*); Oregon and California

Petition History

On March 7, 2014, we received a petition dated March 7, 2014, from The Center for Biological Diversity, Oregon Wild, Friends of Del Norte, Oregon Coast Alliance, The Native Plant Society of Oregon, The California Native Plant Society, The Environmental Protection Information Center, and Klamath-Siskiyou Wildlands Center (the petitioners), requesting that silvery phacelia be listed as an endangered or threatened species and, if applicable, critical habitat be designated for this species under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we

find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the silvery phacelia (*Phacelia argentea*) based on Factors A and D. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the silvery phacelia, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Southern Hog-Nosed Snake as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0063 under the Supporting Documents section.

Species and Range

Southern hog-nosed snake (*Heterodon simus*); North Carolina, South Carolina, Georgia, and Florida

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from The Center for Biological Diversity, requesting that 53 species of reptiles and amphibians, including the southern hog-nosed snake, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the southern hog-nosed snake (*Heterodon simus*) based on Factors A and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the southern hog-nosed snake, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Spotted Turtle as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R5–ES–2015–0064 under the Supporting Documents section.

Species and Range

Spotted turtle (*Clemmys guttata*); Connecticut, Delaware, Florida, Georgia, Illinois, Maine, Maryland, Massachusetts, Michigan, Pennsylvania, New Hampshire, New York, North Carolina, Ohio, South Carolina, Vermont, Virginia, and West Virginia

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the spotted turtle, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the spotted turtle (*Clemmys guttata*) based on Factors A, B, D, and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the spotted turtle, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Weller's Salamander as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0065 under the Supporting Documents section.

Species and Range

Weller's salamander (*Plethodon welleri*, 1931); North Carolina, Tennessee, and Virginia

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity, requesting that 53 species of amphibians and reptiles, including the Weller's salamander, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial scientific or commercial information indicating that the petitioned action may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0065 under the ''Supporting Documents'' section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the Weller's salamander or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Evaluation of a Petition To List the Western Spadefoot Toad as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R8–ES–2015–0066 under the Supporting Documents section.

Species and Range

Western spadefoot toad (*Spea hammondii* or *Scaphiopus hammondii*); California, United States; Northwestern Baja California, Mexico

Petition History

On July 11, 2012, we received a petition dated July 11, 2012, from the Center for Biological Diversity requesting that 53 species of reptiles and amphibians, including the western spadefoot toad, be listed as endangered or threatened and critical habitat be designated under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a). This finding addresses the petition.

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted for the western spadefoot toad (*Spea hammondii* or *Scaphiopus hammondii*) based on Factors A and E. However, during our status review we will thoroughly evaluate all potential threats to the species.

Thus, for the western spadefoot toad, the Service requests information on the five listing factors under section 4(a)(1) of the Act, including the factors identified in this finding (see Request for Information, above).

Evaluation of a Petition To List the Wingtail Crayfish as an Endangered or Threatened Species Under the Act

Additional information regarding our review of this petition can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0067 under the Supporting Documents section.

Species and Range

Wingtail crayfish (*Procambarus (Leconticambarus) latipleurum*); Florida

Petition History

On January 6, 2014, we received a petition dated January 6, 2014, from the Center for Biological Diversity, requesting that the wingtail crayfish be listed as an endangered or threatened species under the Act. The petition clearly identified itself as such and included the requisite identification information for the petitioner, required at 50 CFR 424.14(a).

Finding

Based on our review of the petition and sources cited in the petition, we find that the petition does not provide substantial scientific or commercial information indicating that the petitioned action may be warranted. We are not initiating a status review of this species in response to the petition. Our justification for this finding can be found as an appendix at *http://www.regulations.gov* under Docket No. FWS–R4–ES–2015–0067 under the ''Supporting Documents'' section. However, we ask that the public submit to us any new information that becomes available concerning the status of, or threats to, the wingtail crayfish or its habitat at any time (see **FOR FURTHER INFORMATION CONTACT**).

Conclusion

On the basis of our evaluation of the information presented under section 4(b)(3)(A) of the Act, we have determined that the petitions summarized above for the Blue Ridge gray-cheeked salamander, Caddo Mountain salamander, California giant salamander, Colorado checkered whiptail, the distinct population segment of North American wild horse, gray wolf, excluding Mexican wolf, in the conterminous U.S., Olympic torrent salamander, Pigeon Mountain salamander, Weller's salamander, and wingtail crayfish do not present substantial scientific or commercial information indicating that the requested actions may be warranted. Therefore, we are not initiating status reviews for these species.

On the basis of our evaluation of the information presented under section 4(b)(3)(A) of the Act, we have determined that the petitions summarized above for alligator snapping turtle, Apalachicola kingsnake, Arizona toad, Blanding's turtle, Cascade Caverns salamander, Cascades frog, Cedar Key mole skink, foothill yellow-legged frog, gopher frog, green salamander, Illinois chorus frog, Kern Canyon slender salamander, Key ringneck snake, Oregon slender salamander, relictual slender salamander, Rim Rock crowned snake, Rio Grande cooter, silvery phacelia, southern hog-nosed snake, spotted turtle, and western spadefoot toad present substantial scientific or commercial information indicating that the requested actions may be warranted. Because we have found that the petitions present substantial information indicating that the petitioned actions may be warranted, we are initiating status reviews to determine whether these actions under the Act are warranted. At the conclusion of the status reviews, we will issue a 12-month finding in accordance with section 4(b)(3)(B) of the Act, as to whether or not the Service believes listing is warranted.

It is important to note that the ''substantial information'' standard for a 90-day finding as to whether the petitioned action may be warranted differs from the Act's ''best scientific and commercial data'' standard that applies to the Service's determination in a 12-month finding as to whether a petitioned action is in fact warranted. A 90-day finding is not based on a status review. In a 12-month finding, we will determine whether a petitioned action is warranted after we have completed a thorough status review of the species, which is conducted following a substantial 90-day finding. Because the Act's standards for 90-day and 12-month findings are different, as described above, a substantial 90-day finding does not mean that the 12-month finding will result in a warranted finding.

References Cited

A complete list of references cited is available on the Internet at *http://www.regulations.gov* and upon request from the appropriate lead field offices (see **FOR FURTHER INFORMATION CONTACT**).

Authors

The primary authors of this document are the staff members of the Branch of Listing, Ecological Services Program, U.S. Fish and Wildlife Service.

Authority

The authority for these actions is the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 *et seq.*).

Dated: June 22, 2015.

Stephen Guertin,

Acting Director, U.S. Fish and Wildlife Service.

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AN ANALYSIS OF DOMINANCE, ITS BEHAVIOURAL PARAMETERS AND POSSIBLE DETERMINANTS IN A HERD OF ICELANDIC HORSES IN CAPTIVITY

by

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ABSTRACT

The applicability of the concept of dominance was investigated in a captive herd of 26 Icelandic horses and 5 ponies of different breeds. Eight out of 20 behaviours possibly related to dominance occurred frequently enough to be investigated in detail. For these eight agonistic behaviours the coverage, the unidirectionality in the exchange, and the degree of transitivity (Landau's linearity index) were calculated. Four offensive behaviours, together with avoidance, were suitable for further analysis with regard to dominance. The patterns of asymmetries with which these behaviours were exchanged were sufficiently similar as to justify the application of the dominance concept and to construct a (nearly) linear dominance hierarchy. The rank order of the castrated stallions was completely linear, the hierarchy of the mares was almost completely linear. The results suggest that offensive and defensive aggressive behaviours should be treated separately and that the concept of dominance is applicable. However, ritualized formal dominance signals between adult horses appear to be (almost) absent. The rank positions of the individuals were correlated with age and residency in the herd but not with height. Middle ranking horses tended to be more frequently in the close vicinity of an other horse than high ranking or low ranking horses. Over and above this correlation at the individual level, it was found that pairs of horses close in rank to each other were more often also spatially close to each other. Being in oestrus did not influence the dominance relationships between mares. For castrated stallions the rank positions were correlated with the age at which they were castrated. This suggests that in male horses experience prior to neutering influences the behaviour afterwards.

KEY WORDS: dominance, rank order, horses, Icelandic horses.

INTRODUCTION

Dominance relationships constitute a major aspect of the social structure of some socially living animals. Often, however, dominance rank orders are constructed without prior investigations as to whether the concept of dominance is valid. This is the case for most of the studies of horse social organization. Following HINDE (1974) our understanding

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of the concept of dominance is that if the main directions of asymmetries in the exchange of a number of behaviours within dyads are identical, the intervening variable 'dominance' can be used to summarise these asymmetries. Subsequently, it is often possible to rank individuals so that a more or less linear rank order results. This constitutes the second aspect of the dominance concept. Thirdly, a formal dominance signal can be assumed to exist when the exchange of this behaviour within each dyad is unidirectional for 100% and if the coverage (the percentage of non-zero dyads) is high enough (*cf.* VAN HOOFF & WENSING, 1987).

Many studies have examined the dominance relationships in both domestic horses (GRZIMEK, 1944; HECHLER, 1971; HOUPT *et al.*, 1978; SERENI & BOISSOU, 1978; HOUPT, 1979; ASA *et al.*, 1979; HOUPT & WOLSKI, 1980; HOUPT & KEIPER, 1982; ARNOLD & GRASSIA, 1982; WOOD-GUSH & GALBRAITH, 1987) and feral horses (EBHART, 1954; TYLER, 1972; CLUTTON-BROCK *et al.*, 1976; FEIST & MCCULLOUGH, 1976; BERGER, 1977; VON GOLDSCHMIDT-ROTHSCHILD & TSCHANZ, 1978; WELLS & VON GOLDSCHMIDT-ROTHSCHILD, 1979; MILLER, 1981; HOUPT & KEIPER, 1982; BERGER, 1986; KEIPER & SAMBRAUS, 1986; RUTBERG & GREENBERG, 1990). KEIPER & RECEVEUR (1992) investigated dominance in Przwalski horses. Almost all authors describe a hierarchy in one way or another; only BERGER (1986) found hardly any stable dominance hierarchy in his feral horse herds, while FEIST & MCCULLOUGH (1976) and VON GOLDSCHMIDT-ROTHSCHILD & TSCHANZ (1978) could only detect a rank order between the males.

Several authors (GRZIMEK, 1944; HOUPT *et al.*, 1978; SERENI & BOISSOU, 1978; ASA *et al.*, 1979; HOUPT & WOLSKI, 1980; HOUPT & KEIPER, 1982) used the rivalry around limited and monopolisable resources to establish the dominance-subordination relationship by using (paired) feeding tests. In such tests a possible influence of other herd members is excluded. HOUPT *et al.* (1978) used paired feeding tests to show that in small herds (up to 9 animals) strictly linear hierarchies were found, while in larger herds (10-11 animals) also triangular relationships were formed. However, dominance relationships found in this way may differ from those found in a free roaming situation and during a longer period of observation.

Unfortunately, authors do not fully agree with regard to the behaviours they use to construct rank orders. For example, HOUPT & WOLSKI (1980) used a mix of offensive and defensive aggressive behaviours. On the other hand, WELLS (1978) and FEH (1988) explicitly state that these two classes of behaviour must be treated separately and SCHILDER (1988) found the same in a detailed analysis of dominance relationships in plains zebra stallions. Many authors (GRZIMEK, 1944; HECHLER,

1971; TYLER, 1972; HOUPT *et al.*, 1978; HOUPT, 1979; WELLS & VON GOLDSCHMIDT-ROTHSCHILD, 1979; HOUPT & WOLSKI, 1980; MILLER, 1981) did not use avoidance behaviour, whereas this was useful in zebras (SCHILDER, 1990). Since aggressive behaviours may well by used by lower ranking individuals against higher ranking ones, the use of aggressive behaviours only in investigating dominance may render an unclear or even invalid picture. This was the reason why in zebra stallions (SCHILDER, 1988) and wolves (VAN HOOFF & WENSING, 1987) aggressive behaviours could not be used to construct a rank order.

To investigate which individual factors might be correlated with the rank position an animal has in the dominance hierarchy it is required that the rank order is (nearly) linear. Landau's linearity index (see MARTIN & BATESON, 1993) can be used to assess the degree of linearity in a set of dominance relationships. If the value of this index exceeds 0.9 the linearity of the hierarchy is sufficiently strong to obtain meaningful correlations between rank positions and individual factors like weight or age. The weaker the linearity of the rank order the less these correlations can be meaningfully interpreted. This problem is encountered in KEIPER & RECEVEUR, 1992.

What different types of individual factors could determine (partially) the rank position in a dominance hierarchy? In the literature the following findings have been reported. Some authors reported that dominance rank correlated with the height of the individuals (HECHLER, 1971, CLUTTON-BROCK *et al.*, 1976, RUTBERG & GREENBERG, 1990). Weight correlated positively with rank in some studies (HECHLER, 1971; CLUTTON-BROCK *et al.*, 1976), but not in others (GRZIMEK, 1945; HOUPT *et al.*, 1978; HOUPT, 1979; HOUPT & WOLSKI, 1980; HOUPT & KEIPER, 1982). Sometimes no correlation with physical characteristics could be shown (ARNOLD & GRASSIA, 1982). Concerning the aspect of age, adult horses are nearly always dominant over immature horses (HECHLER, 1971; CLUTTON-BROCK *et al.*, 1976; HOUPT *et al.*, 1978; HOUPT, 1979; ARNOLD & GRASSIA, 1982; KEIPER & SAMBRAUS, 1986). Also, in many studies (GRZIMEK, 1944; HECHLER, 1971; CLUTTON-BROCK *et al.*, 1976; WELLS & VON GOLDSCHMIDT-ROTHSCHILD, 1979; KEIPER & SAMBRAUS, 1986), the age of adult horses correlated positively with their rank position. In the study of HOUPT *et al.* (1978) age was correlated with rank in 6 out of 9 herds. However, age was not correlated with rank in the studies of FEIST & MCCULOUGH (1976), HOUPT & KEIPER (1982) and ARNOLD & GRASSIA (1982). In the last study a group of horses was introduced into the herd during the study. All these new horses, irrespective of their ages, figured in the bottom part of the rank order. This suggests that residency in the herd could also be a determinant of rank position.

A last factor that might influence rank position is castration. LINE *et al.* (1985) showed that there is a minimal difference in the effect of pre-(<3 yr.) and post-(>3 yr.) pubertal castration in male horses on their sexual and aggressive behaviour. But the total frequency of these behaviours is much lower when castrated. Since pre-castration experience may influence later behaviour, the age at which the stallions were castrated may determine the amount of experience and therefore, may influence the position in the rank order.

In the present study we begin with analysing the patterns of asymmetries in behavioural exchanges with the aim to detect behaviours that represent dominance at the dyadic level and whether dominance can be used as an intervening variable. Subsequently, we investigate for each of these behaviours to what degree it allows the construction of a linear rank order. To this end we calculate for each of the dyadic interaction matrices the Landau linearity index, a directional consistency index, the number of tied dyads and the coverage. Using the behaviours that satisfy these criteria, a highly linear dominance rank order among 21 horses (excluding the juveniles, one deviant mare and the non-Iceland horses) can be constructed. Finally, we investigate if and to what degree the factors age, sex, height, residency in the herd, age at castration, oestrus of a mare and the possession of a young foal are related to the rank position attained or to changes therein. We also investigate whether rank and rank difference correlate with kin, proximity and mutual allogrooming.

MATERIALS AND METHODS

The study group

The herd was established in 1971 and contained all sex-age classes except for an intact stallion. There were 26 Icelandic horses (6 castrated stallions (geldings), 16 mares, 2 juvenile stallions and 2 juvenile mares) and 5 ponies of different breeds (table I). Many of these horses were kin. They lived at the 'Breidablik' farm, Tilburg, The Netherlands. The horses in the herd were living together outside, 24 hours a day the whole year round. The herd could always use a shed (20 × 20 m) and a transition corridor (400 × 8 m). They had (sometimes limited) access to a pasture (140 × 100 m) depending on the food availability. There were five of these pastures in a row, each used in turn for 1-3 weeks. The total area was 6.3 hectare. The herd had always free access to running water provided by automatic drinking bowls. It also had free access to extra mineral supplies. The water bowls and mineral supply could be monopolized.

The observations were made when the herd was allowed to graze in one of the pastures and had done so for at least an hour. The behaviour of the animals was sampled between April 1984 and September 1984 during 433 hours of observation. Some of the adult animals were irregularly used for riding, or were absent for some weeks for breeding purposes. The presence and absence due to riding, out of sight in the shed, breeding, *etc.* of the animals was registered. In all dyads the data have been corrected for temporary absence of individuals.

In order to obtain a homogeneous sample the five non-icelandic ponies were excluded from the analyses. Observations on the juvenile animals were not used for the analyses because we were especially interested in dominance, its behavioural parameters and its possible determinants in adult horses. It has been shown by many horse ethologists that adult horses are (nearly) always dominant over immature horses (HECHLER, 1971; CLUTTON-BROCK *et al.*, 1976; HOUPT *et al.*, 1978; HOUPT, 1979; ARNOLD & GRASSIA, 1982; KEIPER & SAMBRAUS, 1986). This was confirmed in the present study (data not published). We also excluded one adult Icelandic mare (v), because she was very probably suffering from a hormone disorder since she was the only mare not seen in season during the study period. Moreover, she had experienced a false pregnancy during the first three months of this period. Her illness also caused deviant behaviour in different respects, for instance a high frequency of being solitary.

Data sampling and analysis methods

The main body of data was collected using focal animal sampling: 100 * 10 minutes per animal or less according to its presence, and additional data were collected using ad lib sampling (ALTMANN, 1974). The data were collected at randomly distributed times during the daylight period. The order of focal animals to be observed changed every day at random. The ad lib data sets included many aggressive and affiliative encounters. The focal animal and ad lib data sets were compared by calculating the rank correlation coefficient between the individual behavioural frequencies (corrected for presence and absence of each horse) found in each data set. This was done for a number of behaviours relevant to this study. The result was that the two data sets resembled each other so much that they could be lumped and treated as one.

For the analyses concerning linearity of rank orders and the influence of possible influential factors the data set was split as follows:

* Data sampled on days during which a young foal (0-3 weeks) was present (foal-days, N = 28) (Note: Three weeks is the mean time that the young foal moves more than two horse lengths from its dam (TYLER, 1972; CROWELL-DAVIS, 1985); also in 75% of the cases its mother is its nearest neighbour (CROWELL-DAVIS, 1985)).

* Data sampled on days during which at least one mare was in season (oestrus-days, N = 52).

* The days without any of these special features were called normal-days (N = 29).

A mare was considered to be in season when she displayed at least twice that day one or more of the following behaviours: *Presenting:* standing in the neighbourhood of a male with straddled hind legs, lifted tail, ears sideways, sometimes with winkling and loss of small amounts of urine (TYLER, 1972; ASA *et al.*, 1979; VON GOLDSCHMIDT-ROTHSCHILD & TSCHANZ, 1978; VON GOLDSCHMIDT-ROTHSCHILD & GLATTHAAR, 1983); *Winkling:* opening and closing of the labia major exposing the clitoris and the vulva, often with losing urine (WARING, 1983). Further, a mare was considered to be in season if she was sexually mounted by a juvenile stallion or a gelding.

The analysis of dominance — Out of the 127 behavioural elements that were recorded, we selected 20 elements that were possibly related to dominance (see table II). Descriptions of these behavioural elements are given in VON GOLDSCHMIDT-ROTHSCHILD & GLATTHAAR (1983) and WARING (1983). Next, we counted for each behaviour the number of relationships covered by that behaviour (the coverage). If the coverage of a behaviour was less than 15% of all possible dyads this behaviour was excluded from further analyses. This left us with 8 behaviours (all with a coverage of at least 30%) for further analysis (table II). These behaviours were: *attack:* fast movement in the direction of an opponent, with ears flattened: *bite:* bite or bite attempt, followed by chase in some cases; *threat to bite:* movement of the head with flattened ears towards the other animal, usually

TABLE I

Attributes of the horses in the study group

sex	*code*	*breed**	*age (yrs)*	*residence (months)*	*height (cms)*	*1st order relative in herd***	*dam of ad., yl., fl. ****	*age at castration*
castrated stallions	D	I	20	104	131	no	–	5
	C§	S	±20	150	94	no	–	1
	H	I	18	61	136	no	–	?
	F	I	14	86	128	no	–	3
	B	I	12	143	133	yes	–	7
	S	I	9	108	135	yes	–	5
	O	I	6	72	138	yes	–	1
juvenile stallions	M§	I	1	13	–	yes	–	–
	T§	I	foal	2	–	yes	–	–
mares	s	I	22	138	132	yes	ad. r, yl. M	–
	l	I	22	141	127	no	no	–
	b	I	19	152	129	yes	ad. B, ad. m	–
	K	I	19	155	125	yes	ad. k, fl. G	–
	U	I	19	124	130	yes	ad. O	–
	v§	I	19	144	123	no	no	–
	f	I	18	61	135	no	no	–
	P	I	18	155	131	yes	ad. d, yl. g	–
	e§	NF	18	137	130	no	no	–
	p	I	15	114	133	yes	ad. S	–
	a§	C	15	13	146	yes	yl. A	–
	c§	S	13	120	95	no	no	–
	d	I	12	143	133	yes	ad. o	–
	k	I	9	109	128	yes	no	–
	t	I	8	97	136	yes	fl. T	–
	m	I	7	85	134	yes	no	–
	E	I	5	61	132	yes	no	–
	r	I	5	61	136	yes	no	–
	o	I	5	61	136	yes	no	–
juvenile mares	A§	NF	1	13	–	yes	–	–
	g§	I	1	12	–	yes	–	–
	G§	I	foal	1	–	yes	–	–

* I = Icelandic horse; S = Shetland pony; NF = New Forest pony; C = Connemara pony.

** This includes son, daughter, mother, brother and/or sister.

*** mother of an other adult (ad.), yearling (yl.) or foal (fl.), e.g. ad. r in the row for mare s means that s is the mother of the adult mare r.

§ Not used for the dominance analyses.

TABLE II

The coverage (% of non-zero dyads) of 20 behaviours possibly related to dominance

Behaviour	*Coverage*	*Used in dominance analyses*
Approach with ears flattened	61	*
Threat to bite	55	*
Bite	54	*
Attack	39	*
Fight	2	
Chase	5	
Drive	6	
Threat to kick	35	*
Kick (kicking back with one hindleg)	32	*
Buck (kicking back with two hindlegs)	32	*
Push	5	
head on back	3	
Walk backwards#	9	
Avoid	87	*
Tooth clapping (snapping)##	10	
Roll over	1	
Urinate over§	<1	
Defecate over§	2	
Non-sexual mount	4	
Rearing	2	

\# Only performed by 8 mares.
Only performed by the 5 juveniles.
§ Only performed by the stallions.

head and neck in one line; *approach with ears flattened* and head held high or horizontally; *threat to kick; kick:* kicking back with one hind leg; *buck:* kicking back with both hind legs; *avoidance:* moving away from an other individual after being approached.

For each of these eight behaviours the interaction frequency matrix was investigated with regard to the following properties (see table III);

I The *coverage, i.e.* the number of dyads with non-zero exchange of the behaviour.
II The *number of tied dyads, i.e.* the number of dyads with equal exchange of the behaviour.
III The *directional consistency, i.e.* the total number of instances the behaviour was performed in the main direction within each dyad divided by the total frequency of the behaviour. This Directional Consistency index (DC-index) ranges from 0 (completely equal exchange) to 1 (complete unidirectionality) (see VAN HOOFF & WENSING, 1987). An equivalent index, namely a direction inconsistency index, has been used by NOË *et al.* (1980) and SCHILDER (1988).
IV The *degree of linearity*, measured by Landau's linearity index *h* (see MARTIN & BATESON, 1993).

For the properties I and III we devised the criterion that the value a behaviour has for that property should be higher than the average value of that property averaged across the eight behaviours (*cf.* SCHILDER, 1988). Similarly, for the property II, the criterion

TABLE III

Properties of eight different dyadic interaction frequency matrices

Behaviour	*Total frequency*	*Coverage (non-zero dyads)*	*Number of tied dyads*	*Directional Consistency index*	*Landau's lionearity index h*	*significance*
Bite	351	119	5	0.72	0.30	p<.01
Threat to bite	334	128	0	0.90	0.46	p<.01
Approach with ears flattened	452	146	2	0.90	0.50	p<.01
Attack	239	96	4	0.95	0.30	p<.01
Threat to kick	185	90	11	0.64	0.12	n.s.
Kick	182	75	17	0.49	0.06	n.s.
Buck	158	70	13	0.52	0.07	n.s.
Avoidance	1423	201	6	0.92	0.86	p<<.001

Total number of dyads for each matrix: 210.

was that the value a behaviour has for this property should be lower than the average value. In table III the values reaching this criterion are underlined. For property IV the criterion was that Landau's linearity index should be statisticaly significant (Appleby, 1983; De Vries *et al.*, 1993). Only if a behaviour satisfied at least two criteria the behaviour was considered to be a possibly suitable indicator of dominance. This was the case for five behaviours: bite, threat to bite, attack, approach with flattened ears, and avoid. If the separate rank orders for these behaviours correlate sufficiently, the relationships between the animals can be summarised in a single hierarchy.

We also investigated the similarities between the patterns of asymmetries in the eight behaviours mentioned in table III by means of two different asymmetry measures for each pair of horses. The first asymmetry measure is defined as: the number of times a behaviour was performed from A to B (f_{AB}) minus the number of times this behaviour was performed from B to A (f_{BA}). By dividing this difference by the sum of f_{AB} and f_{BA} a normalised index of asymmetry is obtained ranging between −1 and +1 (*cf.* Schilder, 1988). We can call this the degree of asymmetry between A and B. A second measure, that only takes the direction of the asymmetry into account but not the degree, is obtained by taking the sign of this magnitude of asymmetry, that is: $\text{sign}(f_{AB}-f_{BA}) = 1$ if $f_{AB}-f_{BA}>0$; $\text{sign}(f_{AB}-f_{BA}) = 0$ if $f_{AB}-f_{BA} = 0$; $\text{sign}(f_{AB}-f_{BA}) = -1$ if $f_{AB}-f_{BA}<0$. Next, for each of these two asymmetry measures, a principal components analysis (PCA; see *e.g.* Tabachnik & Fidell, 1989) has been done with the eight behaviours as variables and the 0.5N(N-1) dyads as cases. Because the asymmetry between A and B is the opposite of the asymmetry between B and A, each dyad is represented only once in the data table (that is: if (A,B) is a case in the data table then (B,A) is not). Table IV presents the results of the PCA based on the signs of the asymmetries. The PCA based on the asymmetry degrees rendered highly similar results and is not shown.

Construction of the final rank order — The following procedure was used to construct the rank order using the five agonistic behaviours that met the above stated criteria.

1) The outcome of the PCA mentioned above shows that the four aggressive behaviours (bite, threat to bite, approach with ears flattened and attack) all have high and quite similar loadings on the first factor. This means that these four behaviours all show a similar pattern of asymmetry across the dyads. Therefore, we decided to add, for

TABLE IV

Factor loadings resulting from a Principal Components Analysis based on the signs of dyadic asymmetries in 8 behaviours

Behaviour	*Factor 1*	*Factor 2*	*Communality*
Bite	54	-17	.33
Threat to bite	73	4	.54
Approach with ears flattened	77	8	.60
Attack	65	-13	.43
Threat to kick	7	71	.51
Kick	-3	76	.57
Buck	-21	82	.71
Avoidance	-67	2	.45
Variance explained	30%	21%	

each dyad, the frequencies of these four aggressive behaviours. Since we are interested in the dominance relationships as such rather than the strength of these relationships, we converted this frequency matrix into a binary relationship matrix. That is, for each dyad, the animal that showed the highest frequency was given a one and the other animal was given a zero. The matrix that contains the avoidance frequencies was also converted into a binary relationship matrix, but now for each dyad the animal with the lowest frequency was given a one rather than the animal with the highest frequency, in order to make this matrix compatible with the binary aggression matrix. We decided to multiply this binary avoidance matrix by two, because avoiding behaviour is in principle a much better parameter for representing the dominance-subordinate relationship than the aggressive behaviours are. This is so, because by giving way to another approaching animal the avoiding animal acknowledges the other's dominance, whereas aggressive behaviour is not necessarily an expression of one's dominance over an other animal (VAN HOOFF & WENSING, 1987; SCHILDER, 1988). This is in accord with the fact that the avoidance matrix has the highest value of Landau's linearity index and a high directional consistency index (table III).

2) The two relationship matrices thus constructed were added. This combined relationship matrix has the advantage that one can easily see which dyads have a clear or less clear dominance relationship (see fig. 1). The matrix contains the scores 0, 1, 2 and 3. A score of 3 means that in that dyad the dominant individual was dominant on basis of two behavioural parameters: aggression given and avoidance achieved. A score of 2 means that the dominant individual was only dominant on basis of avoidance achieved. In this matrix the horses were ordered according to the number of dominated animals. If, however, in the order thus obtained, the dyadic relationship between two adjacent individuals did not agree with this order then these two individuals were reversed (the 'flipping heuristic', ROBERTS, 1990; DE VRIES *et al.*, 1993). The result of this rearrangement procedure is a matrix in which the sum of scores below the diagonal is approximately minimized (*cf.* SCHEIN & FOHRMAN, 1995). This rendered the final rank order. All different steps in this procedure were done using MatMan, a program for the analysis of sociometric matrices (DE VRIES *et al.*, 1993).

In this way, rank orders were constructed for the complete group as well as for males and females separately (see figures 1, 2 and 3). Finally, a rank order for the complete

group was constructed using the method of CLUTTON-BROCK *et al.* (1986), which excludes the influence of age.

The relationship between rank and other factors — Because of the different types of data and the constraints of some statistical tests, a number of different tests had to be used to detect relationships between dominance rank position and several factors that may influence that position. First, all rank orders were tested for linearity by a test proposed by APPLEBY (1983) using the MatMan program (DE VRIES *et al.*, 1993). Correlations between the positions held in these rank orders and the factors (expressed in years), height (in cm at the withers), residency in the herd (in months) and the age the geldings had at castration were calculated. For these comparisons the Spearman rank correlation coefficient (R_s) was used (LEE & LEE, 1982). We wanted to know whether having a newborn foal influenced the rank of the mother and the occurrence of aggressive behaviour. To investigate the latter we calculated a relative aggression score (= Number of aggressive acts/observation time) for the normal-days and for the foal-days and compared these. Another question to be answered was whether the rank positions of the adult offspring were correlated with those of their mothers. For comparisons between rank position and the relative aggressiveness of (adult) offspring (N = 9) and their dams (N = 8) the linear Pearsons correlation coefficient (Rp) was used (LEE & LEE, 1982).

The relationships between rank differences and social or kin bonds were analysed using matrices of allogrooming, proximity and kinship. *Allogrooming* was defined as mutual interactive nibbling between two horses, mainly at each others mane, withers, back and tail base. This behaviour occurred between almost all animals of all sex-age classes. When two animals were within two horse lengthts from each other they were considered to be in each other's *proximity*. Proximity between each pair of horses was recorded every half hour by a scan sample. *Kinship* was defined as a familiar relationship an animal could have knowledge of: mother-offspring, grandmother-grandschild, brother-sister via the mother. Stallion offspring relationships were not included. The correlation between the rank differences between each pair of horses and the social or kin bonds between them is assessed by means of the Mantel test (SCHNELL *et al.*, 1985). To obtain the significance probabilities we employed a permutation procedure which respects the interdependencies of the values within rows and columns of these matrices. The dual normalization procedure described in FREEMAN *et al.* (1992) was used to correct for individual variation in the tendency to allogroom or to be in the proximity of any other horse. We used the program MatMan (DE VRIES *et al.*, 1993), to calculate dually normalized matrices and subsequently perform permutation Mantel tests.

RESULTS

Applicability of the concept of dominance

According to the first criterion stated in the previous section, eight behaviours occurred sufficiently often to be used for assessing dominance. The values the four parameters (coverage, number of ties, directional consistency and linearity) took for the eight selected behaviours as described above are shown in table III. Five behaviours satisfied, at least in two cases, the criterion that a value for a behaviour should be better than the average value for that specific property. None of the behaviours showed a Landau's $h > 0.9$, which is generally taken to denote a strongly linear hierarchy (MARTIN & BATESON, 1993). On the other hand, application of the linearity test (APPLEBY, 1983)

showed that for five behaviours there was a significant deviation from non-linearity. *Avoidance* showed the strongest linearity. The results of the Principal Component Analysis (table IV) clearly showed that two groups of behaviours emerge: offensive aggressive behaviours (*bite, threat to bite, approach with ears flattened* and *attack*) and *avoidance* on the one hand, and defensive aggressive behaviours (*kick, buck* and *threat to kick*) on the other hand. The first group of aggressive behaviours are considered to be of an offensive nature, because of the strong approaching tendencies involved. This group is negatively correlated with *avoidance.* The second group of aggressive behaviours is considered to have a more defensive nature, because these behaviours may be shown while retreating. This is in line with observations made by WELLS (1978), WELLS & VON GOLDSCHMIDT-ROTHSCHILD (1979), FEH (1988) and RUTBERG & GREENBERG (1990). Retreating is almost impossible during biting attempts, because these include necessarily a forward movement. Moreover, when using the hind legs the horse already is in a favourable position to withdraw. With regard to the contexts in which aggression occurred, we can say that aggression occurred on the pasture but was more common around the drinking bowls, the mineral supply and in the transition corridor. These last three situations have in common that there is limited availability in the sense that only one animal at a time could use the supply, or, in case of the corridor, could use the space.

The separate rank orders for these five behaviours correlated significantly with each other (table V), so that the use of dominance as an intervening variable (*cf.* HINDE, 1974) is justified and one hierarchy can be constructed, using the combined relationship matrix as described in the Methods section above. Figure 1 presents this relationship matrix based on the dyadic aggression and avoidance relationships. This combined relationship matrix contains only 28.75 circular triads out of the maximum of 385 circular triads possible in a matrix of size 21. This corresponds to a highly significant Laundau's linearity index h of 0.925 ($p<<0.01$). There were only 5 blank relationships. Because of the strong linearity present in this set of relationships, we may conclude that a (nearly) linear rank order indeed exists among the 21 Icelandic horses and it is therefore justified to construct this (nearly) linear rank order. In the matrix of fig. 1 the animals have been arranged according to this (nearly) linear rank order following the procedure described above in the Methods section. For the dominance relationships among the mares, the value of Landau's linerarity index turned out to be also very large, namely 0.95 (n = 15; $p<<0.01$). The rank-ordered dominance relationships matrix for the mares is presented in fig. 2. For the geldings even a strictly linear hierarchy was found (Landau's h = 1; n =

TABLE V

Spearman rank correlations between five rank orders

Bite	bite				
Threat to bite	0.85	thr. to bite			
Approach flattened ears	0.84	0.92	appr.		
Attack	0.84	0.92	0.87	attack	
Avoidance	0.87	0.93	0.94	0.87	avoid

All correlations are highly significant ($p<0.01$).

	f	P	b	l	K	D	p	s	B	d	U	S	k	F	m	t	Q	o	r	E	H
f	*	3	3	2	3	3	3	3		3	3	3	3	3	3	3	3	3	3	3	3
P		*	2	3	2		3	3	3		2	2	3	3	3	3	3	3	2	3	3
b			*	2	3	2	3	2	2	2	3	2	3	2	3	2	3	3	3	3	3
l	1		1	*	3	1	1	1	3	1	3	1	3	3	3	1	3	3	3	3	3
K					*	3	3		3	3	2	2	3	2	3	3	3	3	3	3	3
D			1	2		*	1			3	3	3	3	3	3	3	3	3	3	3	3
p							*	3	3	3	3	3	3	3	3	3	3	3	3	3	2
s					3			*	2		3	2	3	2	3	3	3	3	2	2	3
B	2					3			*	3	1		3	3	3	3	3	3	3	3	3
d			1	2				3		*		3	3	3	3	3	3	3	3	3	3
U									2	[illegible]	*	3	3	3		2	3	2	2	3	2
S												*	2	3	3	3	3	3	3	3	3
k													*	2	3	3	3	3	3	3	3
F													1	*	3		3	3	3	3	3
m															*	3	3	3	3	3	3
t														2		*	3	2		2	3
Q																	*	3	3	3	3
o																		*	3	3	3
r																			*	2	1
E																			1	*	3
H																					*

Fig. 1. Dominance relationship matrix for 21 male and female horses based on offensive aggressive behaviour and avoidance behaviour. The individuals are arranged in hierarchical order. The stallions are underlined. 3: the row individual is dominant over the column individual on the basis of aggression given and avoidance achieved. 2: the row individual is dominant over the column individual on the basis of avoidance archieved. 1: the row individual is dominant over the column individual on the basis of aggression given.

	f	P	b	l	K	p	d	s	U	k	m	t	o	r	E
f	*	3	3	2	3	3	3	3	3	3	3	3	3	3	3
P		*	2	3	2	3	2	3	2	3	3	3	3	2	3
b			*	2	3	3	2	2	3	3	3	2	3	3	3
l	1		1	*	3	1	1	1	3	3	3	1	3	3	3
K					*	3	3		2	3	3	3	3	3	3
p						*	3	3	3	3	3	3	3	3	3
d			1	2			*	3		3	3	3	3	3	3
s					3			*	3	3	3	3	3	2	2
U									*	3		2	2	2	3
k										*	3	3	3	3	3
m											*	3	3	3	3
t												*	2		2
o													*	3	3
r														*	2
E														1	*

Fig. 2. Dominance relationship matrix for 15 female horses based on offensive aggressive behaviour and avoidance behaviour. Same legend as fig. 1.

	B	D	S	F	O	H
B	*	3	3	3	3	3
D		*	3	3	3	3
S			*	3	3	3
F				*	3	3
O					*	3
H						*

Fig. 3. Dominance relationship matrix for 6 male horses based on offensive aggressive behaviour and avoidance behaviour. Same legend as fig. 1.

6; $p = 0.022$). The dominance matrix for the geldings is presented in fig. 3. These three (nearly) linear hierarchies will be used in the subsequent analyses, where we investigate which factors might be related to these rank orders.

Factors related to rank

Rank and sex — The top five places of the rank order of the complete group were taken by older mares; the first gelding (D) takes the 6th place. The rank positions of the males are interspersed with those of the females. It is noteworthy that the relative number of circular triads is highest (*i.e.*, Laundau's h is smallest) when the intra-sexual dominance relationships of the females (fig. 2) and the males (fig. 3) are combined with the inter-sexual relationships into one set (fig. 1). This means that some intersexual dominance relationships are not transitive. Note, for instance, the remarkable subordinate position of the top ranking female f to the top ranking male B (top ranking in the male hierarchy, that is). Note also that the α and ß male B and D in the male hierarchy have reversed rank positions in the complete hierarchy. This is due to the fact there are two mares (p and s) who dominate B but not D, and there is also one mare (l) who is subordinate to D but not to B. It is evident that the dominance relationships between the top ranking males and the high ranking females are not transitive.

Rank and age — Age correlated positively with rank in the linear rank order of the complete group ($n = 21$) and in the rank order of the females ($n = 15$; see table VI). However, age was not correlated with rank in males. A female rank order constructed excluding the influence of age (see Clutton-Brock *et al.* (1986) for methods) showed a different picture. This rank order was not significantly correlated with the linear rank order ($Rs = 0.15$, $n = 21$, n.s.). Thus, age is an important rank determining factor, at least for the females.

Rank and height — Height correlated negatively with the linear rank order of the complete group (21 horses). So, the smallest horses were highest in rank. On the other hand, height correlated negatively ($Rs = -0.60$, $n = 21$, $p<0.01$) with age also. That is, younger animals are larger than the older ones. Height did not correlate significantly with rank in the separate female and male rank orders.

Rank and residency — Rank position was correlated positively with the time of residence in the herd.

Rank and relative aggression and relative submission — The rank position in the hierarchy was, surprisingly, not or very weakly correlated with the relative aggressiveness of the individuals. This means that not the overall amount of aggressive acts performed determines the rank of an

individual, but rather to whom this aggression was directed. Relative submissiveness, on the other hand, correlated significantly with rank.

Rank and age at castration — The male rank order was correlated with the age at which they were neutered ($Rs = 0.97$, $n = 6$, $p<0.05$). That is to say, the older a male was when being neutered the higher his rank position. Below we will show that age at castration in our study herd is strongly related to the amount of sexual experience and to the possibilities to develop male displays towards other (castrated) stallions. Therefore, we can say that not the castration age as such determines the rank order among the castrated males, but rather that the amount of socio-sexual experience of a castrated stallion determines its rank.

Icelandic horses are, in this herd but also on Iceland and at other studs, always living outside in a group. From this herd all sexual experiences of all males are known. The gelding that ranked highest in the male hierarchy (B) was castrated when 7 years old and had for two years his own herd of mares. He sired four of the other herd members. The second ranking gelding (D), who has a higher rank position in the full herd but is dominated by B, had only little sexual experience: as a five year old he had one mare for a few weeks. None of the other geldings have had any sexual experience. All Icelandic horses, except H and F, have lived in 'free roaming' batchelor herds until 5 years old. From H it is only known that he was castrated on Iceland, most likely as yearling. He lived mainly in a small mixed herd. F and C were kept in mixed herds after neutering. So in this herd, age at castration is strongly related to the amount of socio-sexual experience.

Rank and being in oestrus and/or having a foal — Two foals were born during this study. Their dams climbed respectively one and three places in the rank order while their foals were less than three weeks old. This provided us with not more than a weak indication that dams temporarily rank higher when having a new-born foal. The relative aggression scores of both dams increased slightly after giving birth to a foal: for one dam from 1.13 to 1.57 and for the other from 0.72 to 1.00. The mean relative aggressiveness ($n = 21$), on the other hand, decreased from 1.71 to 1.14. No influence of oestrus on the rank position of the mares concerned was evident.

The rank positions of a mare and her adult offspring — The rank positions of mares correlated positively with those of their adult offspring ($Rp = 0.88$). The relative aggressiveness of the dams and those of their adult offspring was also correlated ($Rp = 0.75$, $n = 8$, $p<0.05$). Since there is no correlation between age of dams and age of their offspring nor between age and relative aggressiveness in the group of adult animals, the possibility of the former correlation being due to the latter is unlikely.

TABLE VI

Spearman rank correlations between rank position in the hierarchy and six individual factors

feature	*group*	*Rs*	*n*	*p*
age	complete group	0.74	21	0.002
	mares	0.75	15	0.002
	geldings	0.09	6	ns
height	complete group	-0.54	21	0.002
	mares	-0.45	15	ns
	geldings	-0.54	6	ns
residency	complete group	0.70	21	0.002
	mares	0.65	15	0.02
	geldings	0.94	6	0.01
rel. aggressiveness	complete group	0.36	21	ns
	mares	0.42	15	ns
	geldings	0.83	6	0.05
rel. submissiveness	complete group	-0.89	21	0.002
	mares	-0.80	15	0.002
	geldings	-0.94	6	0.01
castration age	geldings	0.97	6	0.01

n = number of individuals; p = probability

Rank, kinship and social bonds — An interesting question is whether the difference in rank positions between each pair of horses is related to the social bond, measured as the frequency of allogrooming interactions between the two horses or as being in each other's proximity. It is also interesting to investigate the possible relation between rank difference and kinship. To this end we constructed the following dyadic matrices: (1) a kinship matrix: this is a 0-1 matrix, where a 1 means kin (via the mother) and a 0 means not kin; (2) a matrix of rank differences, which indicates how many horses separate any given pair in the hierarchy (see the lower triangular half of the matrix presented in fig. 4), (3) a frequency matrix of mutual allogrooming, and (4) a proximity matrix, which contains for each pair of horses the frequency with which these two horses were within two horse lengths from each other (see the upper triangular half of the matrix presented in fig. 4). All matrices are symmetric. For each pair of these matrices we calculated the Pearson's correlation coefficient and its statistical significance, using a permutation test which respects the interdependencies of the values within rows and columns of these matrices. The MatMan program (De Vries *et al.*, 1993) was used to perform these permutation Mantel tests.

	f	P	b	l	K	D	p	s	B	d	U	S	k	F	m	t	Q	o	r	E	H	Total
f	*	0.5	2.0	1.0	0.0	0.0	2.0	1.5	3.5	0.5	0.0	1.5	1.0	2.0	0.0	1.0	1.5	2.5	0.5	0.0	1.5	22.5
P	0	*	1.0	1.5	2.0	1.5	1.5	3.3	2.0	1.1	1.0	1.8	4.3	1.8	1.7	1.0	2.5	0.8	1.0	0.8	0.5	31.5
b	1	0	*	2.5	1.8	0.8	5.0	5.3	2.5	3.8	2.0	0.8	10.	1.0	4.0	1.8	2.3	2.0	2.0	2.3	1.0	54.2
l	2	1	0	*	1.5	0.5	4.0	3.0	2.0	5.5	1.5	3.5	2.5	4.0	3.5	3.5	3.0	4.0	2.5	2.0	1.0	52.5
K	3	2	1	0	*	3.3	0.5	2.7	1.0	2.3	6.5	1.5	2.3	0.5	2.3	3.3	0.3	1.3	0.5	1.0	0.5	35.1
D	4	3	2	1	0	*	2.3	0.3	4.0	1.3	0.5	5.0	1.0	5.8	1.7	0.8	2.8	0.8	0.8	0.5	1.0	35.7
p	5	4	3	2	1	0	*	4.0	8.0	5.8	3.0	8.3	4.3	3.5	2.3	0.8	3.0	1.0	1.0	1.3	0.5	62.1
s	6	5	4	3	2	1	0	*	2.3	3.0	4.0	0.7	5.3	0.7	0.3	2.3	4.3	1.0	3.0	1.3	0.0	48.3
B	7	6	5	4	3	2	1	0	*	6.3	1.5	9.0	5.3	5.0	2.3	1.5	9.5	1.5	1.3	0.8	1.0	70.3
d	8	7	6	5	4	3	2	1	0	*	4.5	5.0	6.3	1.0	2.7	0.8	4.0	2.5	1.8	2.3	1.5	61.9
U	9	8	7	6	5	4	3	2	1	0	*	1.0	1.0	0.0	0.5	3.0	1.5	3.0	0.0	2.0	0.0	36.5
S	10	9	8	7	6	5	4	3	2	1	0	*	2.7	3.3	4.7	0.3	11.	4.0	1.0	0.5	1.0	66.9
k	11	10	9	8	7	6	5	4	3	2	1	0	*	1.0	7.3	3.0	6.3	1.3	3.0	3.0	1.5	72.7
F	12	11	10	9	8	7	6	5	4	3	2	1	0	*	2.7	1.0	5.3	2.0	1.5	1.5	1.5	45.1
m	13	12	11	10	9	8	7	6	5	4	3	2	1	0	*	4.3	6.7	4.0	1.3	2.3	0.5	55.1
t	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	*	1.3	2.5	2.0	2.5	0.5	37.2
Q	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	*	4.0	2.5	1.5	2.0	75.6
o	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	*	5.3	5.5	1.5	50.5
r	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	*	8.8	1.0	40.8
E	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	*	0.5	40.4
H	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	*	18.5
Tot	190	171	154	139	126	115	106	99	94	91	90	91	94	99	106	115	126	139	154	171	190	

Fig. 4. This figure shows two triangular halfs of two symmetric matrices. Below the diagonal: matrix of rank differences (*i.e.*, the number of individuals in between each two rank positions). Above the diagonal: matrix of proximity frequences. The column totals give the marginal totals of the full matrix of rank differences. The row totals give the marginal totals of the full matrix of proximity frequences.

The upper panel of table VII shows the results of these tests. It turns out that kinship is not related in any way to difference in rank positions or to one of the social bond measures: proximity and allogrooming. Rank difference, on the other hand, is strongly correlated with proximity (r = -.290, p<.001), that is: the closer two horses are in rank to each other the more frequently they are in each other's proximity. For the correlation between rank difference and allogrooming not more than a weak trend was found (r = -.117, p = .09). Not unexpected, proximity and allogrooming were strongly correlated (r = .491, p<.001).

Horses may differ in their tendencies to get involved in allogrooming interactions with any of the other horses. Similarly, they may differ in their propensities to be in the proximity of any of the other horses. A disadvantage of the Mantel test is that it does not take this individual variation into account. For instance, the correlation between rank

difference and proximity obtained above by the Mantel test could be the result of a correlation between an animal's deviation from the mean rank position and its tendency to be in the proximity of any of the other horses. To overcome this problem, FREEMAN *et al.* (1992) suggested the use of an iterative proportional fitting procedure (BISHOP *et al.*, 1975, pp. 97-101) to fit homogeneous margins to observational data matrices like the proximity and allogrooming matrices. By this procedure the matrices are dually normalized; that is, for each pair of horses a value is obtained that represents the number of allogrooming acts (or being in proximity) they would be involved in if all the marginal totals were equal. Subsequent Mantel tests using these normalized matrices are thus corrected for individual variation in behavioural tendencies. To eliminate non-diagonal zero cells a constant of 0.25 was added to these cells before the iterative proportional fitting procedure was applied, as suggested by FREEMAN *et al.* (1992). The dually normalized matrices were calculated by means of a specially written extension of the program MatMan (DE VRIES *et al.*, 1993).

The lower panel of table VII presents the results of the Mantel test using these normalized matrices. The correlations between kinship and the other variables as well as the correlation between proximity and allogrooming did not change much. However, the correlation between rank difference and proximity dropped from a highly significant value of -.290 ($p<.001$) to a just significant value of -.143 ($p = 0.04$). This decrease is apparently due to the strong negative correlation of -.637 that existed between an animal's total sum of rank differences (indicating its deviation from the mean rank position) and its tendency to be in the proximity of any of the other horses (*i.e.*, the correlation between the column totals in fig. 4 and the row rotals in fig. 4). In other words, top ranking and bottom ranking horses stayed less frequently in the close vicinity of another horse than middle ranking horses did. "It's lonely at the top as well as at the bottom." There is one clear exception, namely the mare U, which occupied the middle rank position 11 (marginal total of 90), but at the same time had a very low frequency of being in another horse's neighbourhood (marginal total of 36.5).

The already rather weak correlation between rank difference and allogrooming (r = -.117) decreased to an insignificant value of -.045 when we performed a Mantel test using the dually normalized matrix of allogrooming. The difference between these two correlation values is not quite understandable to us, because the correlation between an animal's deviation from the mean rank position and its general tendency to be involved in allogrooming interactions is only -.031. However, there existed large differences between individuals to get involved in allogrooming, and therefore we felt that the application of the

TABLE VII

Matrix Correlations between kin, rank difference, proximity and social bond

		Correlation r	p-value#	
kinship	rank difference	.063	>.25	ns
kinship	proximity	.061	>.25	ns
kinship	allogrooming	.041	>.40	ns
rank difference	proximity	-.290	<.001	**
rank difference	allogrooming	-.117	.09	tr
proximity	allogrooming	.491	<.001	**
kinship	rank difference	.063	>.25	ns
kinship	proximity	-.003	>.40	ns
kinship	allogrooming	0.82	>.25	ns
rank difference	proximity	-.143	.04	*
rank difference	allogrooming	-.045	>.40	ns
proximity	allogrooming	.468	<.001	**

Upper panel: the dyadic frequency matrices (proximity and allogrooming) are not corrected for individual differences in their propensities for each of these social behaviours.
Lower panel: the proximity and allogrooming matrices are dually normalized by means of fitting homogeneous margins to these matrices (FREEMAN *et al.*, 1992), thereby correcting for individual differences.
The p-values are two-tailed and based on a permutation test in which the rows and columns of one of the two matrices are simultaneously permuted 10.000 times.
tr = trend $.05 < p < .10$; * = $.01 < p < .05$; ** = $p < .001$.

normalization method by which this individual variation is taken into account was required. Anyway, the conclusion can be drawn that rank difference and allogrooming are not significantly correlated.

DISCUSSION

The applicability of the dominance concept

In this paper we started with analysing what behavioural exchanges reflect dominance relationships in horses. The results demonstrate that only a very limited set of behaviours reflected dominance in such a way that they could be used to construct a rank order. An important result is that we were unable to construct a (nearly) linear rank order when defensive aggressive behaviours (threats or kicks with the hind legs) and offensive aggressive behaviours (threats of attacks with the head) were combined. This result is in line with those of WELLS (1978), FEH (1988) and RUTBERG & GREENBERG (1990) on horses and of SCHILDER (1988, 1990) on zebras. It suggests that the habit of combining behaviours of

an offensive nature with those of a relatively more defensive nature is unjustified. This conclusion is reinforced by the results of a PCA of the asymmetries in the exchange of eight behaviours, in which the factor loadings of the offensive and defensive behaviours are high on two different factors. The fact that kick and buck show a low degree of transitivity and a relatively low directional consistency suggests that they were used defensively against ranking opponents as well as (offensively?) against lower ranking opponents. On the basis of these results we recommend to keep behaviours involving the head and those involving the rear separated, and to take into consideration avoidance behaviour when investigating dominance in horses.

Just as with zebras (SCHILDER, 1988), the conclusion can be drawn that the concept of dominance is applicable to a certain degree. Also comparable to zebras is that horses do not appear to use formal dominance signals as has been shown for chimpanzees (NOË *et al*, 1980) and wolves (VAN HOOFF & WENSING, 1987). These two species use highly ritualized displays which are (almost) completely unidirectionally exchanged within each dyad and are being shown in a large number of dyads. Such displays could therefore be considered as indicators of a formal status rank order. In horses toothclapping and ceremonies involving defecating/urinating over faeces/urine of other individuals could be regarded as dominance related ritualized displays. However, in our study group toothclapping was only performed by the juveniles, which is in agreement with other observations (WARING, 1983). Defecating/urinating ceremonies were alost exclusively performed by adult stallions and were for that matter almost absent in this study (see table II). Therefore, these behaviours could not be used in investigating dominance between adult horses. None of the other behaviours showed sufficient unidirectionality and high enough coverage to be designated as a formal dominance signal. In fact, the behaviour which reflects best the dominance relationship between two horses is avoidance. This is completely in line with the finding that the more two horses are separated in rank the less frequently they are within two horse lengths from each other. This means that if subordinate non-juvenile horses get involved in a conflict with a higher ranking horse, they can only give ground or defend themselves, but are unable to present a display (with the possible exclusion of tooth-clapping) by which they acknowledge the dominance status of the other, thereby possibly reducing its aggression.

Rank and its possible determinants

The hierarchy of the horses does not consist of two disjunct parts, in which all males or all females would occupy the higher positions and

the horses of the other sex would occupy the lower rank positions. In the (nearly) linear rank order of the complete group the rank positions of males and females are interspersed (fig. 1). It turned out that the few triangular dominance relationships that existed involved intersexual relationships. The hierarchies of the males and females separately were more linear than the combined hierarchy (figs. 2, 3).

In general, the rank position in the dominance hierarchy correlated strongly with the factors age, residency and castration age and less with the factor height. This can be interpreted by saying that the rank position of an adult horse appears to be determined by the amount of social experience acquired by the horse, rather than by its actual physical strength/height. This interpretation is strengthened by the fact that the rank of the castrated stallions was not correlated with age but with the age at which they were castrated. That is, geldings with a relatively high age at castration have a high rank. This could be a result of differences in the remaining hormone levels due to differences in surgical techniques, but these differences are usually very minor (VOITH, 1979). More likely it is an effect of the differential behavioural experiences the animal had before castration. For horses there are no specific reports on this issue available. LINE *et al.* (1985) state that there is no difference in sexual and aggressive behaviour in pre- and post-pubertal castrated male horses, but they do not refer to other behavioural experiences. A study by HART & HART (1985) showed that about 50% of the dogs that were neutered because of excessive aggression towards other dogs continue to attack dogs after castration. This suggests that experience may play a role here also. For the horses in the present study, hormones as well as the possibilities to mate influenced the socio-sexual experiences of the castrated stallions. This in turn determined strongly the rank order among the castrated males.

We were amazed by the fact that an effect of residency was still detectable, although the mean time of residency was 9.5 years (range: 5-12 years). Residency was, however, in most individuals related to age. The use of the ranking method developed by CLUTTON-BROCK *et al.* (1986) could be a solution to separate these effects. Unfortunately, this method resulted in a lot of ties, which made this rank order unfit for further analysis.

Our results possibly can be best compared with studies involving groups of horses that are together for at least several years (for example BERGER, 1986 and KEIPER & SAMBRAUS, 1986) rather than with studies in which horses do not know each other for a long time (as for example in the studies of HOUPT *et al.*, 1978 and HOUPT & KEIPER, 1982). The reason for this is that the influence of the factor 'social experience' may be different.

Obviously, hormones also influence the social behaviour of both females and males in times of oestrus. However, in this study, being in oestrus did not have an observable effect on the rank of the female.

The rank positions of dams and their mature offspring were correlated and both correlated with relative aggressiveness. No correlation was found between age of dams and their offspring nor between age and relative aggressiveness. Therefore, the high rank positions of offspring of an aggressive mother are most likely due to aggressivenes of the offspring itself. Some other authors (TYLER, 1972; WELLS & VON GOLDSCHMIDT-ROTHSCHILD, 1979; HOUPT, 1979; HOUPT & WOLSKI, 1980) also showed a relationship between the rank position of dams and their adult offspring. BEAVER & AMOSS (1982) described cases in which aggressive behaviour was correlated with naturally elevated serum testosterone in mares. If there are hereditary factors involved, this would explain why foals tend to be as aggressive as their mothers. But, of course learning factors may be involved too (HOUPT, 1981).

Finally, we found that rank strongly influenced the proximity structure of the horses. At the individual level, it turned out that horses with a rank position close to the mean rank tended to stay more frequently in the proximity of other horses than high ranking or low ranking horses did. Over and above this effect at the individual level, we also found that pairs of horses close in rank to each other tended to stay close in each other's physical proximity. We did not find that rank or rank difference influenced the allogrooming relationships between the horses. Neither did we find any correlation between kinship and rank difference or kinship and the social bond measures (proximity and allogrooming). We conclude that rank and rank difference have a strong direct effect on the interindividual distances between the horses, but probably not on the social bonds between them.

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THE IMPORTANCE
OF SOCIAL RELATIONSHIPS
IN HORSES

CHAPTER 5

Differences in social behaviour between late pregnant, post-partum and barren mares in a herd of Icelandic horses

VanDierendonck, M.C., Sigurjonsdottir, H., Colenbrander, B., and Thorhallsdottir, A.G., Applied Animal Behaviour Science 89(3-4): 283-297, 2004.

ABSTRACT

Group dynamics and the social behaviour of mares were investigated in the periods before and after foaling during 2 non-consecutive years, in a herd of Icelandic horses consisting of adult mares and geldings and juvenile fillies, geldings and colts. The horses were observed 24-hours a day for 5-6 weeks; i.e. a total of 1483 hours. There were significant changes in the social behaviour and interactions of mares after foaling, compared both to their own behaviour pre-partum and to that of barren control mares. While the frequency of allogrooming dropped during the study period in all mares, it dropped significantly more in foaling than in control barren mares. However, while the absolute allogrooming frequency was strongly correlated with reproductive state, it was still remarkably consistent within an individual adult mare between the study years. After foaling, mares with foals separated off into a distinct subgroup, whereas barren control mares increased interaction with the rest of the herd; it is presumed that separation served to keep the foals at a safe distance from the more boisterous geldings and sub-adults; alternatively, it could have been the result of mutual attraction between the foals. In general, the linear dominance order was correlated strongly with age, and the top-ranking mares were older mares that had not yet begun a senescent loss of physical condition which resulted in a later slight drop down the hierarchy. There was no consistent relationship between dominance rank and the pattern of preferred recipients of affiliative interactions, while familiarity was a more important determining factor than kinship in the selection of a preferred partner for affiliative interactions. The preferred partner for proximity was often the same individual as that for allogrooming, suggesting that proximity was a result of an active process rather than of passive acceptance. The presence of adult geldings in the herd did not seem to change the social behaviour of mares as compared to that described for feral horses, and did not appear to negatively affect behaviour during parturition, mare-foal bonding or subsequent maternal care. In short, maintenance of breeding mares in a herd of mixed age and sex did not interfere with normal species-specific behaviour or with the development of the stable, long-lasting mare-mare bonds that are central to maintaining a stable herd. It is concluded that such a system could be a welfare-friendly alternative to typical modern husbandry systems, as long as adequate space is available.

Keywords
Horses; social behaviour; parturition; allogrooming; mares; foals; geldings.

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INTRODUCTION

In many equine husbandry systems, the behaviour of horses is influenced negatively by the absence of opportunities for social contact, unhindered locomotion or foraging, and this is confounded by inappropriate feeding (Mills and Clarke 2002). In principle, abnormal behaviours could be prevented or ameliorated by the adoption of more "natural" husbandry systems, such as keeping mares and geldings in groups rather than individually. However, while group housing systems provide extensive opportunities for social contact and free locomotion (Mcgreevy 1997, Lebelt 1998, Mcbride and Long 2001), imposed and unstable social grouping can lead to problems such as extreme submission and/or aggression possibly leading to injury (Kolter 1984, Zeitler-Feicht 1996, Zeitler-Feicht and Prantner 2000, Bachmann and Stauffacher 2002, Mills and Clark 2002). However, these domesticated groups still differ markedly from the natural forms of social organisation, namely harems, multiple male bands or bachelor bands, described in the literature (Klingel 1975, Linklater 2000). In general, domesticated or managed groups of horses consist either of broodmares (and their foals), young stock (usually in year cohorts) or adult mares plus adult castrated males (geldings). And while extensive research has been performed on the behaviour of individuals in both breeding mare and young stock groups (Estep et al. 1993, Barber and CrowellDavis 1994, Weeks et al. 2000, Christensen et al. 2002a, 2002b), little data is available on complex groups with both (sub)adult geldings and breeding and non-breeding mares (Sigurjónsdóttir et al. 2003).

Important parameters for evaluating the stability and structure of an equine social group include aggression, dominance, allogrooming and proximity. Allogrooming seems to be a very useful behaviour to strengthen the bond between individuals, especially mares (Feist and McCullough 1976, Kimura 1998, Feh 1999), since it may help to reduce tension between animals (Spruijt et al. 1992, Feh and Mazieres 1993) as well as being a way of 'cleaning' or 'scratching'. The relationship between close proximity and allogrooming is, however, still under debate (Clutton- Brock et al. 1976, Jezierski and Gebler 1984, Wood-Gush and Galbraith 1987, Kimura 1998, Roberts and Browning 1998, Sigurjónsdóttir et al. 2003). Close proximity could be the result of individual initiatives such as allogrooming, or of passive acceptance. Proximity is usually defined in terms of 'nearest neighbour' scores, but occasionally as distances between individuals (Linklater et al. 2000). Every individual has a 'flight zone' and a 'personal space'; the area immediately around the horse in which only close companions are tolerated (for review see Mills and Nankervis 1999). For the study of proximity relationships, biologically meaningful information can best be obtained by evaluating which individuals are within a short distance (personal space) of each other, and less meaningfully by nearest neighbour scores.

In addition to allogrooming and proximity, dominance plays an important role in the social organisation of equine groups (Rutberg and Greenberg 1990, Vandierendonck et al. 1995, Roberts and Browning 1998, Sigurjónsdóttir et al. 2003). Often, similarly ranked animals form especially close social bonds (become 'preferred partners') (VanDierendonck et al. 1995). However, while rank and age are important to subgroup formation within a feral population (Kaseda et al.1995, Kimura 1998), duration of co-residence (VanDierendonck et al. 1995), familiarity and kinship (Roberts and Browning 1998) are also major

factors contributing to strengthened social relationships between horses (Sigurjónsdóttir et al. 2003). Certainly, mares tend to have stronger bonds with familiar individuals or close kin (Estep et al. 1993, Barber and CrowellDavis 1994). It follows that a given individual will usually allow others with specific characteristics with regard to kinship, familiarity, duration of residence and sex within its flight or personal space.

The relationship between geldings and, in particular, foaling mares has not been examined extensively (Wood-Gush and Galbraith 1987, VanDierendonck et al. 1995, Asa 1999). In the closest feral counterpart of this type of herd, the multiple male band, mares are more frequently harassed and have less resting time, and therefore tend to be in poorer body condition than mares in harems (Linklater 2000). The breeding performance of mares in multiple male bands is also worse than in herds with a single harem stallion. However, it is not known if the presence of adult geldings has a similar detrimental effect.

Parturition and suckling are associated with short and long-term physiological and behavioural changes, related primarily to the demands of caring for a foal. These behavioural changes can also profoundly affect the dam's relationship with other group members. Self-imposed social isolation of the dam and her newborn foal serves to prevent others from interfering with the process of bonding between the newborn foal and its dam in the first few days after birth (Klingel 1975). Indeed, within a herd of broodmares, mares that foal are more likely to make changes in their affiliative relationships after foaling than before and, after foaling, mares are generally less affiliative with other herd mates (Estep et al 1993).

In the current study, which is part of a larger study (Sigurjónsdóttir et al. 2003), the behaviour of adult mares around the time of parturition was observed in a group of domestic horses that also included sub-adult (1-4 year old) mares, yearling stallions and sub-adult and adult geldings (but no adult stallions). This husbandry system has been used extensively for many centuries in Iceland, but is virtually unheard of in mainland Europe and has not been studied in great depth. In order to better understand the desires/needs of parturient mares and to determine the basic requirements of such a group management system during the foaling season, changes in the social behaviour of mares before and after parturition and differences to barren control mares were investigated.

ANIMALS, MATERIALS AND METHODS

During the foaling periods (April to mid-June) of 1997 and 1999, social behaviour was studied in pastured groups of Icelandic horses. The studied groups comprised adult mares (pregnant and non-pregnant) and geldings, sub-adult mares and - geldings (1-4 year olds) and yearling stallions (Table 1). Eighteen individuals (including 11 of the adult mares) were observed in both of the study years.

Table 1
Sex, age and reproductive status of the horses in the study groups.

	1997 n	1999 n	1997 mean ± sd age (years)	1999 mean ± sd age (years)
Pregnant mares*(≥ 6 yrs)	12	14	14.8 ± 3.3	16.4 ± 3.7
Post partum mares*	7	13**	13.9 ± 3.6	16.5 ± 3.8
Barren control mares (≥ 6 yrs)	5	4(5)**	15.0 ± 3.8	16.0 ± 7.0
Adult Geldings (≥ 4 yrs)	5	4	6.2 ± 3.0	13.5 ± 3.1
Sub Adult Males (1-4 yrs)	6	5	1.3 ± 0.5	2.8 ± 1.3
Sub Adult Females (1-4 yrs)	6	4	2.2 ± 0.8	3.3 ± 1.0
Foals	7	13		

* During the observation periods of 1997 and 1999, 7 and 14 mares foaled, respectively
** One foal died soon after birth and its dam was, therefore, moved from the pregnant group to the barren (no foal) control, instead of the post-partum, group after parturition

The animals studied were part of a herd of 80-100 horses that was free-ranged all year round in a mountainous, river-dense area exceeding 1200 hectares in Southeast Iceland. In winter and spring, supplementary silage was provided once daily. During the observation periods only, the animals under study were corralled within an 8-hectare sub-enclosure, to facilitate detailed observation. Shelter within the sub-enclosure was more than adequately provided by landscape elements.

In 1999, 8 animals (including 4 adult mares) from two neighbouring farms were added to the group observed in 1997, with the aim of introducing familiarity as a variable to the social interactions. Seven foals (2 colts and 5 fillies) were born in 1997 and 13 (5 colts, 8 fillies) in 1999. All horses, except one from 1997 and the 8 animals introduced in 1999, were born in the herd and all adult mares were multiparous. The degree of kinship between pairs of animals was calculated by means of co-ancestry over 5 generations, as described by Hartl and Clark (1997).

Observation and recording of behaviour patterns

The observation methods consisted of All Occurrence (AO) observations of 99 predefined behavioural elements, which included affiliative and dominance elements. In addition, in 1997, the AO observations were alternated every half hour with 15-minute focal animal (FA) observations of adult mares, and spatial scan (SS) samples. If a foal was born, preference was given to collecting FA and AO samples rather than SS samples. The ethogram

used was described by Schilder (1990) and modified according to McDonnell and Haviland (1995). The FA and AO data were recorded on a Psion handheld computer(s) using 'The Observer' software*, and transferred daily to a laptop computer. Since there was 24-hour daylight in Iceland, the data were collected continuously, 24 hours a day. To prevent any structural bias in recordings, the observers worked in 8-hour shifts with one overlapping hour at each shift change, so that shift changes were not at the same time of the 24 hr clock every day. Each observer covered day and night periods equally. Some observation time was lost due to unfavourable weather conditions or human body maintenance requirements. Regular inter-observer reliability sessions were performed. In both years, the first foal was born 5 days after starting the baseline observations. Observation was continued for a total of 488 hours in 1997 and 828 hours in 1999, and 666 spatial samples were recorded.

Data analyses

The data from the adult breeding mares was analysed with respect to reproductive status, i.e. pregnant versus post-partum. In addition, a barren mare was assigned randomly to each foaling mare, as a control. The social interactions of the control mare were then grouped according to the pre and post-partum periods of the associated foaling mare, to control for the effect of season; these mares will be referred to accordingly as pre-foaling controls and post-foaling controls. Because foals were born at different dates, mares were observed for different lengths of time in the post-partum period. For this reason, all behavioural dyadic interactions were recalculated from absolute values to comparable frequencies per 24 hours per individual (or dyad) per period. When analysing the proximity samples, the observations made when the animals were feeding on silage were not included because the supplementary feeding enticed the animals into closer proximity than when grazing; as a result only 534 out of the 666 spatial samples were analysed. The proximity data were expressed as a percentage: the number of recordings divided by the number of opportunities (which was not the same for all dyads because of the different foaling dates) that individuals of a given dyad were within 2 horse-lengths of each other.

Dominance relationships and rank orders were determined on the basis of five behavioural elements, namely submission and 4 offensive behaviours (ears back, threat to bite, bite, and attack), as described by VanDierendonck et al. (1995). Relative aggressiveness of an individual was calculated as the frequency of the four offensive behavioural elements per 24 hours.

Data was analysed using a combination of MatMan® (DeVries et al. 1993), The Observer® (both from Noldus Information Technology, Wageningen, The Netherlands) and MVSP® software (Kovach Computing Services, Pentraeth, Wales). Statistical analyses were performed using the following tests: (partial) row-wise tau matrix permutation test (2000 permutations) for matrix comparisons (DeVries 1993), Wilcoxon signed ranks test for related samples, Mann-Whitney U test for unrelated samples, Spearman's Rank Correlation (Rho) and Pearson's correlation coefficients for rank correlations, Landau's 'h' (DeVries 1995) for linearity, Preferred partners were identified using Standardised Residuals (Siegel and Castellan 1988).

The small number of samples from individuals that performed less than [the mean mi-

nus the standard deviation] amount of a particular behaviour were excluded from the analysis of that behaviour.

The proximity results were plotted after a Principal Components Analysis (PCA: Manly 1994) of the standardised residual (SR) results per dyad. These SR results were expressed as the percentage of the highest value in each matrix of standardised residuals. Each individual was considered to be a single case.

Hypothesis matrices were all corrected for structural zeros and constructed for sex and age, mare reproductive state, kinship, familiarity (based on the place where the animals were located before joining the herd) and dominance rank distance.

RESULTS

Dynamics of allogrooming

Frequencies

Allogrooming was performed an average of 2.6 ± 0.83 times per individual adult mare per day in 1997 and 4.8 ± 2.03 in 1999 (mean ± standard deviation). The allogrooming frequency decreased significantly after the time of parturition in both foaling mares and in their control (barren) herd-mates (Fig 1a, b); the decrease was however significantly greater for the former (Table 2). In addition, while the allogrooming frequency of pregnant mares did not differ from that of the pre-foaling controls, that of post-partum mares was significantly lower than for post-foaling control mares (Table 2: Mann Whitney U test 1997: $p < 0.003$; 1999: $p < 0.05$).

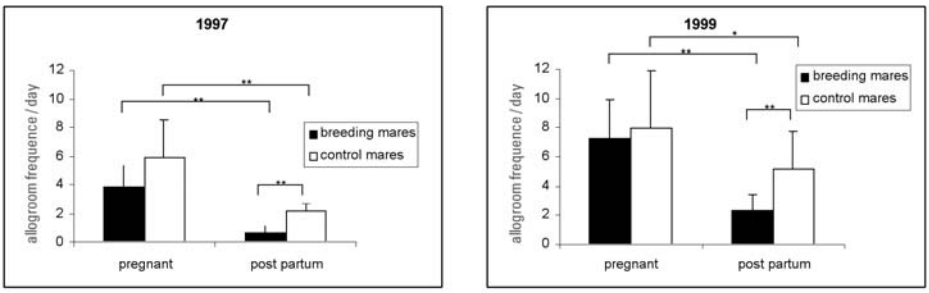


Figure 1a,b: Daily allogrooming frequencies for individual, adult breeding and control (barren) mares (mean ± sd) in 1997 (1a) and 1999 (1b).
*, ** indicates where frequencies differ significantly (* $p < 0.05$; ** $p<0.01$) (dependent subgroups via Wilcoxon's Signed Rank test; independent subgroups via the Mann Whitney U test).

Table 2
Comparison of allogrooming frequencies in pregnant, foaling and barren mares 1997 and 1999

			1997			1999		
		Tests*		p	n1, n2		p	n1, n2
Pregnant mares vs	**Post-partum mares**	**WSR**	-2.37	0.02	12, 7	-3.18	0.001	14, 13
Pre-foaling controls vs	**Post-foaling controls**	**WSR**	-2.03	0.04	5, 5	-1.83	ns (0.07)	4, 5
Pregnant mares vs	**Pre-foaling controls**	**MWU**	17	ns	12, 5	22	ns	14, 4
Post-partum mares vs	**Post-foaling controls**	**MWU**	0	0.003	7, 5	12.5	0.05	13, 5

Barren control mares were classified as pre-foaling controls and post-foaling controls, respectively, before and after the time of foaling in their matched foaling mare.
*tests: WSR = Wilcoxon Signed Ranks test; MWU = Mann Whitney U test

The allogrooming frequency of specific mares was similar in both years, as long as they were in the same reproductive state (Wilcoxon Signed Ranks test $Z = -1.60$, $n = 9$, $p = 0.11$). Allogrooming was performed preferentially between mares of the same reproductive status (pregnant, post-partum or barren: Kendall row-wise correlations for 1997: $\tau_{rw} = 0.16$, $n = 29$, $p < 0.001$; 1999: $\tau_{rw} = 0.13$, $n = 36$, $p < 0.001$).

Preferred allogrooming partners

All mares had at least one preferred allogrooming partner. The preferred partners for each individual were identified from their individual allogrooming frequencies and defined as a dyadic relationship with a Standardised Residual of >1.96, provided the analysed mare had an overall allogrooming frequency exceeding [the group mean – 1 sd]. In 78% of cases in 1997 and 75% in 1999, both members of such a dyad were each other's preferred partner. Preferred partnership was fairly consistent over the 2 study years, indeed of the 23 partnerships identified in 1997 ($N_{max} = 110$), 21 (= 91.3%) were still partnerships in 1999.

Dynamics in Proximity

Overall proximity frequencies

Proximity occurred preferentially among mares of the same reproductive status (pregnant, post-partum or barren: Kendall row wise correlation for 1997, $\tau_{rw} = 0.34$ ($n = 29$, $p < 0.001$). In control mares, the frequency of proximity decreased significantly during the foaling season (Mann Whitney U test for pre- vs post-foaling control mares: $U = 2.02$, $p < 0.03$); by contrast no such post-parturient decrease was observed for foaling mares. However, although the frequency of proximity to another animal was higher for foaling than barren mares after the time of parturition (Mann Whitney $U = -2.68$, $p < 0.001$), the foaling mares maintained proximity only with their own foal, and their distance from other adult mares actually increased (Fig. 2: "proximity to animals other than own foal"; for pregnant vs post-partum, Wilcoxon Signed Rank = 2.37, $p < 0.02$: for post partum vs control, Mann Whitney $U = -2.84$, $p < 0.003$).

For parturition itself, most mares separated themselves from the group by at least 50-100 meters, but within 4-8 hours of the birth of the foal they had returned to the periphery of the herd.

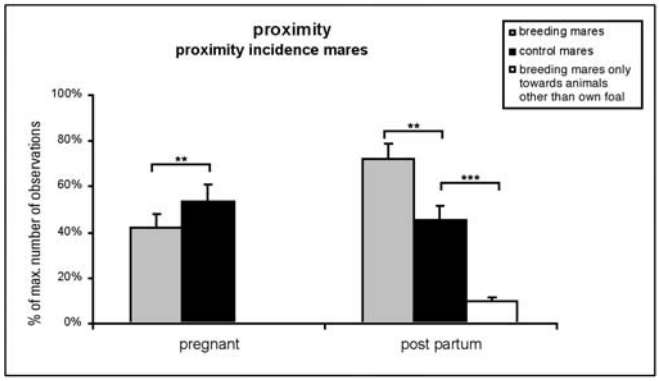


Figure 2: Mean (± sd) frequencies with which adult breeding and control (barren) mares were within 2 horse lengths of each other (proximity scores). For foaling mares, the additional column represents the frequencies that a mare was proximate to an animal other than her own foal.
, * indicates significant differences between proximity frequencies within a group of mares (** p< 0.01; *** p<0.001) (dependent subgroups compared using Wilcoxon's Signed Rank test; independent subgroups using the Mann Whitney U test).

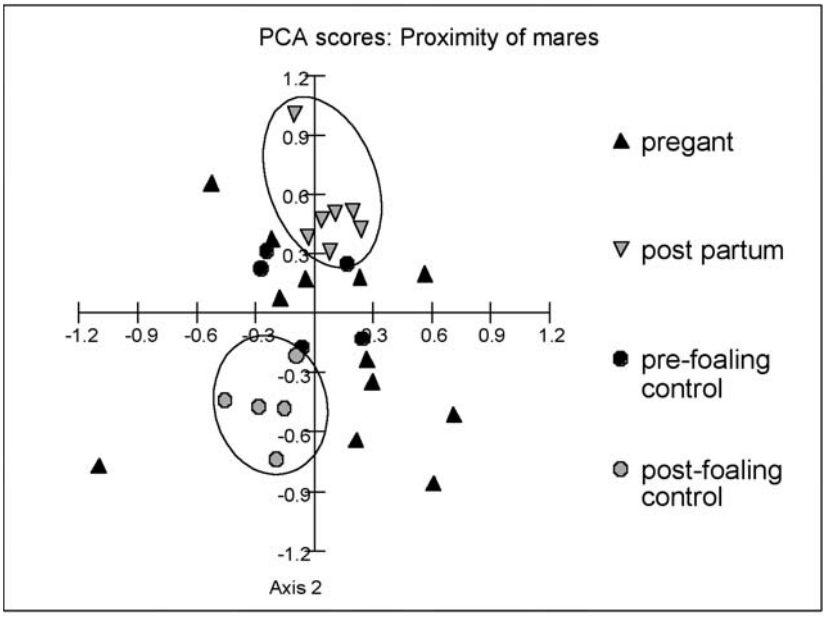


Figure 3: Principal Component Analysis to compare proximity scores between individual adult breeding and control (barren) mares. The clustering of mares indicates the clear spatial separation of the mares with foals from the barren mares, after the time of foaling. Prior to foaling, the barren and pregnant mares were intermingled.

Preferred proximity partners

All mares had at least one preferred partner with whom they had a close spatial partnership. In 59% of cases, there was a clear reciprocal proximity relationship between dyads of mares that were each other's preferred partner. There was no distinction between pregnant

and barren mares with regard to the formation of preferred partnerships in proximity. Following parturition, however, there was a spatial separation between the mares with foals and the barren controls (Fig. 3), i.e. the foaling mares and the barren controls separated into sub-groups (indicated by the large circles in fig 3). With the first two axes 35.2% of the variance was explained, with the first three, 44.8%. Since all cases are individuals, the axes represent the spatial distribution.

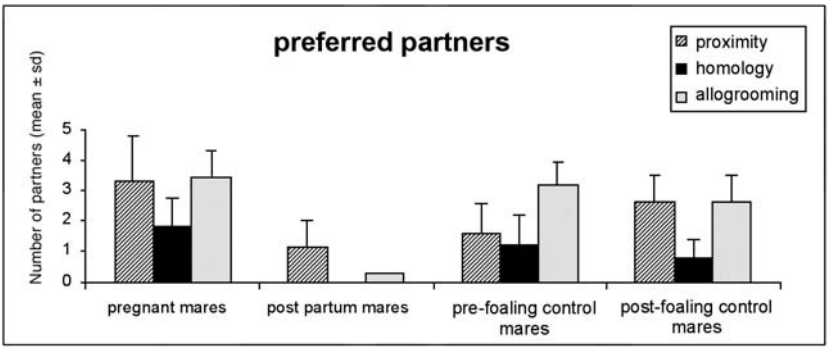


Figure 4: The relative number (mean ± sd) of preferred partners for allogrooming or proximity. Homology was when the same adult mare was the preferred partner for both allogrooming and proximity.

Comparison of allogrooming and proximity results

Dyadic proximity frequencies and allogrooming frequencies overlapped significantly (τ_{rw} = 0.32, n = 29, p < 0.001). For pregnant and pre-foaling control mares, there was considerable homology of the preferred partner for allogrooming and proximity (55.0% and 75.0%, respectively). By contrast, for foaling mares and post-foaling control mares, respectively only 0 and 30.8% of the proximity and allogrooming dyads were the same (fig 4).

Kinship and familiarity

Allogrooming and proximity were significantly related to kinship (allogrooming 1997, τ_{rw} = 0.17, n = 29, p < 0.001; 1999, τ_{rw} = 0.28, n = 36, p < 0.001: proximity 1997, τ_{rw} = 0.19, n = 29, p < 0.001). However, because there were no unfamiliar individuals in 1997, familiarity could only be investigated in 1999: in fact, familiarity had a stronger impact on the dyadic allogrooming relationships than kinship (partial Kendall row wise correlation between kinship and allogrooming frequency controlled for familiarity ($\tau_{rw;XY.Z}$) = -0.03, n = 36, p < 0.84: partial correlation between familiarity and allogrooming frequency controlled for kinship $\tau_{rw;XY.Z}$ = 0.38, n = 36, p < 0.001).

Dominance

Rank order

For all mares, the position in the dominance hierarchy was remarkably consistent across the 2 study years (comparing those animals present in both years: n = 21, Spearmans Rho = 0.958, p < 0.002). However, the position of individuals in the dominance hierarchy often changed in the period immediately after foaling, but there was no consistency in the direction of change in the dominance rank (from 5 ranks lower to 6 ranks higher than the position during pregnancy; n = 20). Possibly because the mares tended to become more aggressive at this time (Wilcoxons Signed Ranks test Z = -1.9, n = 20, p = 0.052).

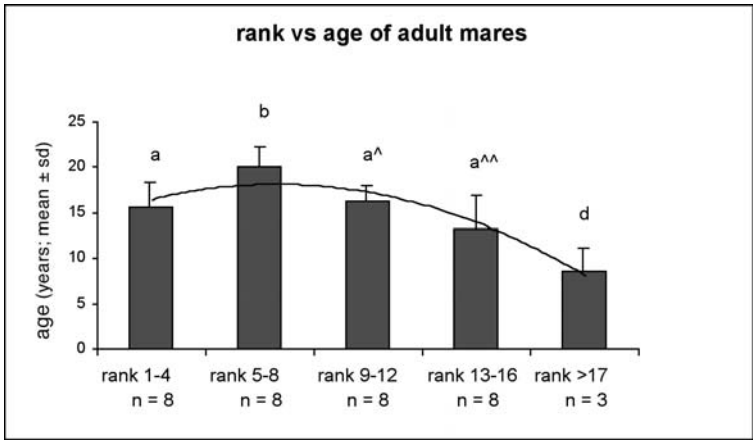


Figure 5. Age of adult mares (mean ± sd) in the various dominance rank categories. [a-d] Different letters indicate columns with mean ages that differed significantly (p < 0.05: Mann Whitney U test). a^ and a^^ indicate groups that tended to differ (a versus a^^, p < 0.09; a^/a^^, p < 0.06). The line of best fit was expressed by the quadratic regression $y = -1.3x^2 + 5.8x + 11.9$ ($R^2 = 0.54$, df = 32) and indicated a dominance hierarchy with the form: older mares > oldest mares > younger mares.

The calculated linear rank order (DeVries 1998) that fitted best the dominance relationships observed had only 9 inconsistencies with a strength of 58 and a linearity index (Landau's 'h') of 0.638 in 1997, and 12 inconsistencies with a strength of 69 and a linearity index 'h' of 0.814 in 1999. The rank positions were significantly related to age, irrespective of reproductive stage (Rho = 0.47, df = 22, p < 0.02: combined over the 2 study years). Relative aggressiveness was inversely related to dominance order among mares in 1999 (Rho = -0.42, n = 32, p < 0.02) but not in 1997 (Rho = 0.1165, df = 24, ns) and rank order was unrelated to the number of foals produced in a mare's lifetime. Although the linear rank order was strongly correlated with age, the top ranking mares were not the oldest (Fig. 5.). In fact, a regression analyses revealed that the data were better represented by a second-degree curve with a peak at around 14-15 years old and a slight decline thereafter, rather than a straight line.

In both study years, closely related horses (as calculated via co-ancestry) were significantly closer in dominance rank than horses with a lower degree of co-ancestry (1997 $\tau_{\rho\omega}$ = 0.219, n = 24, p < 0.001 and 1999: $\tau_{\rho\omega}$ = 0.300, n = 32, p < 0.001, no control for seasonality). However, closeness in dominance rank was not related to allogrooming frequency, spatial proximity or reproductive state.

DISCUSSION

In the observed herd of adult and sub-adult mares and geldings, and yearling stallions, mare-mare relationships were very stable and involved long lasting bonds that persisted after foaling and across the non-consecutive study years. There was a stable individual characteristic of the frequency of expression of various social behaviour elements and on dominance rank position over the years, although absolute frequencies of social behaviour and rank of a broodmare were also influenced strongly by its reproductive state. In general, frequencies of social interaction with other herd members decreased markedly in the period following parturition. Age, kinship and familiarity were also important factors that influenced the choice of partners for social interaction. These results suggest that, within a large mixed-sex domesticated herd, mare–mare dyadic relationships play a critical role in maintaining the intra-band bonds that help ensure the long term survival of the band, as has previously been reported for feral horses (Linklater 2000; Wernicke and VanDierendonck 2003), thus irrespective of the presence of castrated or intact stallions.

Allogrooming

Daily allogrooming frequency per individual mare did not differ between the study years, as long as the mares were compared in the same reproductive state in both years. Furthermore, while there was a seasonal effect on the frequency of allogrooming, the decrease noted across the observation period was considerably more marked in foaling than in control mares. As previously reported, the seasonal decrease of the allogrooming frequency coincided with the shedding of the winter coat (Tyler 1972, Kimura 1998). During the period of observation after foaling, the breeding mares did not return to their pre-partum allogrooming frequencies, but they did remain faithful to their preferred allogroom partners. This contradicts previous studies in which broodmares were reported to return to their pre-foaling allogrooming rates already within 3-4 weeks after parturition, but with different partners (Estep et al. 1993). The differences between these brood mares and the studied herd observations could be explained by differences in the sex-age composition of the herd, breed- and climate differences, or how the potentially confounding seasonal factors were dealt with during the observations and analysis of the different studies. Finally, although allogrooming behaviour of adult mares was similar over the study years, the absolute frequency was strongly influenced by reproductive state, a finding that should be taken into account when comparing allogrooming behaviour between situations.

Proximity

Following parturition, dams and their foals formed a distinct subgroup that was separated spatially from both post-foaling control mares and the remaining pregnant mares; by contrast the pregnant and pre-foaling control mare subgroups overlapped considerably. Furthermore, the mares and their foals had very different spatial patterns in relation to other herd members as well as those of barren control mares. Specifically, the barren mare, but not the dam and foal, subgroup overlapped spatially with the geldings and sub-adults. The increased interaction with males was emphasised by the more frequent recording of oestrous behaviour in the control mares during the period after foaling of the broodmares, than ei-

ther earlier in the year or in the recently foaled mares themselves (unpublished observations). Klingel's bonding theory (Klingel 1975) may explain why pregnant mares separate themselves from the herd for a period of several hours around the time of parturition, namely to allow the foal to recognise and 'imprint' on its dam. In addition, sub-group formation by the foaling mares could be a means of avoiding the often boisterous activities of the sub-adults and geldings. In this respect, sub-adult males played more than once an hour, while adult mares did not play at all (Sigurjónsdóttir et al. 2003), and adult mares displayed threat behaviour towards geldings and sub-adults 5 times more often than towards other adult mares (unpublished observations). All of this suggests that the biological function of the sub-grouping of dams and foals is to facilitate protection of those foals; alternatively, it is possible that sub-group formation is stimulated by a mutual attraction between the foals.

Overlap between allogrooming and proximity results

There was a considerable overlap between the allogrooming interactions and proximity, in terms of both frequency and preferred partnerships. Up to 75% of preferred partners were the same for both allogrooming and proximity, suggesting that proximity was a result of active individual action rather than passive acceptance as also found by Kimura (1998).

Dominance

Increasing age was significantly correlated to dominance ranking. However, in contrast to the studies that have postulated a linear relationship between age and dominance (Van-Dierendonck et al. 1995, Kimura 1998), the dominance hierarchy was best described as; older mares > oldest mares > younger mares. Interestingly, previously high-ranking mares seemed to retain "respect" from most other herd members even when they had passed into a senescent physical decline. The negative relationship between relative aggressiveness and dominance rank suggested that high ranked mares don't need to perform visible acts of aggression to maintain their position, this echoed previous observations in feral horse groups (Rutberg and Greenberg 1990).

Mares tended to become more aggressive after parturition, as recorded previously for mares in feral groups (Rutberg and Greenberg 1990) but contrary to previous reports for domesticated horses (Estep et al. 1993). In the current study, the increase in aggressive behaviour was primarily in the form of 'interventions', during which dams defended their foals or other mares appeared to try to prevent the approach of herd members towards a very recently foaled dam. Future analysis of acts of 'intervention' may help to clarify the significance of this behaviour.

Social relationships, kinship and familiarity

Allogrooming frequencies between dyads of mares were correlated strongly with kinship. However, when unfamiliar animals were introduced into the group, familiarity was a more powerful predictor of the frequency of affiliative interactions than kinship. This supported previous observations that mares in particular are more attracted to familiar than to related herd mates (Monard et al. 1996, Linklater 2000). The significance of kinship versus familiarity regarding mare partnership preferences could be studied in more depth by observing further changes as the residence time of the introduced animals increases. Interestingly,

mares of similar dominance position were usually more closely related than those that were further apart in rank.

In contrast to previous reports (Monard and Duncan 1996, Monard et al. 1996, Roberts and Browning 1998, Kimura 1998), there was no relationship between dominance rank and other social preferences. In particular, the studied Icelandic horse mares did not form subgroups based on rank or age, as was reported for mares in the feral Yururi herd (which also included geldings: Kimura 1998). In addition, although the presence of adult geldings and sub-adults did appear to influence the spatial and aggregative behaviour of the broodmares, there was no evidence to suggest that the mares formed bonds specifically to reduce direct aggression or harassment by male animals, as has been postulated by Linklater et al. (1999). The long-term stability of both affiliative social behaviour and dominance order was reminiscent of the important role of mare-mare bonding in harem stability in feral horse herds (Feist and McCullough 1976), and was not noticeably influenced by the presence of adult geldings. Furthermore, there were no ethological or physical indicators of a detrimental effect of the presence of geldings on the welfare of either the broodmares or their foals (e.g. no stereotypic behaviours, extreme aggressive or submissive behaviour, injuries or attempts at infanticide).

CONCLUSION

Parturition and motherhood changed temporarily the nature and frequency of social interactions between a mare and other herd members. However, there was no evidence that any other group members (other mares, geldings or youngsters) consistently harassed or disturbed mares or their foals either during or in the period after parturition. On the other hand, since mares with foals chose to separate off into a distinct sub-group after parturition, space is an important requirement when mares are expected to foal within such a complex social group. Nevertheless, maintenance of domestic horses within these mixed herds during the foaling season did not prevent the expression of characteristic species-specific behaviour, and it allowed the development of long-term stable relationships suggesting that, given sufficient space, this system represents a welfare friendly alternative to modern horse husbandry systems. By extrapolation, it is concluded that maintaining horses in mixed groups at pasture or in loose yards could provide a relatively natural social environment in which those horses could adequately express normal species-specific patterns of behaviour (Brambell 1965). Most modern husbandry systems entail a high degree of social isolation, whereas the results of this study and those of Bachmann et al. (2003), Cooper and McGreevy (2002), Christensen et al. (2002a) emphasise the significance of stable social contact.

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Interventions in social behaviour in a herd of mares and geldings

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ABSTRACT

Social dynamics and maintenance of social cohesion were studied by analysing social interventions in two groups of horses consisting of adult mares, their offspring, adult geldings and sub-adults. The animals were observed for a total of 1316 h. All relevant dyadic and triadic social interactions, including initial behaviour, possible intervention and outcome, were recorded. The main question was: do horses use interventions in affiliative interactions to safeguard their social network?

Horses were significantly more likely to intervene in allogrooming or play interactions that involved a preferred partner. The stronger the preferred association in allogrooming, the higher the likelihood the intervener took over allogrooming with an initial dyad member. Interveners originating from two newly introduced groups ($n = 3$ and 5), intervened significantly more often when a member of their own group allogroomed with an unfamiliar horse. In play, no correlation with unfamiliarity was found. Overall, the intervening horses stopped more than half of the initial allogrooming interactions, and a third of all interactions. Therefore, social facilitation cannot sufficiently explain interference behaviour. This study shows that maintaining relationships with preferred partners is important to horses and has implications for equine husbandry and management.

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1. Introduction

Feral horses (*Equus caballus*) are social animals. However, in most husbandry systems domestic horses are kept confined and solitary (Holmes, 1839; Schimmel, 1914; Linklater, 2000; Waring, 2003; VanDierendonck and Goodwin, 2005). These domestic husbandry systems are often regarded as a way to assure individual physical health, because they facilitate individually adjusted management procedures. However, these systems disregard the basic social and behavioural needs of horses, which may result in abnormal behaviour.

Horses that live in groups show affiliative as well as agonistic interactions within dyads or triads (three individuals). The two most important equine affiliative behaviours are allogrooming, performed by all horses, and play, mainly shown by males of all ages, but also, to a much lesser degree, by fillies and sub-adult mares (Monard and Duncan, 1996; Monard et al., 1996; McDonnell and Poulin, 2002; Sigurjonsdottir et al., 2003; Waring, 2003). Spatial proximity is also shown to have a passive acceptance component as well as an active preference to be close to certain individuals when grazing and resting (Sigurjonsdottir et al., 2003).

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Interventions in dyadic interactions are an intriguing aspect of social life. An animal may actively interfere in an ongoing dyadic interaction with the apparent aim of altering the interaction. Only after the initially approaching animal has actually become involved in the interaction between the other two, it is clear that the dyadic interaction has turned into a triadic one. This interference may be part of a deliberate strategy to gain an advantage or resource or may be a haphazard coincidence. Studies on triadic interactions amongst animals have predominantly involved non-human primates (Harcourt and de Waal, 1992). References to non-primates are sparse. Examples include studies of interventions in zebras (Schilder, 1990), coalitions and alliances among feral horses (Berger, 1986; Linklater and Cameron, 2000), and within-group alliances among dolphins (Connor and Whitehead, 2005). Handbooks on horse behaviour rarely refer to intervention behaviours (Mills and Nankervis, 1999; McDonnell, 2003; Waring, 2003; McGreevy, 2004; Zeitler-Feicht, 2004).

Fundamental knowledge on the importance of social bonds in domesticated horses is still lacking. Do domestic horses actively maintain their social networks by interfering in ongoing interactions that could jeopardize their current social bonds? Knowledge on the nature of bonds that horses form and their intervention behaviour can be used to improve animal welfare, optimise animal housing, and reduce the risk of injury. Although various aspects of the relationships between horses in a domestic setting have been studied intensively (e.g. review by Waring, 2003), investigations on interventions in domestic horses are lacking. We are only aware of one study on equine interventions (Schilder, 1990) which concerned zebras in semi-captivity. Competition around reproduction and food were important determining factors in zebra's. However, these factors can only be of minor importance for pastured domesticated horses.

In the present study we present a qualitative and quantitative account of the nature and structure of intervention behaviour in two groups of domestic horses. Our main question is: do domestic horses use interventions as a social instrument to maintain their own bonds within a network, i.e. to safeguard their social network. To this end, we identified the circumstances in which domestic horses use interventions, and whether they do so systematically. We also assess the hypothesis that social facilitation might be the cause of intervention behaviour. The results extend our knowledge on what matters to horses with respect to their social environment and help identify factors to be taken into account for stress reduction in horse husbandry and management.

2. Animals, materials and methods

2.1. Study site and period of study

During the foaling period, from the beginning of May to mid-June in 1997 and 1999, social and specifically intervention behaviour was studied in a pastured group of Icelandic horses. The animals under observation were part of a herd of 80–100 horses that free-ranged all year round in a mountainous area exceeding 1200 ha at Skaney Farm in the western part of Iceland. In winter and spring, adequate supplementary feeding was provided daily. To facilitate detailed observation, the animals under study were corralled within an 8-ha sub-enclosure, during the observation periods. Shelter within the sub-enclosure was provided by landscape elements. Daily mean ambient temperatures ranged between 4 and 10 °C. Long daylight hours allowed night observations and in general it was possible to observe the horses continuously for 24 h a day, weather permitting. Observations covered 488 h in 1997 (from May 6 till June 11) and 828 h in 1999 (from May 3 till June 14).

2.1.1. Animals

In both years, approximately half of the herd consisted of breeding mares (most of them were pregnant at the beginning of the study) and the other half a combination of adult geldings, sub-adult mares, sub-adult geldings and yearling stallions (Table 1). During the study, 7 foals were born in 1997 and 13 in 1999. Of the 30 horses in 1997 and 31 horses in 1999 of 1 year and older, 18 individuals (including 11 of the adult mares) were observed in both years. In 1999, 8 animals (including 4 adult mares) from two separate neighbouring farms (3 horses (L group) and 5 horses (B group)) were added to introduce 'unfamiliarity' as a variable in social interactions. All horses, except one in 1997 and the eight animals introduced in 1999, were born in the resident herd and all adult mares were multiparous.

2.1.2. Observation methods

Data were collected by the method "All Occurrences" of predefined behavioural elements, which included affiliative, dominance and intervention elements (Tables 2 and 3). All data were recorded on a Psion handheld computer using 'The Observer' software® 5.0, (Noldus Information Technology, 2004) and transferred daily to a laptop computer. Regular inter-observer reliability sessions were performed. To prevent any systematic bias in recordings, the observers worked in 8-h shifts with one overlapping hour at each shift change, so that shift changes gradually changed over time. Some observation time was lost due to weather conditions (i.e. fog) or computer failure.

For each intervention, the animals and their roles were registered. The animal approaching a pair of horses that were socially engaged and initiated a triadic interaction was defined as 'intervener'. If the intervener aimed its behaviour clearly towards one of the two initial animals involved in an interaction, that animal was called the 'target' and the other one the 'recipient' (cf. Schilder, 1990). Interventions by a dam to stop other animals approaching her own newborn foal were not recorded. The main elements of the ethogram describing intervention behaviours are presented in Table 2. Other intervention behaviours included threaten to bite, bite, threaten to kick and kick without the intervener positioning him/herself between the two initial animals.

For analyses, social behavioural elements were categorised in six mutually exclusive groups (cf. Schilder, 1990) as shown in Table 3.

2.1.3. Data analyses

For each intervention, the sex, age and familiarity of the animals were registered as well as their specific role was determined (intervener, recipient, target). Interactions with foals as interveners were not included in the analyses.

An initial behaviour was considered to be 'taken over' when one of the initial animals was displaced and the intervener continued the initial behaviour with the other animal. An intervention was considered to have 'stopped' the initial behaviour when one of the following behavioural elements was recorded and the initial behaviour was not 'taken over': stop allogrooming, stop play, initiate new behaviour.

The mares were considered to be in oestrus when they showed at least two of the following behaviours within 24 h: sexual mounting, winking, presenting or copulation. The oestrus was considered to end on the last day one of these behaviours was recorded.

For each intervention the affiliative or associative strength (quantified by means of standardized residuals (SR)) of each horse with respect to

Table 1
Number of animals (present in each year)

	1997	1999
Adult mares, pregnant	12	14
Adult mares, barren	5	4
Adult geldings	5	5
Juvenile geldings	2	2
Juvenile mares	4	4
Juvenile stallions (<1 year)	3	1

Table 2
Description of intervention behaviour elements

Intervention behaviour	Description
Choose interposition	An animal positions itself between two or more animals
Keep interposition	An animal keeps itself between two or more moving animals
Push in between two animals	An animal pushes itself between two individuals standing close to each other
Intervention via any other behaviour	Any other action than above that (1) prevents an interaction between two other animals from starting, continuing or (2) changes its character (e.g. intervener bites one of the initial dyad without choosing interposition)

Table 3
Categories of social behavioural elements, with certain specific items

Category	Behaviours
Affiliative	(Initiate) allogrooming; nose play, (initiate to) play
Agonistic	Attack, threat with ears flat, (threaten to) bite, (threaten to) kick, fight, chase, herding
Approaching	Approach, follow, nose-to-nose, nose-to-side, inspect foal, stand alert
Sexual	Nose-to-genitals, (attempt to) mount, present mare, winking, intromission
Neutral	Leave, stand, suckle, blocking mare, vocalise,

Ethogram elements based on Schilder (1990) and McDonnell (2003).

each other horse was determined as described by VanDierendonck et al. (2004). Standardized residuals were calculated on the basis of the frequencies of allogrooming, playing and proximity during the whole observation period and corrected for structural zeros, using the software program MatMan (Noldus Information Technology, 2003; De Vries et al., 1993). SR's are computed according to the formula: (observed frequency − expected frequency)/(√expected frequency). For each horse, the individuals with a SR > 1.96 (being the nominal 2.5% cut-off value for a standard normal deviation measure) were considered preferred partners of that horse. For each intervention, the social dominance rank of each animal was assessed according to the procedure described in VanDierendonck et al. (1995). For 1997 and 1999 data, the same five behaviours from VanDierendonck et al. (1995) were used to assess the dominance rank order most consistent with a linear hierarchy using the I&SI method (De Vries, 1995, 1998).

For each intervener, the total relative frequency of allogrooming, play, nose-to-nose contact and mounting of its preferred partners was calculated. This allows assessing the proportion of interventions in dyads in which at least one horse was a preferred partner, in relation to the total number of opportunities to intervene the individual did have. For each intervener the proportion of interventions, i.e. the 'number of interventions/total frequency in which an intervention could take place' was calculated.

To express the relative contribution by a particular sex–age group in each of the roles to the total amount of interventions, the following deviation measure was used: z = (observed frequency − expected frequency)/(√expected frequency).

Data concerning triads in which at least one of the participants was in oestrus were analysed separately, since it is unclear whether the intervention was induced by reproductive or other factors (Schilder, 1990).

Data were analysed using the software programs MatMan® (De Vries et al., 1993) and The Observer® 5.0 and SPSS® (v11.5). Statistical analyses performed included the Shapiro–Wilks test for normality, Wilcoxon-signed ranks test for related samples (WSR) and the Mann–Whitney U-test for unrelated samples (MWU); the Monte Carlo procedure with a Bonferroni-corrected critical significance level of 0.005 was used for the familiarity comparison in allogrooming interventions. The Chi-square goodness-of-fit test was used in cases of non-parametric group comparisons in which expected values could be calculated. All tests were two-tailed with the critical level of significance α set to 0.05.

3. Results

3.1. Frequencies of different interventions and distribution of interveners amongst the social classes

Over both years combined, adults and sub-adults intervened 824 times. Interventions were most frequent in affiliative interactions (36%), followed by interventions in approaching interactions (28%) and neutral (15%) or unspecified (13%) initial interactions. Interventions were least frequent in agonistic interactions (2%). In 138 interventions, at least one of the participants was in oestrus. Interventions unaffected by oestrus behaviour (n = 686) took place in allogrooming (162×, 24%), play (105×, 15%), whereas in 317 cases (46%) the interventions were directly related to the birth of a foal. In the remaining 102 cases (15%) the interventions took place in other interactions.

The initial interaction was completely stopped by the intervener and not 'taken over' in 35% (292×) of all 824 cases. In interventions in allogrooming and play (neither during oestrus nor related to the birth of a foal) (n = 267), the interaction was altered (not just stopped) by 'taking over', in 28% of the cases.

On an average the horses intervened 16.6 ± 2.19 (mean ± S.E.M.) times. All animals showed this behaviour at least once and the maximum was 63 times: kurtosis 1.41 and skewness 1.30. The Shapiro–Wilks test for normality was 0.886, df = 46, $p < 0.0001$. There were differences between social groups when frequencies of interventions are analysed with respect to the nature of the interaction: in general, adult mares intervened less than the other sex–age classes. However, around parturition, the sex–age distribution of the interferers drastically reversed (Fig. 1; for all comparisons $\chi^2 > 13.5$; df = 3; $p < 0.01$).

3.2. Interventions not related to parturition or oestrus

3.2.1. Interventions in allogrooming

Of the 37 different interveners involved in the 162 interventions in allogrooming, 9 did so only once. Interveners were significantly more likely to intervene in allogrooming dyads which included at least one preferred partner of the intervener: (WSR test, $z = -5.21$, n = 37, $p < 0.0001$). Indeed, only three of the 162 interventions in allogrooming, did *not* include a preferred partner of the intervener.

Adult mares intervened significantly less than other sex–age classes (1997: $\chi^2 = 37.3$, df = 3, $p < 0.001$; 1999

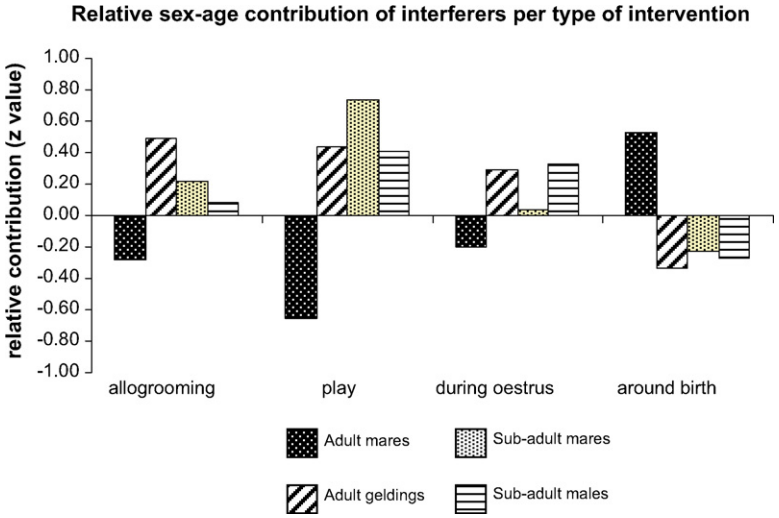


Fig. 1. Relative contribution of the different sex–age groups of the interveners to interventions in allogrooming, play, during oestrus and around birth in 1999 as an example of the differences between the relative contributions of the adult mares compared to the other subgroups. Bar above horizontal: involved in interventions more than expected; bar below horizontal, involved in interventions less than expected.

$\chi^2 = 49.7$, df = 3, $p < 0.001$), whereas the difference between the adult geldings and the sub-adults was less obvious (1997: $\chi^2 = 5.7$, df = 1, ns; 1999: $\chi^2 = 13.8$, df = 1, $p < 0.02$).

3.2.1.1. Outcome of interventions in allogrooming. In 30% of the interventions in allogrooming, the intervener displaced one of the initial dyad members and continued allogrooming with the other (i.e. took over allogrooming). In 15% of the cases the initial animals continued allogrooming, whereas in 47% the allogrooming was stopped entirely by the action of the intervener (in 4% the horses changed to play interaction, whereas in 5% the outcome of the intervention was unspecified).

Presence of a preferred partner of the intervener in the initial dyad led to a higher proportion of interventions resulting in cessation of the initial interaction as compared to the proportion of interventions that resulted in continuation of the originally interacting dyad (WSR test, $z = 2.12$, $n = 31$, $p = 0.034$).

Allogrooming was taken over significantly more often when one of the initial actors was more strongly preferred by the intervener compared to when this preference was less strong (Mann–Whitney test, $n_1 = 22$, $n_2 = 31$, $z = -2.14$, $p = 0.03$; Table 4).

Table 4
Outcome of the intervention in allogrooming related to the strength[a] of the association between the intervener and the initial dyad members

Outcome of intervention	*n*	# interveners	Mean strength[a] mean ± S.D.
Taken over	50	22	14.1 ± 11.6[b]
Continued by original dyad	25	16	9.9 ± 9.3
Stopped	80	31	7.4 ± 6.7[b]

[a] Mean strength = mean Standardized Residual (SR) of intervener with both the initial animals.
[b] Values differ significantly, $p = 0.03$.

3.2.1.2. Does dominance influence interventions in allogrooming?. Irrespective of the outcome, more interventions in allogrooming took place when the animals in the initial interaction were both higher in rank than the intervener ($\chi^2 = 7.65$, df = 2, $p < 0.05$). 'Taking over allogrooming' occurred significantly more often when both initial animals were ranked higher than the intervener ($\chi^2 = 6.28$, df = 2, $p < 0.05$) as compared to when one or both of the initial animals were lower than the intervener. Rank had no influence when the intervention was stopped ($\chi^2 = 5.64$, df = 2, $p = 0.06$) or when the initial dyad continued allogrooming ($\chi^2 = 1.0$, df = 2, $p > 0.5$).

3.2.1.3. Does unfamiliarity influence interventions in allogrooming?. In Table 5, impact of unfamiliarity on interventions is presented. For each of the nine different combinations of familiar and unfamiliar involvees in the triads a Monte Carlo procedure was used to test whether the observed and expected frequencies differed (Table 5). To control the type I error rate for this set of nine tests a Bonferroni-corrected critical significance level of 0.005 was used. In both of the introduced groups (L and B) a significantly higher frequency of interventions was observed when one of the initial dyad members was familiar and the other unfamiliar (Table 5, columns in italic face, LL- and BB-). Besides, in group B, interventions in pairs of familiar animals occurred significantly more often than predicted by chance (column BBB).

3.2.2. Interventions in play

In 32.1% of the interventions in play ($n = 105$), play was stopped, in 24.5% the intervener displaced one of the initial dyad members and started to play with the other (take over play), in 25.5% of the cases the initial animals continued playing, and in 17.9% the outcome was unspecified. Of the 24 different interveners, only 3 were adult mares (with a total of 5 interventions). Adult geldings and sub-adults interfered about equally often in

Table 5
Observed and expected frequencies of interventions when the initial allogrooming animals are both familiar, both unfamiliar or one familiar and one unfamiliar to the intervener

Familiarity is based on the herd origin of the animals (>1 year):
S residential herd ($n = 22$), L herd ($n = 3$) and B herd ($n = 5$)
The first letter in each of the 3 letter column headings represents the origin of the intervener
SSS/LLL/BBB — three familiar animals
SS-/LL-/BB- — intervention in a dyad of a familiar and an unfamiliar animal
S–/L–/B– — intervention in a dyad of unfamiliar animals

	SSS	*SS-*	S–	LLL	*LL-*	L–	BBB	*BB-*	B–	N_{tot}
Observed frequency	40	*14*	4	1	*5*	10	6	*13*	11	104
Expected frequency	51.7	*20.7*	3.0	0.03	*0.8*	9.7	0.3	*4.3*	13.5	104
p value[a]	0.03	*0.11*	0.77	0.10	*0.001*	1.00	0.0001	*0.0001*	0.56	

Columns in *italic face*: combinations in which an intervener is familiar with one animal.
[a] The critical significance level is set to 0.005 (Bonferroni procedure for multiple tests).

play (1997: $\chi^2 = 0.05$, df = 1, ns; 1999: $\chi^2 = 2.9$, df = 1, ns).

Of the 105 interventions, 77 took place when at least one animal was a preferred play partner of the intervener ($n = 28$) (mean ± S.D.: 0.20 ± 0.42 interventions per animal per day), a much higher frequency than for allogrooming (0.049 ± 0.037 interventions per animal per day). There was a significant preference to intervene in initial play dyads in which at least one animal was a preferred play partner of the intervener (WSR test, $z = -3.89$, $n = 24$, $p = 0.0001$). The strength of association between the intervener and either of the other animals was much lower in play than in allogrooming interventions (play: mean SR = 3.21 ± 2.62; allogrooming: mean SR = 8.27 ± 6.38). Males played significantly more than young mares; up to 38.9% of the interventions were performed by mares interrupting play-dyads which 91.6% of the time included at least one male.

3.2.2.1. Influences of rank interventions in play. In contrast to interventions in allogrooming, neither rank ($\chi^2 = 2.55$, df = 2, $p = 0.28$) nor unfamiliarity ($\chi^2 = 0.3$, df = 2, $p = 0.98$) influenced intervention preferences.

3.2.3. Interventions in other social interactions

Interventions rarely ($n = 11$) took place in aggressive interactions, and unfamiliarity was not an influencing factor ($\chi^2 = 2.57$, df = 2, $p = 0.28$). Rank also had no significant influence ($\chi^2 = 0.18$, df = 2, $p > 0.9$).

Interventions, which occurred when one horse was approaching another, mainly took place when the behaviour elements 'approach' ($n = 11$), 'nose-to-nose' ($n = 13$) and 'nose-to-side' ($n = 7$) were performed. Behaviours where both initial horses behaved reciprocal, such as nose-to-nose, were more likely to invoke interventions when non-familiar animals were involved ($\chi^2 = 7.2$, df = 2, $p < 0.05$), whereas behaviours which involved more active participation by one individual (such as 'approach' and 'nose-to-side') did not ($\chi^2 = 1.18$, df = 2, $p = 0.55$).

3.3. Interventions in relation to parturition or oestrus

3.3.1. Interventions after parturition

Interventions ($n = 317$) in which a dam and her newborn foal were recipients of an intervention directed towards animals in their direct vicinity mainly took place in the first few hours after the birth. Characteristics of the triads in these interventions differed significantly from chance ($176.7 > \chi^2 > 20.7$; df = 3; $p < 0.001$) for all sex–age classes for all three roles (Fig. 1). Interventions around birth also differed in the initial type of behaviour compared to the 'normal' interventions: for comparison, the relative proportion of the various categories of initial behaviours around birth and during oestrus is given in Table 6.

Interventions around birth differed strongly from the other interventions in frequency, sex–age distribution of the interveners, distribution of the initial behaviours, as well as the specific situation under which they occurred. These results will be fully discussed in another paper.

3.3.2. Interventions influenced by oestrus

Most of the 138 intervened initial interactions which occurred when at least one of the three horses involved was in oestrus could be categorised either as meeting interactions (33.3%) or sexual interactions (23.9%) (Table 6). The adult mares in oestrus were in the majority of cases only recipients, while the sub-adult mares in

Table 6
Distribution of initial behavioural categories in which was intervened around birth of a foal and during oestrus

Interventions	n	Initial behaviour categories[a]					
		Affiliative (%)	Aggression (%)	Meeting (%)	Sexual (%)	Neutral (%)	Unspecified (%)
Around birth[b]	317	0.0	1.6	49.9	0.9	24.6	23.0
During oestrus[c]	138	19.6	1.4	33.3	23.9	8.0	13.8

[a] See Table 3.
[b] Around birth = interventions just after foaling, directed towards animals in the immediate vicinity of the dam and new born animal.
[c] During oestrus = interventions while at least one partner was in oestrus.

oestrus were involved in all 3 roles. Of the 7 adult oestrus mares who did intervene, a skewed distribution emerged: 3 performed 78.9% of the interventions.

Adult geldings acted as interveners in 13.8% of cases, were recipients in 10.2% and targets in 29.9% of cases. Again, some were very active while others were not.

When an oestrus mare was involved, there was no preference to intervene in an initial dyad which had at least one preferred (allogrooming) partner: WSR test $z = -1.49$, $n = 12$, $p = 0.136$. In contrast, when no oestrus mare was involved, the presence of a preferred partner strongly influenced the probability of interfering in allogrooming and play interactions.

In 34.8% of the interventions with an oestrus mare involved the initial interaction stopped. Sexual interaction (either by the original mounting animal or the intervener) was the outcome in only 3.6% of the cases.

4. Discussion

Interventions were a fairly regular component of the social dynamics in the herd and often concern affiliative interactions. The frequency of interventions is also relatively high during oestrus and around foaling. The interventions frequently altered ongoing or beginning interactions, especially when preferred partners of the interferer were interacting, or when familiar individuals were interacting with unfamiliar ones.

4.1. The role of preferred partnerships in interventions

Allogrooming is one of the major affiliative interactions in equines and has primarily a social function (Feist and McCullough, 1976; Schilder, 1990; Spruijt et al., 1992). The function of allogrooming is to enhance social bonding and to reduce social tension between group members (Waring, 2003; Feh and de Mazieres, 1993). It is accompanied by changes in physiological parameters, such as a decrease in heart rate and cortisol levels (Feh and de Mazieres, 1993; Mcbride et al., 2004).

Sigurjonsdottir et al. (2003) showed that preferred allogrooming and play partnerships were present within the adult mare group, the adult gelding group and the sub-adult group. In this paper, adult geldings and sub-adults were more active in all three intervention roles than were adult mares, illustrating differences in social dynamics between these groups. Sub-adult animals are more flexible in forming social relations and developing preferred partnership relations than adult mares.

In affiliative interactions such as allogrooming and play, the interveners interrupted significantly more often when one of their preferred partners was part of the initial dyad, as was also observed in zebras (Schilder, 1990). This contrasts to interventions in allogrooming and play during oestrus, which were not influenced by the presence of a preferred partner. Thus, in light of the possible functions of allogrooming, interveners might be indeed actively safe-guarding their own social bonds (Schilder, 1990), since new relationships can threaten existing relationships.

These results clearly indicate the importance of allogrooming for preferred partnerships in social bonding. In allogrooming, the strength of the preferred partnerships between the intervener and one of the initial members positively correlates with the frequency of taking over allogrooming. Interventions apparently have a corrective or preventive function, as suggested earlier by Schilder (1990): when an individual observes a preferred partner allogrooming or playing with another animal, this may threaten the relation between the observing individual and the preferred partner. The observing individual may then interfere in the interaction and thus prevent a potential weakening of its bond with that preferred partner. In contrast, zebras intervened mainly in agonistic interactions. This can be probably explained by the fact that among these zebras, the harems were very small compared to the herds in the present study. So the zebras had a much smaller number of potential (preferred) partners. Additional factors to explain differences could be the absence of a stallion in the present study, the different competition regime or an increased need to reduce stress for the introduced animals.

It is not the relatively low rate of interventions, compared to the opportunities offered, which is of interest but rather the fact that interventions almost exclusively occurred when at least one of the horses involved in the affiliative dyadic interaction was a preferred partner of the intervener. This affirms the social role of affiliative interactions as well as the social importance of interventions. Social facilitation as a driving force can be rejected because the majority of interventions in affiliative behaviour resulted in breaking up the initial interaction. Since there were hardly any interventions in agonistic interactions, protection of specific individuals is unlikely; so a remaining possible function is the protection of the existing bond with the preferred partners. A possible explanation of the relatively low frequency could be that the interveners probably only intervened when the allogrooming took place in close proximity to the intervener.

Based on intervention studies in other mammals, interventions could be expected in courtship interactions. However, in this study there were no adult stallions and thus also no male reproductive competition. Oestrus mares were of particular interest to the yearling stallions and to some geldings. Surprisingly mounting was done not only by yearling stallions (259×) but also by the other animals (304×). During 'courtship', yearling stallions were never seen courting their own (lactating) dam, but very frequently courting other oestrus mares. Remarkably, several highly pregnant mares not only mounted (79×) but also intervened during courtship (26×, by 7 different adult mares). The mounting of adult mares could not have been confused with dominant mounting (Feist and McCullough, 1976), since the mounting was preceded and followed by typical courtship-like behaviours, while dominant mounting is normally preceded by aggressive interactions and 'head-on-hindquarters'. Data on female non-dominant mounting have only been reported incidentally (Feist and McCullough, 1976; Asa et al., 1979; Waring, 2003), but seems to be common in Jennies (McDonnell, 1998).

All adult mares in this study were multiparous, and bred by a free running stallion. This might explain the sometimes-persistent interest a mare did have for the gelding of her choice, provoking courtship. In the zebra population, interference by youngsters during stallion-mare courtship was explained as a protection of their dam from a perceived threat by the stallion (Schilder, 1990).

4.2. Consequences for horse husbandry

The results from this study suggest that physical positive interactions such as grooming and play are of great importance for horses to establish and maintain social bonds. Facilitating the development and maintenance of these relationships could benefit the welfare of domesticated horses. To show this conclusively experimental studies are required.

This study also showed that affiliative relationships are not merely a one-to-one issue, but may be influenced by the presence of other animals. Interventions probably facilitate the stability of mare–gelding groups.

Based on this study, it is also recommended to first familiarise new horses to each other to create (temporal) preferred relationships, before they are introduced into a resident group.

5. Conclusion

The active protection of preferred affiliative relationships, by means of interventions, shows the high motivation of the horses to safeguard their social network. Social interactions and the formation of preferred relationships in domesticated horses should be facilitated by husbandry and management systems, to ensure their welfare.

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The effects of surgical and chemical castration on intermale aggression, sexual behaviour and play behaviour in the male ferret (*Mustela putorius furo*)

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Abstract

The ferret (*Mustela putorius furo*) has become an increasingly popular pet animal, yet little is known about their behaviour and welfare. Inter- and intra-specific aggression and the intense musky scent are the two main reasons why male ferrets are normally surgically castrated. However, it is this neutering that is thought to play an important role in the development of hyperadrenocorticism, a hyperfunctioning of the adrenal cortex due to neoplastic changes, a common disease in domestic ferrets. An alternative to surgical castration might be the slow releasing GnRH agonist deslorelin implant (chemical castration).

The present study aimed to examine the effects of the deslorelin implant and surgical castration on the occurrence of intermale aggression, sexual behaviour and play behaviour in male ferrets. Therefore, 21 male domestic ferrets received either an implant containing deslorelin ($n = 7$), a placebo implant ($n = 7$), or were surgically castrated ($n = 7$).

Our data showed that: (1) chemical castration with the GnRH agonist deslorelin results in a decrease in the occurrence of aggressive behaviour between male ferrets both in the presence and absence of a receptive female. In addition, our data showed that a deslorelin castration had more effect on the reduction of aggression than surgical castration; (2) sexually motivated behavioural patterns were reduced in the deslorelin and surgical castrated group in the male–female confrontation; (3) the deslorelin group, and to a lesser extent the surgically castrated group, had a higher incidence of play behaviour in comparison to intact males in the intermale confrontation tests. Therefore, deslorelin chemical castration is a suitable alternative

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for surgical castration, and may even be preferred due to the serious medical problems associated with surgical castration in ferrets. Especially the increase of play behaviour might be indicative for an improved welfare of the ferrets.
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Keywords: Ferret; Castration; Intermale aggression; Play behaviour; Welfare

1. Introduction

The ferret (*Mustela putorius furo*) has become an increasingly popular pet animal, yet little is known about their behaviour and welfare. In veterinary practice, two topics are reported regularly: (1) inter- and intra-specific aggression (e.g. Applegate and Walhout, 1998; Staton and Crowell-Davis, 2003; Quesenberry and Carpenter, 2004, p. 83) and (2) the intense musky scent of the males (Quesenberry and Carpenter, 2004, p. 83). In pet industry, these two mentioned characteristics of ferrets often result into repetitive rehousing, sheltering, or even euthanasia. Additionally, aggressive interactions in mustelids can be violent with a high risk of injuries (Poole, 1966, 1973, 1974; Dunstone, 1993). Thus, frequent aggressive interactions between socially housed pet ferrets may seriously decrease their welfare.

In the Netherlands, male ferrets are normally surgically castrated before the age of 1 year (see Schoemaker et al., 2000) to reduce interspecific and intraspecific aggression and to reduce the musky scent they spread. However, it is this castrating that is thought to play an important role in the development of hyperadrenocorticism, a common disease in the domestic ferret. There are several reasons to assume that continuously high concentrations of especially luteinising hormone (LH) can result in adrenocortical hyperplasia, proliferation of cells in the adrenal cortex, or tumorigenesis, formation of tumors, associated with hyperadrenocorticism (see Schoemaker et al., 2000, 2002; Wagner et al., 2001).

Little is reported on the effects of castration on aggressive behaviour in ferret so far. The only available study is of Staton and Crowell-Davis (2003) who reported no difference in aggressive behaviour between surgically castrated and intact male pairs. However, they mentioned their small numbers of pairings as a possible explanation for this lack of significance. Though some analogical studies on dogs found that surgical castration decreases aggression (e.g. Beaver, 1983; Wright and Nesselrote, 1987) the evidence on the effectiveness of castration on the aggression of male ferrets seems scarce, if not unavailable. In general, decreased levels of plasma LH and testosterone concentration are presumed to result in a decrease of intermale aggression and of sexual behaviour (Baum, 1976; Baum et al., 1983; Carroll et al., 1988; Lambert and Baum, 1991; Giammanco et al., 2005).

A potential alternative for surgical castration in the male ferret may be an implant containing the long acting and slow releasing GnRH (gonadotrope releasing hormone) agonist deslorelin (Suprelorin®, Peptech Animal Health, Sydney, Australia) (chemical castration). The effect of a GnRH agonist implant consists of two phases. During the initial (acute) phase, LH plasma concentrations increase, simultaneously resulting in an initial rise of the plasma testosterone concentration (D'Occhio et al., 2000). Depending on the species and the administered dose of GnRH agonist, the course of the second, chronic phase can be different. In the stallion implantation of deslorelin leads, after the initial elevation of LH and testosterone, to a decrease of LH and testosterone below the levels of the control animals around day 13 after implantation

(Johnson et al., 2003). After implantation of dogs with deslorelin, plasma testosterone concentrations decreased within 6–25 days below 1 ng/ml. This concentration was maintained for 3 months to 2.7 years (Trigg et al., 2001). In the bull, GnRH agonist deslorelin treatment leads to a down regulation of the pituitary function, indicated by the decrease of pulsatile release of LH. Plasma testosterone concentration, in contrast, is elevated (Ronayne et al., 1993; D'Occhio and Aspden, 1996; D'Occhio et al., 2000).

Thus, although not fully conclusive, deslorelin implants may decrease levels of LH in the male ferret, which can result in a decreased risk of adrenocortical hyperplasia or tumorigenesis, and a decrease of intermale aggression and sexual behaviour.

Although the GnRH agonist deslorelin might be an alternative for surgical castration, there are no reports on the effect of deslorelin on behaviour in ferrets. Therefore, our first aim was to examine the effects of the deslorelin implant on the occurrence of intermale aggression, sexual behaviour and play behaviour in male ferrets. A second aim was to assess the effect of surgical castration on aggression in male ferrets in general, as in the literature little is reported on such effects. Therefore, the behaviour of the males from one group of ferrets with a deslorelin implant (chemically castrated) was compared to one group of surgically castrated ferrets and to one group of intact (placebo implanted) ferrets. The ferrets were observed in three experiments of pair-wise confrontations (''confrontation tests'').

It was hypothesized that plasma concentrations of both LH and testosterone will decrease after placement of the deslorelin implant in ferrets, resulting in a decrease of intermale aggression and sexual behaviour (Baum, 1976; Baum et al., 1983; Carroll et al., 1988; Lambert and Baum, 1991; Giammanco et al., 2005). Additionally, it was hypothesized that the reduced aggression would result in a less stressful environment and hence, in an increase of play behaviour. Increased incidences of play might indicate on increased welfare (see e.g. Hinton and Dunn, 1967; Fagen, 1981; McCune, 1992; Grier and Burk, 1992; Broom and Johnson, 1993; see for opioid release during play: Vanderschuren et al., 1995a,b; see for a discussion on play behaviour and welfare in farmed mink: Vinke, 2004; Vinke et al., 2005). Additional effects of the treatments on hormone levels (plasma testosterone and FSH), testis volumes, histological examination of the testes and a control for differences in musky scents are presented elsewhere (Schoemaker et al., 2008).

2. Materials and methods

All experiments were performed in adherence to the legal requirements of the Netherlands (Animal Experimentation Act, 1977) and have been approved by Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

2.1. Animals, housing and experimental groups

Twenty-one commercially bred male ferrets between the age of 1 and 2 years were individually housed in outdoor suspended wire meshed home cages (dimensions: 88 cm (L) × 30 cm (W) × 46 cm (H)) with a nest box (dimensions: 29 cm (L) × 34 cm (W) × 23 cm (H)) filled with pea straw. Pellets (FerRet®, Hope Farm, Woerden, The Netherlands) and water were available *ad libitum*.

For the experiment, the 21 ferrets were randomly assigned to three groups ($N = 7$ per experimental group):

- *Placebo group*. Group of males with a placebo implant (placebo control group; mean [±S.D.] body weight: 1.5 ± 0.1 kg; range: 1.3–1.7 kg).

- *Deslorelin-implant group*. Group of males with a deslorelin implant containing 9.4 mg deslorelin (chemically castrated experimental group; mean [±S.D.] body weight: 1.5 ± 0.1 kg; range: 1.3–1.7 kg).
- *Castrate group*. Group of males that were surgically castrated (surgically castrated control group; mean [±S.D.] body weight: 1.4 ± 0.2 kg; range: 1.0–1.6 kg).

The female ferrets that were used as test stimuli in the confrontations tests 2 and 3 (see Section 2.3) were housed in exactly the same way as the male ferrets mentioned above. In their standard housing conditions males and females were visually and physically separated by three empty cages between the groups.

2.2. Start procedures

2.2.1. Vaccination and physical control

All ferrets underwent a weekly procedure of blood sampling and testis measurements (see for detailed information: Schoemaker et al., 2008). During one of these standard checks they were vaccinated against Canine Distemper Virus with Nobivac Puppy DP (Intervet Nederland B.V. Boxmeer, The Netherlands).

2.2.2. Castration

The ferrets of the 'castrate group' were surgically castrated 7 weeks before the behavioural observations started. Anaesthesia was induced in all ferrets by a mask with 4% isoflurane (IsoFlo™, Abbott Animal Health, Hoofddorp, The Netherlands) in 100% oxygen and maintained at 2% isoflurane during the procedure. Analgesia was provided with carprofen 4 mg/kg (Rimadyl®, Pfizer, Capelle a/d IJssel, The Netherlands) and buprenorphine 0.01 mg/kg (Temgesic®, Schering-Plough, Utrecht, The Netherlands).

The total testis volume of one ferret in the placebo implant group did not increase and was comparable with that of ferrets in the deslorelin-implant group. Based on histological evaluation of the left testis (an arrest of spermatogenesis at the level of meiosis), this ferret was diagnosed with hypogonadism, i.e. a condition in which insufficient testosterone is produced, unrelated to this study. This ferret was therefore removed from the dataset and was not used for further statistical analyses.

2.2.3. Implants

The animals of the 'deslorelin-implant group' and 'placebo group' received the implants on the same day that the surgical castrations were performed in the 'castrate group'. The deslorelin implants were manufactured by Peptech Animal Health, Sydney, Australia and contained 9.4 mg deslorelin (Suprelorin®). The implants, either deslorelin or placebo, were administered subcutaneously in the interscapular space using an implanting device provided with the implants. The skin at the injection site was then sealed with tissue glue (Vet-Seal®, B. Braun Medical, Emmenbrücke, Switzerland). The implants were placed at the same time as the blood collection.

2.3. Experimental methods and treatments

Subsequently, three ''confrontation tests'' were carried out to evaluate aggressive behaviour, play behaviour and male sexual behaviour. All males from each experimental group were confronted with a male of the same experimental group in an experimental test area both in the absence (confrontation test 1) and in the presence of a receptive female (confrontation test 2). The subjects were, if necessary, marked with CTC (chlorotetracycline) fluorescent spray to facilitate individual recognition. In confrontation test 3, the sexual motivation of the males was tested in a male–female confrontation.

2.3.1. Test area

The test area for the confrontation tests was a roofed outdoor dog kennel (dimensions: 150 cm (L) × 115 cm (W) × 200 cm (H)). This test area was separated from the standard housing conditions of the ferrets by a wooden door. The area was divided into two compartments by a transparent plastic plate with

Table 1a
Ethogram used in experiments 1 and 2 to score the male ferret behaviour (adapted from Poole, 1974, 1978)

Main pattern	Contains	Description
Offensive behaviour	Positions for attack	Orientation to reach a position from which an attack may be launched
	Attack	Movement into a position from which the neck or ear regions of the opponent may be bitten. Attacker crosses over its opponent's back to reach the far side of the neck so that the attack is generally from above
	Neck bite	Bites opponent in the neck and holds
	Bite other	Bites to other places (for example: flank, belly, anal)
	Drag	Grips opponent by the neck with teeth and pulls it around (neck bite is implied)
	Shake	Animal bites in a body part of another animal and shakes violently with the head up and down and from right to left (neck bite is implied)
	Oblique attack with physical contact	Approach to the opponent from the side or behind. The attacker's head is turned away from its adversary and its back arched attacker pushes its flank against its adversary and may roll on to it
	Position for lunge attack	Orientation to a position from which a lunge attack van be launched
	Lunge attack with contact	Lunges towards the opponent directing the open mouth towards the near side of the opponent's neck and touches opponent's fur, sometimes results in a bite
Defensive behaviour	Ward off	Flank is directed towards an adversary, the head turned away so that the convex side of the neck is presented to the opponent. Generally in response to an attack
	Extricate	Any movements made by an animal to free itself from its opponent's bite. May include rolling, kicking, scratching with forepaws, brief attacks
	Extricate with vocalization	As for extricate but the animal makes high pitch vocalization that sound like screaming
	Defensive	Faces the opponent with back arched
	Defensive threat	As for defensive but accompanied with vocalizations (vocalizations accompanying different levels of intensity are hiss, scream)
	Snap	Biting movements made without any attempt actually to bite the opponent
	Passive	The animal that is being bitten makes no attempts to free itself from the opponents grip
	Scream	Animal vocalizes with high pith tones
Social behaviour (other)	Investigate ano-genital	Sniffing another animal on ano-genital region
	Investigate neck–nose	Sniffing another animal in the neck/ear region
	Investigate nose–nose	Sniffing another animal on the nose
	Nose push	Animal pushes with nose into the flank of the opponent

Table 1a (*Continued*)

Main pattern	Contains	Description
Play behaviour	Locomotory play	Play behaviour consisting out of gallop forward (bouncing jerky gait), gallop away, leap over the opponent, jump on the opponent
	Rough and tumble play	Play behaviour by which the animal may roll over, pushes with paws, ward of with paw, inhibited chin biting
Other behaviour	Back away	Movement away from the opponent whilst remaining facing it
	Face	Facing the opponent
	Evade	Movements away from the opponent (in step), while not facing opponent
	Flee	Movement away from the opponent (in gallop) while not facing opponent
	Follow	Follows opponent while he backs away/evades (walking)
	Chase	Animal follows opponent while he backs away/evades (galloping)
	Approach	Animal increase proximity to opponent
	Explore	Walking around, sniffing and scratching at the walls or floor
	Explore box (only in experiment 2)	Sniffing, scratching, climbing on the box with the female
	Prostration	Flattens itself on the ground with head down, front paws pointing forward and hind legs splayed out on the ground
	Defecating	Animal defecates or urinates
	Rubbing	Animal rubs the ground or wall with face or neck and may roll over
	Fur shake	Animals shakes off water of fur
	Grooming	Animal licks or rubs its pelt with forepaws and tongue
	Coughing	Animal coughs/sneezes
	Alert	Animal actively observes surrounding

The behavioural pattern 'explore box' was only used in experiment 2.

small holes in it (diameter of each hole: 1 cm; placed in three rows: one 5 cm from the ground, one 40 cm from the ground and one in between; the holes were 8 cm apart). The holes made it possible for the ferrets to smell each other before the actual confrontation. After every confrontation test, the area was cleaned intensively with water to get rid of the urine and faeces of the previous pair of ferrets.

2.3.2. Confrontation tests

The confrontation tests, which lasted 10 min each, were carried out between 8:30 and 16:00 h during May. In experiments 1 and 2, each subject was tested in pairs with all males from the same group except its direct neighbours, which resulted in three tests per male (one animal of each group was tested four times because of the number of ferrets used). Neighbours were never paired with each other, to rule out possible influences of familiarity (see Poole, 1973). Pairs of every group were tested at random times throughout 8 consecutive days (except for the weekend) to rule out circadian changes in behaviour, or influences related to the standard management procedures. The total number of tests was 87 (8–9 tests per day): 33 in confrontation test 1, 33 in confrontation test 2 and 21 in confrontation test 3. All tests

Table 1b
Additional behavioural patterns for the ferret ethogram used for the observation of the male's sexual motivated behaviour in experiment 3

Main pattern	Contains	Description
Male sexual behaviour	Neck grip	Male bites the female in the neck and holds (NB: see comment in Section 2.4.1)
	Mounting	Male holds female with a neck grip and tries to mount her
Other behaviour	Intervention	Observers intervenes on the moment the male has mounted or is trying to mount the female

were recorded on video by a digital camera (Panasonic NV-GS15EG) that was mounted above the test area.

2.3.2.1. Procedures of experiment 1: confrontation test male–male without receptive female. To evaluate the spontaneous aggressive behaviour and play behaviour between male ferrets, a first confrontation test was carried out in the absence of receptive female ferret. Each 10 min confrontation test started as soon as two interaction partners were placed in the testing area, separated by the transparent plastic plate. After 2 min the plate was removed and full physical contact was possible during 8 min, after which the experiment was ended and the ferrets were returned to their cages (an unpublished pilot experiment showed that at least 5 min are necessary to observe interactions between the ferret males, therefore, we decide for a full contact period of 8 min). Three females were used as test stimuli in the confrontation tests 2 and 3. The receptiveness of each female was assessed each day by controlling the swelling of the vulva. These females were randomly used during the confrontation tests.

2.3.2.2. Procedures of experiment 2: confrontation test male–male in the presence of receptive female. To enhance the potential aggressive motivation of the male ferrets, a second confrontation test was carried out in the presence of a receptive female ferret. The female in oestrus was placed in a transport box (plastic box of 40 cm (L) × 30 cm (W) × 30 cm (H) with several open spaces through which olfactory stimuli and some visual stimuli could be exchanged) in the middle of the testing area in order to elicit more competition between the males.

The test started as soon as one male–male pair was placed in the testing area with the transparent plastic plate to separate them. After 2 min the plate was lifted and a receptive female ferret in a transporting box was placed in the middle of the testing area. The experiment was ended after 8 min and the subjects were returned to their cages.

2.3.2.3. Procedures of experiment 3: male sexual behaviour in a male–female confrontation. In this third experiment the male sexual behaviour was evaluated. Each of the male ferrets ($N = 21$) was confronted with a female ferret in oestrus. The test started as soon as the subjects were put in the testing area separated by the plastic transparent plate for 2 min, after which the plate was removed and 8 min full physical contact was possible. To avoid the risk of ovulation induction in the females, intervention took place if a male mounted the female for pelvic thrusting.

2.3.3. Behavioural observations

Table 1a shows the ethogram used to score the behaviour in the male–male interactions in experiments 1 and 2. Table 1b shows the additional ethogram used to score the sexual behavioural patterns in the male–female interactions in experiment 3. The observed behavioural patterns were mutually exclusive.

2.4. Data analysis and statistics

2.4.1. Data analysis

The videos were analysed afterwards by one observer. The observer was ''blind'' with regard to the experimental condition, but cues in the recordings often allowed an educated guess. The behavioural patterns were scored with the use of a focal animal sampling method with The Observer 5.0 software (Noldus Information Technology, Wageningen, The Netherlands). The tape was played at normal speed.

Only the behaviour in the 8 min of true interaction was scored (i.e. after the plastic transparent plate was removed). For a more accurate statistical analysis, the data of some separate behavioural patterns were lumped together into a behavioural class. The behavioural classes were defined as follows:

(1) '*Total offensive and defensive*' consists the mean duration of the sum of all offensive and defensive behaviour patterns, that is: passive, defensive, defensive threat, extricate, extricate with scream, position for lunge attack, lunge attack, oblique attack, shake, drag, neck bite, attack, position for attack and scream. Defensive behaviour is often evoked by aggressive behaviour of the opponent, so combining these two behavioural classes leads to an overall insight in agonistic behaviour. Two behavioural sub-clusters which prominently present aggression are elucidated in more detail:
   (a) '*Total offensive*' is the combined behavioural sub-class of the total durations from position for attack, attack, neck bite, drag, shake, oblique attack, position for lunge attack and lunge attack. These behavioural patterns represent all the *offensive behaviours* ('initiating the aggressive interaction').
   (b) '*Total neck bite*' is the combined behavioural sub-class consisting out of the total durations of neck bite, drag and shake. These three behavioural patterns are reported as the most aggressive in the behavioural repertoire of ferrets and polecats (Staton and Crowell-Davis, 2003; Poole, 1974, 1978).
(2) '*Play*' is the combined behavioural class of locomotory play and rough and tumble play.
(3) '*Social*' combined the patterns ano-genital-, nose–neck- and nose–nose-investigation.

To evaluate the male sexual behaviour in experiment 3, not all behavioural patterns that were scored during the observation were used for further statistical analyses. We added the most important relevant behavioural patterns to the ethogram: neck gripping and mounting (Baum, 1976; Baum et al., 1983; Carroll et al., 1988; Lambert and Baum, 1991). As we decided to interrupt the copulations, the pattern *pelvic thrusting* was not observed in this study. We analysed neck gripping, mounting, the classes 'total offensive and defensive', 'play' and 'social' and the latency period in neck gripping. The latency in neck gripping was supposed to reveal to the motivation of the animals to perform reproductive behaviour. Although 'neck biting' and 'neck gripping' are visually undistinguishable, we prefer in experiment 3 the term 'neck gripping' to stress the sexual context of the behavioural patterns which is in line with Baum (1976), Carroll et al. (1988) and Lambert and Baum (1991).

2.4.2. Statistics

To compare the three experimental groups of animals the average of the three (or four) observations per subject were used as statistical unit for further analysis. All data were analysed using SPSS 12.0 for Windows. As a Kolmogorov–Smirnov-test showed that most variables were not normally distributed, non-parametric tests were used for statistical analysis.

A Kruskal–Wallis-test (KW) was used to test for overall differences between the three experimental groups. Mann–Whitney *U*-tests (MWU) were conducted for post hoc testing between two experimental groups if the KW test had shown overall significant differences. All statistics were two-tailed tested. Results are given in mean ± S.E.M. and are considered non-significant when $p > 0.05$.

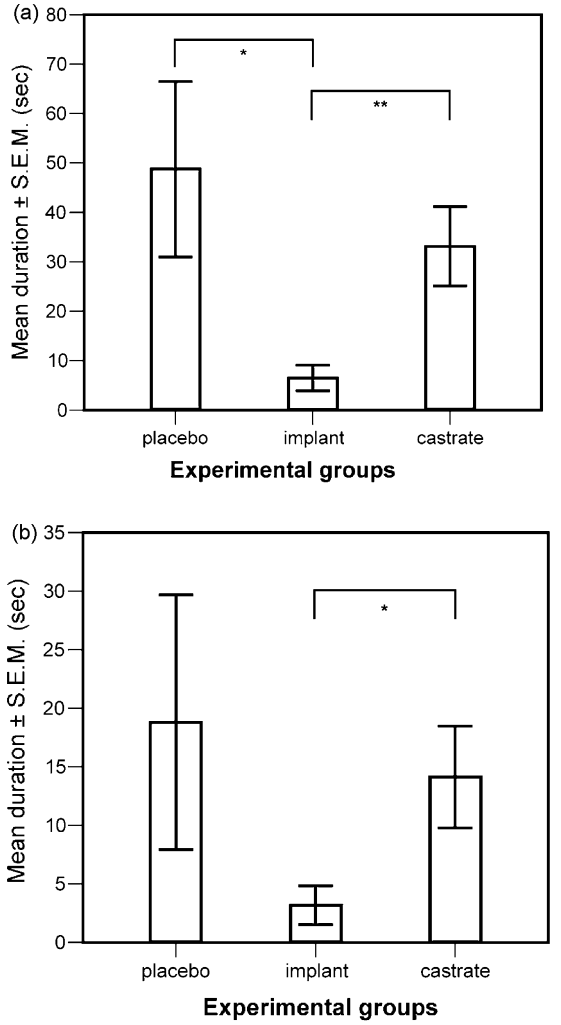


Fig. 1. Total mean duration (±S.E.M. in s) of two different behavioural parameters (classes: a, 'total offensive and defensive'; b, 'total neck bite') in experiment 1 (*$p \leq 0.05$; **$p \leq 0.01$).

3. Results

3.1. Experiment 1: male–male confrontation without receptive female

For the behavioural class 'total offensive and defensive' the results showed an overall significant difference between the three experimental groups (KW: $\chi^2 = 8.95$, d.f. = 2, $p \leq 0.01$). The deslorelin-implant group showed significantly less 'total offensive and defensive' behaviour than both other groups (deslorelin-implant group: 6.49 s ± 2.58 *versus* placebo group: 48.73 s ± 17.75 and castrated group: 33.15 s ± 8.01; MWU: $U \geq 4.00$, $p \leq 0.05$) (Fig. 1a).

To elucidate the level of the actual initiation of the aggressive interactions in more detail, the sub-class 'total offensive behaviour' was analysed separately. The results showed an overall

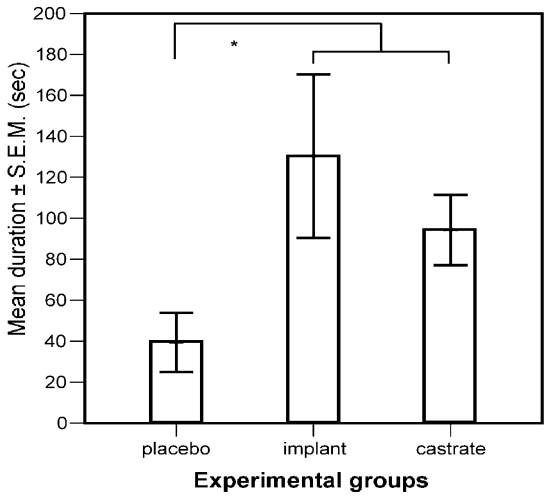


Fig. 2. Total mean duration (±S.E.M. in s) of the behavioural class 'play' in experiment 1 (*$p \leq 0.05$).

significance ('total offensive behaviour': KW: $\chi^2 = 7.36$, d.f. = 2, $p \leq 0.05$). Post hoc analyses of this sub-class showed that deslorelin-implant animals expressed significantly less offensive behaviour than castrated animals (deslorelin-implant group: 5.46 s ± 2.48 S.E.M. *versus* castrated group: 22.78 s ± 4.76; MWU: $U = 4.00$, $p \leq 0.01$). No significant differences were found for the comparison between the deslorelin-implant group and the placebo group (MWU: $U = 9.00$, $p = 0.09$). Furthermore, no significant differences were found between the placebo and castrated group neither for the behavioural classes 'total offensive and defensive', nor for the class 'total offensive behaviour' (MWU: $U > 15.00$, $p > 0.39$).

Fig. 1b shows the total mean duration of the behavioural sub-class 'total neck bite'. A separate analysis of this sub-class was chosen to show the level of three of the most prominent aggressive patterns (see Section 2.4.1). Overall between-groups analyses showed that there was a significant difference (KW: $\chi^2 = 6.09$, d.f. = 2, $p \leq 0.05$): the deslorelin-implant group showed a shorter total duration of neck biting behaviour than castrated animals (i.e. deslorelin-implant group: 3.2 s ± 1.67 *versus* castrated group: 14.1 s ± 4.35; MWU: $U = 6.00$, $p \leq 0.05$). No significant differences were found between the deslorelin-implant group and the placebo group (MWU: $U = 9.00$, $p = 0.09$) or between the placebo group and the castrated group (MWU: $U = 18.00$, $p = 0.67$).

Fig. 2 shows that in particular the ferrets in the placebo group played less. An overall significant difference between the groups was found for the total mean duration of the class 'play' (KW: $\chi^2 = 6.14$, d.f. = 2, $p \leq 0.05$), whereby the post hoc testing showed that the placebo group played significantly less than both the deslorelin-implant group and castrate group (placebo group: 39.42 s ± 14.50 S.E.M. *versus* deslorelin-implant group: 130.33 s ± 39.92 and castrate group: 94.28 s ± 17.16, respectively; MWU: $U \geq 6.00$, $p \leq 0.05$). No significant differences were found between the deslorelin-implant group and the castrate group (MWU: $U = 23.00$; $p = 0.85$).

For the class 'social' a trend towards significance was found for an overall group difference (KW: $\chi^2 = 5.68$, d.f. = 2, $p = 0.06$). Finally, in experiment 1, no significant differences were found between the three experimental groups for the separate behavioural patterns as mentioned in the ethogram (Table 1a).

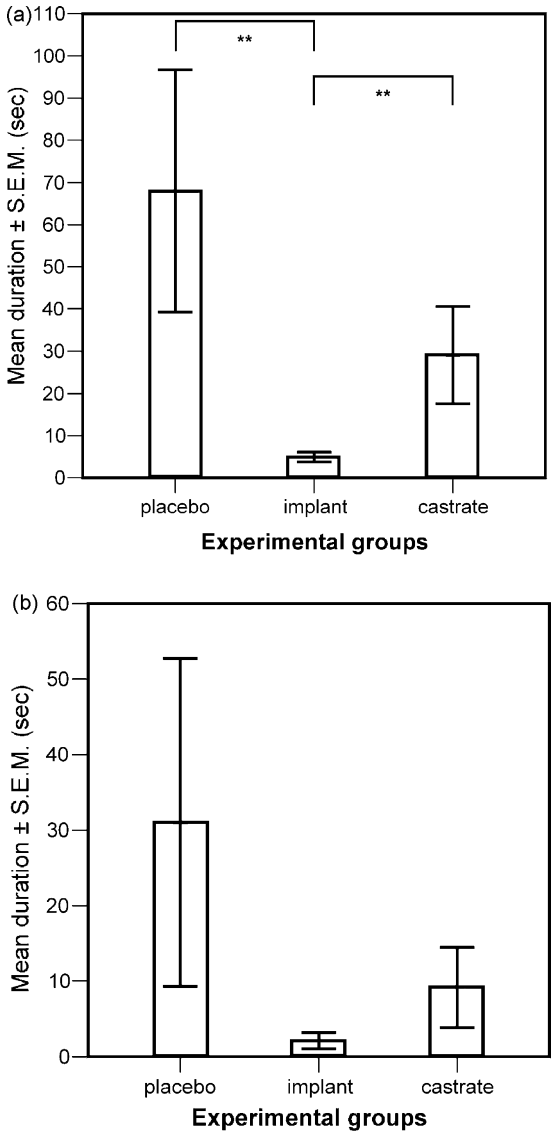


Fig. 3. Total mean duration (±S.E.M. in s) of two different behavioural parameters (classes: a, 'total offensive and defensive; b, 'total neck bite') in experiment 2 (**$p \leq 0.01$).

3.2. Experiment 2: male–male confrontation in presence of a receptive female

An overall significant difference between the groups was found for the behavioural class 'total offensive and defensive' (KW: $\chi^2 = 12.59$, d.f. = 2, $p \leq 0.01$), whereby the deslorelin-implant group again showed less offensive and defensive behaviours (4.93 s ± 1.21) compared to both the castrated animals and placebo animals (castrate group: 29.08 s ± 11.47 and placebo group: 67.99 s ± 28.76, respectively; MWU: $U \geq 1.00$, $p \leq 0.01$, see Fig. 3a). Furthermore, no

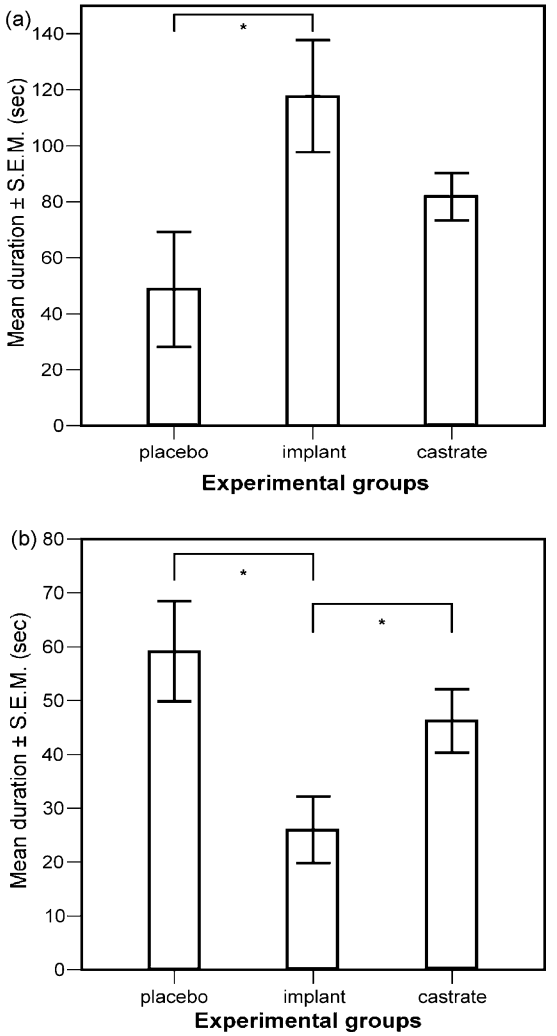


Fig. 4. Total mean duration (±S.E.M. in s) of the behavioural classes 'play' (a) and 'social' (b) in experiment 2 (*$p \leq 0.05$).

significant differences were found between the three experimental groups for either of the behavioural sub-classes 'total offensive' and 'total neck bite' ('total offensive': KW: $\chi^2 = 4.93$, d.f. = 2, $p = 0.09$ and 'total neck bite': KW: $\chi^2 = 2.51$, d.f. = 2, $p = 0.29$, see Fig. 3b).

Fig. 4a shows the duration of play behaviour in the three groups. Statistical testing showed that there was an overall significant difference for the behavioural class 'play' (KW: $\chi^2 = 6.09$, d.f. = 2, $p \leq 0.05$): deslorelin-implant animals played significantly more than the animals of the placebo group (deslorelin-implant group: 117.78 s ± 19.95 *versus* placebo group: 48.71 s ± 20.54; MWU: $U = 7.00$, $p \leq 0.05$). No significant differences were found in the class 'play' between the animals of the placebo group and the castrate group (MWU: $U = 9.00$, $p = 0.09$).

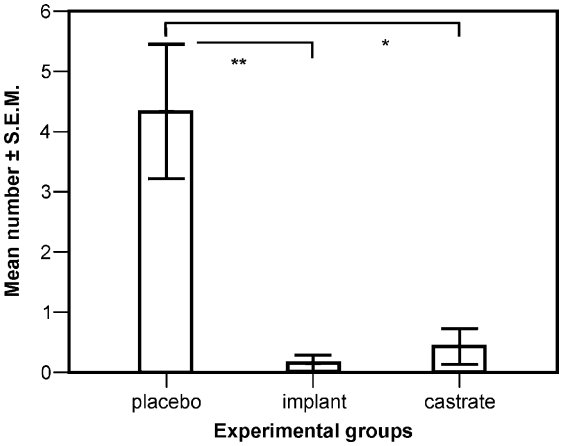


Fig. 5. Mean number of mounts (±S.E.M.) performed by the ferret males in experiment 3 (*$p \leq 0.05$; **$p \leq 0.01$).

For the class 'social' an overall significant difference was found between the experimental groups (KW: $\chi^2 = 7.35$, d.f. = 2, $p \leq 0.05$), whereby the deslorelin-implant group did significantly less social investigations than both the placebo group and the castrate group (deslorelin-implant group: 26.01 s ± 6.19 *versus* placebo group: 59.15 s ± 9.29 and castrate group: 46.23 s ± 5.85; MWU: $U \geq 5.00$, $p \leq 0.05$, Fig. 4b).

In experiment 2, no significant differences were found between the experimental groups for the separate behavioural patterns as mentioned in the ethogram (Table 1a), except for the pattern 'follow': subjects of the castrate group followed their interaction partners significantly more compared to both the deslorelin-implant and the placebo group (MWU: $U \geq 7.00$, $p \leq 0.05$).

3.3. Experiment 3: male sexual behaviour in a male–female confrontation

The placebo animals mounted the females more than the males from the deslorelin-implant and castrated group (mean number of mounts: placebo group: 4.33 ± 1.12 *versus* deslorelin-implant group: 0.14 ± 0.14 and castrate group: 0.43 ± 0.30; KW: $\chi^2 = 9.92$, d.f. = 2, $p = 0.007$). This difference was significant between the placebo group compared to both the deslorelin-implant group (MWU: $U = 4.00$, $p \leq 0.01$) and the castrated group (MWU: $U = 4.50$, $p \leq 0.05$, see Fig. 5).

Fig. 6 shows that there was an overall difference in the total mean duration of neck gripping between the three experimental groups (KW: $\chi^2 = 6.90$, d.f. = 2, $p \leq 0.05$). Post hoc analysis showed that the males from the castrate group showed a significantly higher total mean duration of neck gripping behaviour toward the females compared to the deslorelin-implant group (castrate group: 30.86 s ± 9.57 *versus* deslorelin-implant group: 9.42 s ± 5.29; MWU: $U = 7.00$, $p \leq 0.05$). No overall difference was found between the three experimental groups in the latency period to neck gripping (KW: $\chi^2 = 2.01$, d.f. = 2, $p = 0.37$).

In Fig. 7 it can be seen that the animals of the placebo group clearly played less compared to the other experimental groups. Overall, this difference was significant (class 'play': KW: $\chi^2 = 12.05$, d.f. = 2, $p \leq 0.01$). Further post hoc analysis showed that the animals from the placebo group played less than both the castrated and the deslorelin-implanted animals (placebo

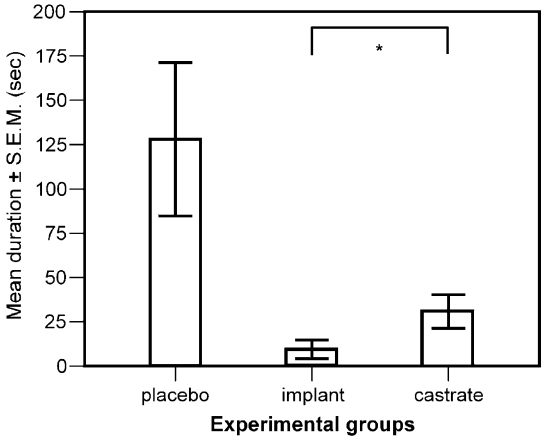


Fig. 6. Total mean duration (±S.E.M. in s) of neck gripping in experiment 3 (*$p \leq 0.05$).

group: 27.30 s ± 11.80 *versus* castrate group: 185.38 s ± 27.30 and deslorelin-implant group: 168.98 s ± 22.59, respectively; MWU: $U \geq 0.00$, $p \leq 0.01$). No differences in play behaviour were found between the castrate group and the implant group (MWU: $U = 22.00$, $p = 0.81$).

Furthermore, no overall significant differences were found in the total mean duration for the behavioural class 'social' (KW: $\chi^2 = 1.35$, d.f. = 2, $p = 0.51$), nor for behavioural patterns that concerned offensive or defensive interactions, except for the defensive behavioural pattern 'ward off', i.e. flank is directed towards an adversary (KW: $\chi^2 = 8.18$, d.f. = 2, $p \leq 0.05$). The deslorelin-implant group showed more ''ward off' behaviour than both the two other experimental groups (deslorelin-implant group: 9.14 s ± 3.03 *versus* placebo group: 0.00 s ± 0.00 S.E.M. and castrate group: 0.96 s ± 0.69; MWU: $U \geq 6.00$, $p \leq 0.05$).

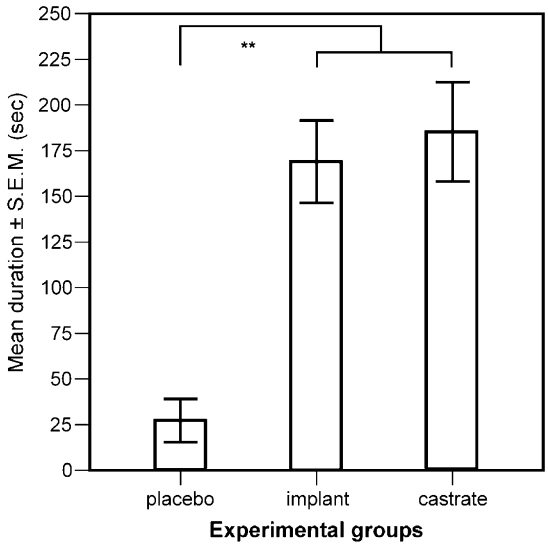


Fig. 7. Total mean duration (±S.E.M. in s) of the behavioural class 'play' in experiment 3 (**$p \leq 0.01$).

4. Discussion

The present study aimed to assess the effects of a chemical castration with a deslorelin-implant in the male ferret, as an alternative for surgical castration, on the occurrence of intermale aggression, sexual behaviour and play behaviour. The main results were that: (1) chemical castration with the GnRH agonist deslorelin results in a decrease in the occurrence of aggressive behaviour between male ferrets both in the presence and absence of a receptive female. In addition, our data showed that a deslorelin castration had more effect on the reduction of aggression than surgical castration; (2) sexually motivated behavioural patterns were reduced in the deslorelin and surgical castrated group in the male–female confrontation; (3) the deslorelin group, and to a lesser extent the surgically castrated group, had a higher incidence of play behaviour in comparison to intact males in the intermale confrontation tests. These findings, especially with regard to the placebo and deslorelin implanted animals, correspond to our expectations that deslorelin will diminish aggressive behaviour, because testosterone and related compounds (estradiol and dihydrotestosterone [DHT]) are important for the regulation of aggressive behaviour in male animals (Giammanco et al., 2005). The study of Schoemaker et al. (2008), which separately presents the hormonal part of the current study, confirms this expected decrease of aggression-related hormones. As one of the major motivations for surgical castration in practice is the reduction of aggression, our finding that the surgical castration barely had an effect on aggression in ferrets is rather surprising. It shows that the decrease of testosterone levels to near-zero levels, as found in both the deslorelin and castrated group (Schoemaker et al., 2008), is in itself not enough to reduce aggression in ferrets. The lack of effectiveness of surgical castration on reducing aggression in ferrets affirms the study of Staton and Crowell-Davis (2003) who reported no difference in aggressive behaviour between surgically castrated and intact male pairs. However, their results were inconclusive because of the low sample size in combination with dichotomous data (whether or not a fight occurred). Also for other species, mainly birds (e.g. Pinxten et al., 2003) but also mammals (e.g. Demas et al., 1999), however, it was found that surgical castration did not reduce aggression. Part of the ineffectiveness of surgical castration might be attributed to species differences, seasonality and age of castration (Trainor et al., 2006). Our findings raise an interesting additional point as in contrast to surgical castration, chemical castration with deslorelin did have an effect on aggression. One of the most likely explanations is that deslorelin as a GnRH agonist decreases the plasma estradiol level, which is also involved in the regulation of aggression (Trainor et al., 2006). Additionally, surgical castration may lead to elevated GnRH and LH levels in the blood, which may result in some testosterone-related compound production in tissues other than the testes. The most logical tissue would be the adrenal gland. As is shown in several studies, ferrets with hyperadrenocorticism have elevated plasma concentration of estradiol, 17-α-hydroxyprogesterone, androstenedione, and dehydroepioandrosterone sulphate (e.g. Rosenthal and Peterson, 1996). Some of these hormones might have an effect on the behaviour of the affected ferret and it is mentioned that ferrets with hyperadrenocorticism are indeed more aggressive and that these symptoms can be decreased by using a GnRH agonist (Wagner et al., 2005). In some other species, androstenedione is also considered to elevate aggressive behaviour (Swiss-Webster mice: Luttge and Hall, 1973; golden hamster: Payne, 1974). It is therefore possible that after a short period the increased levels of LH after surgical castration will lead to elevated plasma concentrations of the mentioned hormones, in particular androstenedione.

Another result of interest is the increased levels of play behaviour in the castrated group and in the deslorelin-implant group in particular. Also this result might be explained by lower plasma

testosterone levels in these two groups (Schoemaker et al., 2008): less testosterone leads to less dominant behaviour, hence less agonistic interactions, which subsequently results in more play behaviour due to less stressful (competitive) conditions. This finding is in agreement with the findings in the literature showing that play behaviour is often seen under familiar and relaxed conditions (e.g. Fagen, 1981; Grier and Burk, 1992; Broom and Johnson, 1993) and does not occur under severe stress (Hinton and Dunn, 1967; McCune, 1992). Additionally, as the actual performance of play behaviour is known to be opioid mediated (Vanderschuren et al., 1995a,b), it is expected that play patterns have rewarding properties (''pleasure'') for the animal. The increased levels of play, hence increased rewarding experiences, can improve the animal's welfare (this in addition to the already discussed decreased level of agonistic patterns) (see also Vinke et al., 2005).

Two aspect of play behaviour should be studied for this species in more detail in future studies: (1) aggressive interactions often resulted from a sudden switch from play behaviour into more aggression-motivated behavioural patterns (i.e. 'play escalation'). Information on this relation is important for the further assessment and evaluation of the welfare of ferrets; (2) the observed ''ward off'' behaviour in experiment 3, which was originally classified under the defensive patterns as based on Poole (1974, 1978). However, during our observations we got more and more the impression that ''ward off'' is also an element in ferret play behaviour, especially in the more relaxed male–female context. This behavioural element ''ward off'' can also be seen in mink play (Vinke, 2002, personal observation). This would also explain why this pattern was significantly higher in the deslorelin-implant group.

The significant differences in the main behavioural classes during the three experiments were all in the same direction: the deslorelin-implant group showed less aggressive interactions, less sexual behaviour and more play behaviour. Moreover, in many cases the differences in some other behavioural categories were in the same direction, but just above the significance level (showing a *trend*). Because of this clear directionality of the results, and hence the relative small impact of a type I error, we refrained from correction for multiple testing, as it would decrease the power considerably as a result of the small sample sizes as used in this study (i.e. $N_{1,2,3} = 6, 7, 7$). Future studies can consider larger sample sizes.

In conclusion: the present study showed that chemical castration with the GnRH agonist deslorelin was more effective in decreasing aggressive behaviour in male ferrets than surgical castration. In comparison to the placebo-treated group both the surgically and chemically castrated group showed more play behaviour and less sexual behaviour. This shows that a reduction of testosterone levels is in itself not enough to reduce aggression in ferrets, but it might affect sexually motivated behaviour and play behaviour. Especially the increase of play behaviour might be an indicator of improved welfare. Taken together, deslorelin chemical castration is a suitable alternative for surgical castration, and may be preferred due to the serious medical problems associated with surgical castration in ferrets.

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Effect of ovariectomy on the course of gestation in the grey squirrel (*Sciurus carolinensis*)

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SUMMARY

In the grey squirrel pregnancy continues in the absence of the ovaries from day 16 of gestation. Since the investigation was performed on squirrels caught in the wild the stage of gestation was estimated from plasma progesterone levels, volume of corpora lutea at ovariectomy and date of parturition for those squirrels which did give birth. Three squirrels ovariectomized in early pregnancy did not give birth. Plasma progesterone levels after ovariectomy were lower than those found in normal pregnant squirrels suggesting that the corpora lutea are a continuing source of progesterone even at a time when pregnancy can continue in their absence.

INTRODUCTION

In mammals such as the mouse, rabbit, hamster and goat, ovariectomy at any time during pregnancy causes abortion or resorption of embryos. In other species including guinea-pigs, cats and women, pregnancy will continue when ovariectomy is performed after a certain stage of gestation. The available evidence has been reviewed by Amoroso & Perry (1977).

In the pregnant grey squirrel the highest levels of plasma progesterone occur after day 30 when the corpora lutea are regressing in size, which may indicate an extra-ovarian source of progesterone. In the present study the effect of removal of the ovaries on the course of gestation has been studied. Since the grey squirrel will not breed in laboratory conditions it was necessary to perform the investigations on squirrels caught in the wild.

MATERIALS AND METHODS

Female squirrels were trapped alive in multicatch traps during the breeding seasons in June 1980 and February 1981. On the day of capture a vaginal smear was taken and a blood sample obtained by cutting off the tip of the tail. Plasma progesterone was then determined by radioimmunoassay (Tait, Pope & Johnson, 1981). Squirrels were diagnosed as pregnant if plasma progesterone concentrations were higher than 3·0 nmol/l. Squirrels were assumed to be in early stages of pregnancy if they showed a metoestrous-type vaginal smear with cornified epithelial cells and many polymorphonuclear leucocytes. The stage of gestation on capture was further estimated from plasma progesterone levels by comparison with levels of female squirrels at known stages of gestation (Tait *et al.* 1981).

Six squirrels diagnosed as being at a stage of mid to late pregnancy were obtained in June 1980. Another six pregnant squirrels including three diagnosed as being in early pregnancy were obtained in February 1981. These 12 squirrels were ovariectomized within one week of capture. For two of these squirrels blood samples were taken and subsequently assayed for progesterone within ten minutes of performing ovariectomy and at weekly intervals thereafter. Following ovariectomy the squirrels were housed individually in weldmesh

cages containing a nest box in a sheltered outside holding. They were exposed to natural climatic conditions except for a cover to protect them from rain. The squirrels were fed on whole wheat grain and had constant access to drinking water. They were examined daily for signs of abortion or birth of young.

Ovariectomy

The squirrels were anaesthetized with a variable mixture of halothane and oxygen. The ovaries were exposed via ventrolateral incisions, the blood supply clamped with artery forceps and removed without ligaturing in order to avoid any damage to the fallopian tubes. At the end of the experiment autopsies were performed on all animals to ensure that no ovarian tissue remained.

Estimation of volume of corpora lutea

At ovariectomy the ovaries were fixed in alcoholic Bouin, embedded in Paraplast (Sherwood Medical), serially sectioned at 8 μm and stained with haematoxylin and eosin. Volume of each corpus luteum (V) was calculated using the formula: $V = (\pi/6)\, d_1 \,.\, d_2 \,.\, d_3$ where d_1 is the product of the number of sections in which a corpus luteum appears and the section thickness, and d_2 and d_3 are the two diameters (μm) measured at right angles in the largest section of the corpus luteum (Rowlands, 1961).

RESULTS

Effect of ovariectomy on continuation of pregnancy

Of the twelve squirrels ovariectomized during pregnancy, nine gave birth to young (Table 1). The squirrels which did give birth were ovariectomized between days 16 and 37 of gestation as calculated from the day of parturition, which was assumed to be day 44 (Smith, 1967). The three squirrels which did not give birth to young were diagnosed as being in early pregnancy at the time of ovariectomy which was performed 2 days after capture. Squirrels numbers 1 and 2 had small corpora lutea in their ovaries which together with the low plasma progesterone levels indicated a gestation of not more than 10 days. The fairly large volume of corpora lutea of squirrel number 3 suggested a pregnancy of more

Table 1. *Estimates of the stage of gestation in pregnant grey squirrels. The day of gestation on which ovariectomy (OVX) was performed was calculated from the observed day of parturition. The day of gestation on which ovariectomy was performed on squirrels 1, 2 and 3 was estimated from the volume of corpora lutea*

Squirrel no.	Plasma progesterone on capture (nmol/l)	Estimated day of gestation on capture	Corpora lutea vol. (mm^3) at OVX (range)	Day of gestation at OVX	Estimated day of gestation at OVX
1	6·4	1–20	0·24–0·26	—	5
2	20·7	1–20	0·45–0·50	—	6–10
3	27·0	6–20	0·64–0·84	—	10+
4*	8·8	1–20	0·69–0·87	16	
5	29·9	6–20	0·60–0·97	18	
6	31·2	6–20	0·60–0·64	20	
7*	20·0	6–20	0·86–0·94	27	
8	49·6	21–25	0·63–0·76	29	
9	45·5	21–25	0·61–0·98	30	
10	46·7	21–25	†	32	
11	82·0	26–30	0·51–0·68	32	
12	123·6	31–35	0·21–0·34	37	

* Weekly blood samples assayed for plasma progesterone.
† Not available.
— Did not give birth.

than 10 days and the plasma progesterone level indicated a pregnancy of not more than 20 days. It was assumed that these three squirrels resorbed their embryos since no evidence of abortion was seen. Plasma progesterone levels of these animals were less than 2·0 nmol/l at autopsy 50 days after capture.

Plasma progesterone levels after ovariectomy

Plasma progesterone levels of squirrels numbers 4 and 7 were 8·8 and 20 nmol/l respectively on the day of capture. Seven days later when ovariectomy was performed the plasma progesterone concentrations obtained ten minutes after operation were 3·2 and 20 nmol/l respectively. Subsequent maximum values of plasma progesterone attained during the continued pregnancy were 87 and 80 nmol/l compared with normal maximum values of 200 nmol/l.

DISCUSSION

The grey squirrel will not breed in laboratory captivity and consequently very little information is available on the course of gestation. Previous studies (Tait *et al.* 1981) suggested that there may be an extra-ovarian source of plasma progesterone in the later stages of gestation, since maximum levels of plasma progesterone were achieved at around day 30 when corpora lutea were regressing in size. In the present study the effect of removal of the ovaries on the course of gestation has been studied.

The length of gestation in the grey squirrel is not known with any accuracy but is usually assumed to be 44 days. This period is based on data from a limited number of observed matings, recording the time between mating and birth (Smith, 1967) or on data recording the time between oestrus and birth (Goodrum, 1940; Brauer & Dusing, 1961). Our observations of squirrels in woodland enclosures based on the time from oestrus to birth support a gestation length of around 44 days and this was used for calculations in the present study.

The stage of gestation was estimated by reference to plasma progesterone levels at the time of capture of squirrels caught in the wild and to volume of corpora lutea at ovariectomy. From the evidence obtained it is clear that pregnancy will continue in the absence of the ovaries after day 16 of gestation. The earliest age during gestation at which the ovaries are essential cannot be concluded with any accuracy. Because of the biphasic pattern of plasma progesterone (Tait *et al.* 1981) the stage of gestation based on plasma progesterone levels is not accurate up to day 20. Nor is it possible to diagnose early pregnancy by palpation because of the extraordinarily strong abdominal musculature in the grey squirrel.

The levels of plasma progesterone appear to decrease after ovariectomy and subsequent levels are much lower than in a normal pregnancy. A similar situation exists in guinea-pigs (Heap & Deanesly, 1966), sheep (Flylling, 1970) and cows (Erb, Gomes, Randel, Estergreen & Frost, 1967). It is therefore tempting to suggest that the corpora lutea normally continue as a source of progesterone even at a time when pregnancy can continue in their absence.

It is not possible to comment on the levels of plasma progesterone necessary to maintain pregnancy in the grey squirrel since plasma progesterone levels were not measured following ovariectomy in those squirrels which did not subsequently give birth. Although a number of questions remain to be answered the present study has demonstrated that the grey squirrel may be added to the list of species in which pregnancy will continue in the absence of the ovaries.

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EVOLUTION IN MENDELIAN POPULATIONS

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THEORIES OF EVOLUTION

One of the major incentives in the pioneer studies of heredity and variation which led to modern genetics was the hope of obtaining a deeper insight into the evolutionary process. Following the rediscovery of the Mendelian mechanism, there came a feeling that the solution of problems of evolution and of the control of the process, in animal and plant breeding

and in the human species, was at last well within reach. There has been no halt in the expansion of knowledge of heredity but the advances in the field of evolution have, perhaps, seemed disappointingly small. One finds the subject still frequently presented in essentially the same form as before 1900, with merely what seems a rather irrelevant addendum on Mendelian heredity.

The difficulty seems to be the tendency to overlook the fact that the evolutionary process is concerned, not with individuals, but with the species, an intricate network of living matter, physically continuous in space-time, and with modes of response to external conditions which it appears can be related to the genetics of individuals only as statistical consequences of the latter. From a still broader viewpoint (compare LOTKA 1925) the species itself is merely an element in a much more extensive evolving pattern but this is a phase of the matter which need not concern us here.

The earlier evolutionists, especially LAMARCK, assumed that the somatic effects of physiological responses of individuals to their environments were transmissible to later generations, and thus brought about a directed evolution of the species as a whole. The theory remains an attractive one to certain schools of biologists but the experimental evidence from genetics is so overwhelmingly against it as a general phenomenon as to render it unavailable in present thought on the subject.

DARWIN was the first to present effectively the view of evolution as primarily a statistical process in which random hereditary variation merely furnishes the raw material. He emphasized differential survival and fecundity as the major statistical factors of evolution. A few years later, the importance of another aspect of group biology, the effect of isolation, was brought to the fore by WAGNER. Systematic biologists have continued to insist that isolation is the major species forming factor. As with natural selection, a connection with the genetics of individuals can be based on statistical considerations.

There were many attempts in the latter part of the nineteenth century to develop theories of direct evolution in opposition to the statistical viewpoint. Most of the theories of orthogenesis (for example, those of EIMER and of COPE) implied the inheritance of "acquired characters." NÄGELI postulated a slow but self contained developmental process within protoplasm; practically a denial of the possibility of a scientific treatment of the problem. Differing from these in its appeal to experimental evidence and from the statistical theories in its directness, was DE VRIES' theory of the abrupt origin of species by "mutations." A statistical process, selec-

tion or isolation, was indeed necessary to bring the new species into predominance, but the center of interest, as with Lamarckism, was in the physiology of the mutation process.

The rediscovery of Mendelian heredity in 1900 came as a direct consequence of DE VRIES' investigations. Major Mendelian differences were naturally the first to attract attention. It is not therefore surprising that the phenomena of Mendelian heredity were looked upon as confirming DE VRIES' theory. They supplemented the latter by revealing the possibilities of hybridization as a factor bringing about an extensive recombination of mutant changes and thus a multiplication of incipient species, a phase emphasized especially by LOTSY. JOHANNSEN'S study of pure lines was interpreted as meaning that DARWIN'S selection of small random variations was not a true evolutionary factor.

A reaction from this viewpoint was led by CASTLE, who demonstrated the effectiveness of selection of small variations in carrying the average of a stock beyond the original limits of variation. This effectiveness turned out to depend not so much on variability of the principal genes concerned as on residual heredity. As genetic studies continued, ever smaller differences were found to mendelize, and any character, sufficiently investigated, turned out to be affected by many factors. The work of NILSSON-EHLE, EAST, SHULL, and others established on a firm basis the multiple factor hypothesis in cases of apparent blending inheritance of quantitative variation.

The work of MORGAN and his school securely identified Mendelian heredity with chromosomal behavior and made possible researches which further strengthened the view that the Mendelian mechanism is the general mechanism of heredity in sexually reproducing organisms. The only exceptions so far discovered have been a few plastid characters of plants. That differences between species, as well as within them, are Mendelian, in the broad sense of chromosomal, has been indicated by the close parallelism between the frequently irregular chromosome behavior and the genetic phenomena of species crosses (FEDERLEY, GOODSPEED and CLAUSEN, etc.). Most of DE VRIES' mutations have turned out to be chromosome aberrations, of occasional evolutionary significance, no doubt, in increasing the number of genes and in leading to sterility of hybrids and thus isolation, but of secondary importance to gene mutation as regards character changes. As to gene mutation, observation of those which have occurred naturally as well as of those which MULLER, STADLER, and others have recently been able to produce wholesale by X-rays, reveals characteristics which seem as far as possible from those required for a directly adaptive evolutionary process. The conclusion nevertheless seems warranted by

the present status of genetics that any theory of evolution must be based on the properties of Mendelian factors, and beyond this, must be concerned largely with the statistical situation in the species.

VARIATION OF GENE FREQUENCY

Simple Mendelian equilibrium

The starting point for any discussion of the statistical situation in Mendelian populations is the rather obvious consideration that in an indefinitely large population the relative frequencies of allelomorphic genes remain constant if unaffected by disturbing factors such as mutation, migration, or selection. If $[(1-q)a+qA]$ represents the frequencies of two allelomorphs, (a, A) the frequencies of the zygotes reach equilibrium according to the expansion of $[(1-q)a+qA]^2$ within at least two generations,[1] whatever the initial composition of the population (HARDY 1908). Combinations of different series are in equilibrium when these are combined at random, but as WEINBERG (1909) and later, in more detail, ROBBINS (1918) have shown, equilibrium is not reached at once but is approached asymptotically through an infinite number of generations. Linkage slows down the approach to equilibrium but has no effect on the ultimate frequencies.

Mutation pressure

The effects of different simple types of evolutionary pressure on gene frequencies are easily determined. Irreversible mutation of a gene at the rate u per generation changes gene frequency (q) at the rate $\Delta q = -uq$. With reverse mutation at rate v the change in gene frequency is $\Delta q = -uq+v(1-q)$. In the absence of other pressures, an equilibrium is reached between the two mutation rates when $\Delta q = 0$, giving $q = v/(u+v)$.

Migration pressure

The frequency of a gene in a given population may be modified by migration as well as by mutation. As an ideal case, suppose that a large population with average frequency q_m for a particular gene, is composed of subgroups each exchanging the proportion m of its population with a random sample of the whole population. For such a subgroup, $\Delta q = -m(q-q_m)$.

The conditions postulated above are rather artificial since, in an actual species, subgroups exchange individuals with neighboring subgroups rather

[1] This statement assumes that there is no overlapping of generations which may bring about some delay in the attainment of equilibrium.

than with a random sample of the whole species and the change in q will be only a fraction of that given above. The fraction is the average degree of departure of the neighboring subgroups toward the population average. The formula may be retained by letting q_m stand for the gene frequency of immigrants rather than of the whole species.

Selection pressure

Selection, whether in mortality, mating or fecundity, applies to the organism as a whole and thus to the effects of the entire gene system rather than to single genes. A gene which is more favorable than its allelomorph in one combination may be less favorable in another. Even in the case of cumulative effects, there is generally an optimum grade of development of the character and a given plus gene will be favorably selected in combinations below the optimum but selected against in combinations above the optimum. Again the greater the number of unfixed genes in a population, the smaller must be the average effectiveness of selection for each one of them. The more intense the selection in one respect, the less effective it can be in others. The selection coefficient for a gene is thus in general a function of the entire system of gene frequencies. As a first approximation, relating to a given population at a given moment, one may, however, assume a constant net selection coefficient for each gene. Assume that the genes a and A tend to be reproduced in the ratio $(1-s):1$ per generation. The gene array $[(1-q)a+qA]$ becomes $[(1-s)(1-q)a+qA]/[1-s(1-q)]$. The change in the frequency of A is $\Delta q = [sq(1-q)]/[1-s(1-q)]$ or with sufficiently close approximation $\Delta q = sq(1-q)$ if the selection coefficient is small.

A second approximation may be obtained by considering the zygotic frequencies. Assume that the types aa, Aa, and AA reproduce in the ratio $(1-s') : (1-hs') : 1$ per generation. The change in the frequency of A to a sufficiently close approximation is $\Delta q = s'q(1-q)[1-q+h(2q-1)]$ (WRIGHT 1929). In the case of selection for or against a complete recessive ($h=0$, s' negative or positive respectively), $\Delta q = s'q(1-q)^2$.

The case of no dominance ($h=\frac{1}{2}$) is the same as the case of genic selection except that the selection against the gene is $s'/2$ instead of s.

The two factor case in which the phenotypes aabb, $aaB-$, $A-bb$ and $A-B-$ reproduce at the rates $(1-s_{ab}):(1-s_a):(1-s_b):1$ respectively yields (for low values of the selection coefficients):

$$\Delta q_A = q_A(1 - q_A)^2[s_a + (s_{ab} - s_a - s_b)(1 - q_B)^2].$$

The frequency of A depends on the frequency and selection of B, becom-

ing independent only if $s_{ab} = s_a + s_b$, that is, if the two series of genes are cumulative with respect to selection. It does not seem profitable to pursue this subject further for the purpose of the present paper, since in the general case, each selection coefficient is a complicated function of the entire system of gene frequencies and can only be dealt with qualitatively. Attention may, however, be called to HALDANE'S (1924–1927) studies of selection rates and of the consequent number of generations required for unopposed selection to bring about any required change in gene frequency under various assumptions with respect to mode of inheritance and system of mating.

Equilibrium under selection

There may be equilibrium between allelomorphs as a result wholly of selection, namely, selection against both homozygotes in favor of the heterozygous type. Putting $\Delta q = s'q(1-q)[1-q+h(2q-1)] = 0$ gives $q = [1-h]/[1-2h]$ as the condition.[2] This includes the case of selection against both homozygotes and also that in favor of them, but examination of the signs of Δq above and below the equilibrium point shows that only the former is in stable equilibrium in agreement with FISHER (1922). The linkage of a favorable dominant with an unfavorable recessive of another series is a case in which selection would be against both homozygotes as JONES (1917) has pointed out, and stressed as a factor in the vigor of heterozygosis. In a population produced by the intermingling of types in which different deleterious recessives have become fixed, there will be a temporary selection in favor of the heterozygotes even without any linkage at all. Unless the linkage is very strong, however, this effect does not persist long enough to have much effect on gene equilibrium. The extreme case of equilibrium of the sort discussed here is, of course, that of balanced lethals, found in nature in Oenothera.

In the two factor case, discussed in the preceding section,

$$\Delta q_A = 0 \text{ if } q_B = 1 - \sqrt{\frac{s_a}{s_a + s_b - s_{ab}}}$$

$$\text{and } \Delta q_B = 0 \text{ if } q_A = 1 - \sqrt{\frac{s_b}{s_a + s_b - s_{ab}}}.$$

[2] The condition can be expressed in a more symmetrical form by using a different form of statement of the selection coefficients. Assume that the rates of reproduction of the three types aa, Aa and AA are as $(1-s_a):1:(1-s_A)$. The value of q at equilibrium comes out $q = \frac{s_a}{s_A + s_a}$ with stable equilibrium only for positive values of the two selection coefficients.

There may be equilibrium here, if s_a and s_b are alike in sign, and s_{ab} is either opposite in sign or of the same sign and smaller, but it is an unstable equilibrium. Of more general importance, perhaps, is the equilibrium reached by a deleterious mutant gene. For mutation opposed by genic selection $\Delta q = -uq+sq(1-q)=0$, $q=1-u/s$. For mutation opposed by zygotic selection (aa, Aa and AA reproducing at rates $(1-s'):(1-hs'):1$ it is easily shown that $q=1-u/hs'$ (WRIGHT 1929), unless h approaches 0. Thus with no dominance, $q=1-2u/s'$, and for selection against a dominant mutation, $q=1-u/s'$. The important case of selection against a recessive is that in which $h=0$. The formula becomes $q=1-\sqrt{u/s'}$. All of these cases are illustrated in figure 1 in which the ordinates show the selection pressure as related to factor frequency, under the different conditions of selection. The intersections with the straight line representing mutation pressure give the points of equilibrium. If deleterious dominant and recessive mutations occur with equal frequency and are subject to the same degree of selection, the frequency of the recessive mutant genes will be greater than that of the dominant ones in the ratio $\sqrt{u/s'}$ to u/s'. The corresponding figure for factors lacking dominance is $2u/s'$, where s' is the selection against the homozygote. These considerations alone should lead to a marked correlation in nature between recessiveness and deleterious effect. This correlation is further increased by the greater frequency of recessive mutation which seems to be a general phenomenon. It is this correlation which gives the theoretical basis for the immediate degeneration which usually accompanies inbreeding, a process which increases the proportion of recessive phenotypes.

The amount to which gene frequency in a subgroup may depart from the species average as a result of local selection held in check by population interchange with other regions may be calculated by solving the quadratic $\Delta q = sq(1-q)-m(q-q_m)=0$. If the local selection coefficient is much greater than the proportion of migration ($s > m$), $q=1-\frac{m}{s}(1-q_m)$ or $-mq_m/s$ depending on the direction of selection, formulae analogous to those for the equilibrium between mutation and selection. If, on the other hand, selection is weak compared with migration ($s < m$), the departure from q_m is small and $q=q_m[1+\frac{s}{m}(1-q_m)]$. This case is doubtless the more important in nature. Large subgroups living under different selection pressures should show gene frequencies clustering about the average according to this expression. The effect of small size of the subgroups in bringing about random deviation in this and other cases is not here con-

sidered. The case in which s and m are of the same order of magnitude may be illustrated by the case of exact equality. Here $q = \sqrt{q_m}$ or $1 - \sqrt{1 - q_m}$ depending on the direction of selection.

Multiple allelomorphs

The foregoing discussion has dealt formally only with pairs of allelomorphs, a wholly inadequate basis for consideration of the evolutionary pro-

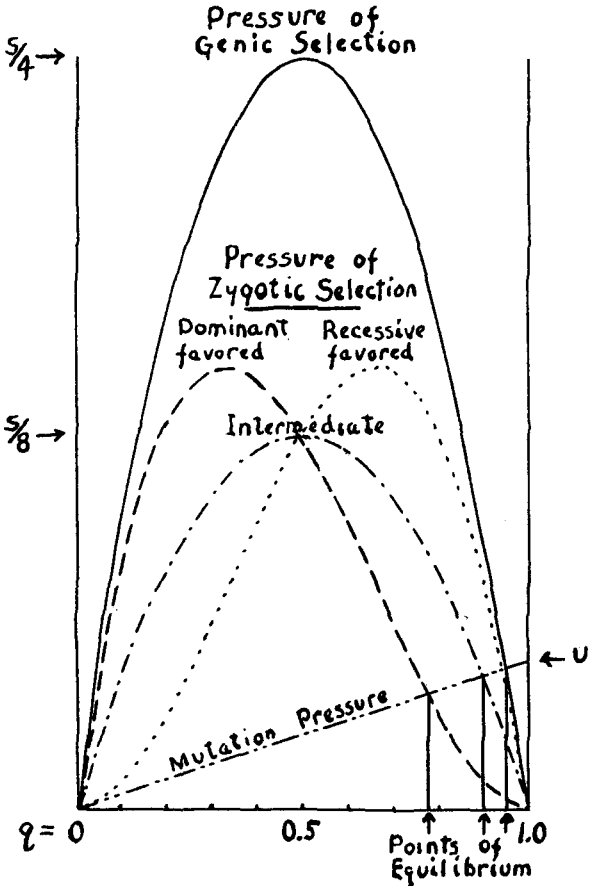


FIGURE 1.—Rate of change of gene frequency under selection or mutation. Genic selection (A, a reproducing at rates 1:1−s); Zygotic selection: dominant (B-, bb at rates 1:1−s), recessive (cc, C− at rates 1:1−s), intermediate (DD, Dd, dd at rates $1:1-\frac{1}{2}s:1-s$), Mutation such that $u = -0.05$ s. Intersections of line of mutation pressure with those for selection pressure determine the equilibrium frequencies.

cess unless extension can be made to multiple allelomorphs. Among the laboratory rodents some 40 percent of the known series of factors affect-

ing coat color are already known to be multiple. The number of multiple series is large in other organisms, for example, Drosophila (MORGAN, STURTEVANT and BRIDGES 1925). It is not unlikely that further study will indicate that all series are potentially multiple. In this case, each gene has a history which is not a mere oscillation between approximate fixation of two conditions but a real evolutionary process in its internal structure. Presumably any particular gene of such an indefinitely extended series can arise at a step from only a few of the others[3] and in turn mutate to only a few. Since genes as a rule have multiple effects and change in one effect need not involve others, it is probable that in time a gene may come to produce its major effects on wholly different characters than at first. Continuing this line of thought, it indeed seems possible that all genes of all organisms may ultimately be traced to a common source, mitotic irregularities furnishing the basis for multiplication of genes.

The relative frequencies of all allelomorphs in a series tend, of course, to remain constant in the absence of disturbing forces. The zygotes reach the equilibrium of random combination of the genes in pairs by the second generation from any initial constitution of the population. The effects of the various kinds of evolutionary pressure on the frequency of each gene may be treated as before by contrasting each gene with the totality of its allelomorphs. In the binomial expression $[(1-q)a+qA]$, A may be understood as representing any gene, and a as including all others of its series. Such treatment, however, requires further qualification with regard to the constancy of the various coefficients. It may still be assumed that the rate (u) of mutational breakdown of the gene in question (A) is reasonably constant, but its rate (v) of mutational origin from allelomorphs must be expected to change. This may be expected to rise to a maximum, as genes closely allied to A in structure become frequent, and to fall off to zero as changes accumulate in the locus. Even at its maximum, however, the rate of formation should in general be of the second order compared with the rate of change to something else, simply because it is one and its alternatives many. Moreover, there is an indication that the genes which become more or less established in a population are not a random sample of the types of mutations which occur. It has been the common experience that mutations are usually recessive. Recessiveness is most simply interpreted physiologically as due to inactivation which may well be the commonest type of mutational change. But the evolutionary process presumably involves in-

[3] Those most closely related genetically, however, need not always be closest in effect. The complete inactivation of a gene in a particular respect may for example occur more freely than a partial inactivation.

crease in activity of genes at least as frequently as inactivation with the consequence that the rate of formation (v) of genes of evolutionary significance becomes negligibly small in comparison with rate of breakdown (u) of such genes. It should be said that FISHER has advanced an alternative hypothesis according to which genes originally without dominance become dominant through a process of selection of modifiers (FISHER 1928, 1929, WRIGHT 1929, 1929a).

The selection coefficient, s, relating to a gene A cannot be expected to be constant if the alternative term a includes more than one gene. The coefficient should rise to a maximum positive value as A replaces less useful genes but should fall off and ultimately become negative as the group of allelomorphs comes to include still more useful genes. But as already discussed, even if A has only one allelomorph, the dependence of the selection coefficient on the frequencies and selection coefficients of non-allelomorphs keeps it from being constant. The existence of multiple allelomorphs merely adds another cause of variation.

Random variation of gene frequency

There remains one factor of the greatest importance in understanding the evolution of a Mendelian system. This is the size of the population. The constancy of gene frequencies in the absence of selection, mutation or migration cannot for example be expected to be absolute in populations of limited size. Merely by chance one or the other of the allelomorphs may be expected to increase its frequency in a given generation and in time the proportions may drift a long way from the original values. The decrease in heterozygosis following inbreeding is a well known statistical consequence of such chance variation. The extreme case is that of a line propagating by self fertilization which may be looked upon as a self contained population of one. In this case, 50 percent of the factors with equal representation of two allelomorphs (that is, in which the individual is heterozygous) shift to exclusive representation of one of the allelomorphs in the following generation merely as a result of random sampling among the gametes. From the series of fractions given by JENNINGS (1916) for the change in heterozygosis under brother-sister mating (population of two) it may be deduced that the rate of loss in this case is a little less than 20 percent per generation. A general method for determining the decrease in heterozygosis under inbreeding has been presented in a previous paper (WRIGHT 1921). It can be shown from this that there is a rate of loss of about 1/2N in the case of a breeding population of N individuals whether equally divided between males and females or composed of monoecious individ-

uals, assuming pairs of allelomorphs. HAGEDOORN (1921) has urged the importance of such random fixation as a factor in evolution.

Another phase of this question was opened by FISHER (1922) who attempted to discover the distribution of gene frequencies ultimately reached in a population as a result of the above process. He studied a number of conditions relative to mutation and selection. He does not state the rate of decrease in heterozygosis (where any) which would follow from the solutions which he reached but this can be deduced very directly from them. It comes out 1/4N for a population of N breeding individuals in the absence of selection or mutation. This is just half the rate indicated by the method referred to above.

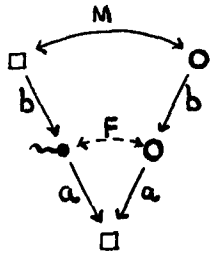


FIGURE 2.

Rate of decrease in heterozygosis

The following symbols and formulae were used in the previous paper in determining the consequences of systems of inbreeding. Primes were used to indicate the number of generations preceding the one in question. Only pairs of allelomorphs are considered here.

M correlation between genotypes of mates

b $(=\sqrt{\tfrac{1}{2}(1+F')})$ path coefficient, zygote to gamete

a $\left(=\sqrt{\dfrac{1}{2(1+F)}}\right)$ path coefficient, gamete to fertilized egg

F $(=b^2M)$ correlation between uniting egg and sperm, also, total proportional change in heterozygosis.

P $(=2q(1-q)(1-F))$ proportion of heterozygosis.

The general formula for the correlation between uniting gametes is easily deduced and has been used as a coefficient of inbreeding in dealing with complex livestock pedigrees (WRIGHT 1922, 1923, 1925, MCPHEE and WRIGHT 1925, 1926),

$$F = \Sigma\left[\left(\tfrac{1}{2}\right)^{n_s+n_d+1}(1 + F_A)\right].$$

Here F_A is the coefficient of inbreeding of any common ancestor that

makes the connecting link between a line of ancestry tracing back from the sire and one tracing back from the dam. The numbers of generations from sire and dam to such a common ancestor are designated n_s and n_d respectively. The contribution of a particular tie between the pedigrees of sire and dam is $(\frac{1}{2})^{n_s+n_d+1}(1+F_A)$ and *the total coefficient is simply the sum* of all such contributions. This formula makes it possible to compare quantitatively the statistical situation in actual populations with that in ideal populations.

In dealing with regular systems of mating the method of analysis consists in expressing the correlation between mated individuals in terms of path coefficients and correlations pertaining to the preceding generation ($M=\phi(a,'b,'M')$ and from this obtaining expression for F in terms of the F's of the preceding generations.

Consider a population in which there are N_m breeding males and N_f breeding females, and random mating. The proportion of matings between full brother and sister will be $1/(N_mN_f)$, that between half brother and sister $(N_m+N_f-2)/(N_mN_f)$, and that between less closely related individuals $(N_m-1)(N_f-1)/(N_mN_f)$. The correlation between mated individuals may be written as follows, giving due weight to these three possibilities:

$$M = a'^2b'^2\left[\frac{1}{N_mN_f}(2+2M') + \frac{N_m+N_f-2}{N_mN_f}(1+3M') + \frac{(N_m-1)(N_f-1)}{N_mN_f}4M'\right].$$

This leads to the following formula for proportional change in heterozygosis since the foundation period:

$$F = F' + \left(\frac{N_m+N_f}{8N_mN_f}\right)(1-2F'+F'').$$

The proportion of heterozygosis may be written, relative to that of preceding generations:

$$P = P' - \left(\frac{N_m+N_f}{8N_mN_f}\right)(2P'-P'').$$

It is to be expected that the proportional change per generation will reach approximate constancy. This rate may be found by equating P/P' to P/P''

$$-\frac{\Delta P}{P'} = \tfrac{1}{2}\left(1+\frac{N_m+N_f}{4N_mN_f}\right) - \tfrac{1}{2}\sqrt{1+\left(\frac{N_m+N_f}{4N_mN_f}\right)^2}.$$

This gives $(1/8N_m+1/8N_f)(1-1/8N_m-1/8N_f)$ as a close approximation even for the smallest populations while for reasonably large ones the form $1/8N_m+1/8N_f$ is sufficiently accurate.

The simplest case is that of continued mating of brother with sister ($N_m=N_f=1$). The rate of loss of heterozygosis comes out $\frac{1}{4}(3-\sqrt{5})$ or 19.1 percent per generation. The formula for proportion of heterozygosis takes the form $P=\frac{1}{2}P'+\frac{1}{4}P''$ as given in the previous paper, with results in exact agreement with those derived by JENNINGS (1916) by working out in detail the consequences of every possible mating from generation to generation.

Another simple case is that in which one male is mated with an indefinitely large number of half-sisters. This is approximately the system of breeding continuously within one herd, headed always by just one male. In this case $N_m=1$, $N_f\doteq\infty$, with rate of loss of heterozygosis of 11.0 percent per generation in agreement with previous results (WRIGHT 1921).

With a relatively limited number of males but unlimited number of females, the rate becomes approximately $1/8N_m$.

An especially important case is that in which the population is equally divided between males and females. Here $N_m=N_f=\frac{1}{2}N$ and the rate of loss is approximately $1/2N$ (or somewhat more closely $1/(2N+1)$) where N is the total size of the breeding population.

It is not, perhaps, clear at first sight that a population of N monoecious organisms, in which self fertilization is prevented, should show a decrease in heterozygosis exactly equal to that in a population of the same size equally divided between males and females. The chance that uniting gametes come from full sisters is $2/[N(N-1)]$, the chance that they come from half sisters is $4(N-2)/[N(N-1)]$ while the chance that they come from less closely related individuals is $(N-2)(N-3)/[N(N-1)]$ giving

$$M=\frac{a'^2b'^2}{N(N-1)}[2(2+2M')+4(N-2)(1+3M')+(N-2)(N-3)4M']$$

$$P=P'-\frac{1}{2N}(2P'-P'')$$

exactly as in the preceding case.

The somewhat arbitrary case in which the gametes produced by N monoecious individuals unite wholly at random is that which can be compared directly with FISHER'S results. The gametes have a chance $1/N$ of coming from the same individual and of $(N-1)/N$ of coming from different individuals. The correlation between uniting gametes may thus be written

$$F = \frac{1}{N}b^2 + \left(\frac{N-1}{N}\right)4b^2a'^2F'$$

$$P = \frac{(2N-1)}{2N}P'.$$

As might be expected, the result does not differ appreciably from that of the preceding case. The rate of loss of heterozygosis is exactly 1/2N instead of merely approximately this figure. The simplest special case is, of course, continued self fertilization in which N = 1 and the formula gives the obviously correct result of 50 percent loss of heterozygosis per generation.

From the mode of analysis it might be thought that the loss in heterozygosis is wholly the consequence of the occasional matings between very close relatives. This, however, is not the case. If instead of random sampling of the gametes produced by the population it is assumed that all individuals reproduce equally and that inbreeding is consistently avoided as much as possible, the percentage of heterozygosis still falls off. The rate of loss is, however, only about half as rapid (approximately 1/4N) in a reasonably large population equally divided between males and females. The cases of N = 2, 4, 8 and 16 have been given previously (WRIGHT 1921).

In dealing with heterozygosis in the foregoing, it has been assumed for simplicity that each locus was represented by only two allelomorphs in the population in question and that either complete fixation or complete loss of a particular gene means homozygosis of all individuals with respect to the locus. But in any case beyond that of self fertilization, more than two allelomorphs may be present and complete loss of the gene no longer implies homozygosis of the locus. The initial rate of loss of heterozygosis in a large population may thus be only 1/4N with gradual approach to 1/2N as the number of loci with only two remaining allelomorphs increases. The rate of decay of the distribution of gene frequencies is 1/2N regardless of number of allelomorphs.

The population number

It will be well to discuss more fully, before going on, what is to be understood by the symbol N used here for population number. The conception is that of two random samples of gametes, N sperms and N eggs, drawn from the total gametes produced by the generation in question (N/2 males and N/2 females each with a double representation from each series of allelomorphs). Obviously N applies only to the breeding population and not to the total number of individuals of all ages. If the population fluctu-

ates greatly, the effective N is much closer to the minimum number than to the maximum number. If there is a great difference between the number of mature males and females, it is closer to the smaller number than to the larger. In fact, as just shown, a population of N_m males and an indefinitely large number of females is approximately equivalent to a population of $4N_m$ individuals equally divided between males and females.

The conditions of random sampling of gametes will seldom be closely approached. The number of surviving offspring left by different parents may vary tremendously either through selection or merely accidental causes, a condition which tends to reduce the effective N far below the actual number of parents or even of grandparents. How small the effective N of a population may be is indicated by recent studies of SMITH and CALDER (1927) on the Clydesdale breed of horses in Scotland, in which they find a steady increase in the degree of inbreeding (Coefficient F) equivalent to that in population headed by only about a dozen stallions. Even more striking is the rapid increase in the coefficient of inbreeding in the early history of the Shorthorn breed of cattle (MCPHEE and WRIGHT 1925).

THE DISTRIBUTION OF GENE FREQUENCIES AND ITS IMMEDIATE CONSEQUENCES

No mutation, migration or selection

On making a cross between two homozygous strains a population is produced in which the members of each pair of allelomorphs in which the strains differ are necessarily equally numerous. The proportional frequency of each allelomorph in unfixed series is $q = 0.50$. In an indefinitely large population, there should be no change in this frequency in later generations (except by recurrent mutation or selection). In any finite population, however, some genes will come to be more frequent than their allelomorphs merely by chance. This means a decrease in heterozygosis, since the proportion of heterozygosis under random mating is $2q(1-q)$, and this quantity is maximum when $q = 0.50$. As time goes on, divergences in the frequencies of factors may be expected to increase more and more until at last some are either completely fixed or completely lost from the population. The distribution curve of gene frequencies should, however, approach a definite form if the genes which have been wholly fixed or lost are left out of consideration. This can easily be seen by considering a case opposite in a sense to that considered above. Suppose that a large number of different mutations occur in a previously pure line. The frequency ratio of mutant to type allelomorphs is initially $(1/2N):(2N-1)/2N$ where N is the number of individuals. The great majority of such muta-

tions will be lost, by the chances of sampling, as FISHER (1922) points out. Those which persist are largely those for which there has been a chance increase in frequency. The distribution curve of frequencies of persisting mutations will thus continually spread toward higher frequencies. There must be a position of equilibrium as far as form is concerned between this situation and that first considered, although a uniform decline in absolute numbers.

As noted above, decrease in heterozygosis takes place in the early generations following a cross without any appreciable fixation or loss of genes. But after equilibrium has been reached in the form of the distribution curve, further loss in heterozygosis must be identical in rate with fixation plus loss.

In simple cases, the equilibrium distribution of gene frequencies can easily be worked out directly. Under brother-sister mating, for example, the following relative frequencies of the 4 possible types of mating involving unfixed factors are in equilibrium although the absolute frequencies of all are falling off 19.1 percent ($=\frac{1}{4}(3-\sqrt{5})$) each generation as new genes enter the fixed states, AA × AA or aa × aa.

Mating	*Relative Frequency*
	Percent
AA×Aa	$7-3\sqrt{5}=$ 29.2
Aa ×Aa	$-22+10\sqrt{5}=$ 36.1
AA×aa	$9-4\sqrt{5}=$ 5.6
Aa ×aa	$7-3\sqrt{5}=$ 29.2
	100.1

Similarly in populations of 2 and 3 monoecious individuals with random union of gametes, the following relative frequencies are in equilibrium although the absolute frequencies are decreasing in each generation by exactly 25 percent and 16⅔ percent respectively verifying the 1/2N of theory.

Gene Frequency	*Class Frequency*	
	Percent	
3A:1a	32	Case of 2 monoecious
2A:2a	36	individuals per gen-
1A:3a	32	eration
Gene Frequency	*Class Frequency*	
	Percent	
5A:1a	18.3	
4A:2a	21.0	Case of 3 monoecious
3A:3a	21.4	individuals per gen-
2A:4a	21.0	eration
1A:5a	18.3	
	100.0	

In order to determine generally the distribution of gene frequencies, consider the way in which genes (A) with frequency q are distributed after one generation of random mating. In a population of N breeding individuals, each of the specified genes will have 2Nq representatives among the zygotes and their allelomorphs $2N(1-q)$. A random sample of the same size will be distributed according to the expression $[(1-q)a+qA]^{2N}$. The contribution of this sample to the frequency class with an allelomorphic ratio of $q_1:(1-q_1)$ will be in proportion to the $2Nq_1$'th term of the above expression and to the number of genes included in the contributing class (f). The sum of contributions from all such classes should give the $2Nq_1$'th term an absolute frequency smaller than its value in the preceding generation (f_1) by the amount $1/(2N+1)$ deduced above. Following is the equation to be solved for f as a function of q.

$$f_1\left(1-\frac{1}{2N+1}\right)=\frac{\lfloor 2N}{\lfloor 2Nq_1 \ \lfloor 2N(1-q_1)}\Sigma q^{2Nq_1}(1-q)^{2N(1-q_1)}f$$

Replacing summation by integration and letting $f=\phi(q)/2N=\phi(q)dq$ we have[4]

$$\frac{\phi(q_1)}{2N+1}=\frac{\lfloor 2N}{\lfloor 2Nq_1 \ \lfloor 2N(1-q_1)}\int_0^1 q^{2Nq_1}(1-q)^{2N(1-q_1)}\phi(q)dq.$$

[3]The cases of 2 and 3 monoecious individuals as worked out by simple algebra suggest an approach to a uniform distribution. As a trial let $\phi(q)=C$. It will be found that this makes the right and left members of the equation identical and is thus a solution.

$$\frac{C}{2N+1}=\frac{C\lfloor 2N}{\lfloor 2Nq_1 \ \lfloor 2N(1-q_1)}\frac{\Gamma(2Nq_1+1)\Gamma(2N-2Nq_1+1)}{\Gamma(2N+2)}=\frac{C}{2N+1}.$$

The case of loss at rate 1/2N should not differ appreciably from that at rate $1/(2N+1)$. It would appear that after a cross, the gene frequencies will spread out from 50 percent toward fixation and loss until a practically uniform distribution is reached. The frequencies of all classes will then slump at a rate of about 1/2N as 1/4N of the genes become fixed and the same number lost per generation. Figure 3 is intended to illustrate this situation.

[4] f must be equated to $\phi(q)/2N$ here, rather than $\phi(q)/(2N-1)$, if the convenient limits 0 and 1 are to be used for integration in place of the limits 1/2N and $(2N-1)/2N$ of the summation with its $2N-1$ terms.

Before finally accepting this solution, however, it will be well to examine the terminal conditions. The amount of fixation at the extremes if N is large can be found directly from the Poisson series according to which the chance of drawing 0 where m is the mean number in a sample is e^{-m}. The contribution to the 0 class will thus be $(e^{-1}+e^{-2}+e^{-3}\cdots)f = \frac{e^{-1}}{1-e^{-1}} f, = 0.582f.$

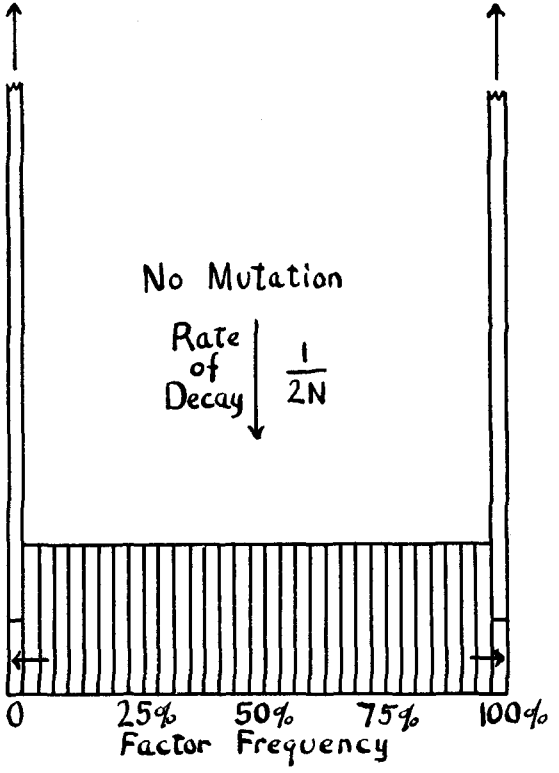


FIGURE 3.—Distribution of gene frequencies in an isolated population in which fixation and loss of genes each is proceeding at the rate 1/4N in the absence of appreciable selection or mutation. $y = L_0 e^{-T/2N}$.

This is a little larger than the $\frac{1}{2}f$ deduced above and indicates a small amount of distortion near the ends due to the element of approximation involved in substituting integration for summation. The nature and amount of this distortion are indicated by the exact distributions obtained in the extreme cases of only 2 and 3 monoecious individuals.

Letting L_0 be the initial number of unfixed loci (pairs of allelomorphs) and T the number of generations we have approximately

Unfixed loci in the T 'th generation $L_T = L_0 e^{-T/2N}$

An analogous formula holds for genes in multiple series, but in this case, as previously noted, the rate of fixation of loci is only half that given above.

The amount of genetic variation with respect to cumulative characters is easily calculated assuming for simplicity pairs of allelomorphs. The contribution of each factor pair to variance, in the case of no dominance, is $2a^2q(1-q)$ where a is the average difference in effect between plus and minus allelomorphs. The general formula for variance in this or any other distribution is thus $\sigma^2 = 2a^2\int_0^1 q(1-q)\phi(q)dq$. In the present case in which $\phi(q) = L$ this reduces to $\sigma^2 = \frac{1}{3}La^2$. PEARSON'S β_2 comes out with a value 2.8 a slightly platykurtic distribution. Since the percentage of heterozygosis for a given factor frequency, q, is $2q(1-q)$, the formula for heterozygosis is the same as that for variance except that a^2 is to be omitted.

Similarly in the case of dominance, the contribution of a single factor pair to variance is $4a^2(1-q)^2(2q-q^2)$ where a is half the average difference in effect between dominant and recessive zygotes. The total variance with perfect dominance is thus in general

$$\sigma^2 = 4a^2 \int_0^1 (1 - q)^2(2q - q^2)\phi(q)dq.$$

In the case of a uniform distribution this gives $\sigma^2 = 8/15\ La.^2$

FISHER (1918) has emphasized the importance of a characteristic of the population which he calls the dominance ratio. He analyzes the variance of characters into three portions, that due to genetic segregation (τ^2) that due to dominance, as something which causes deviations of the phenotype from the closest possible linear relation with the genotype (ϵ^2), and that due to environment. Assuming environment constant, $\sigma^2 = \tau^2 + \epsilon^2$. The simple formulae for the correlations between relatives, to be found if there is random mating and no dominance, must be modified, if dominance is present depending on the value of the dominance ratio defined as ϵ^2/σ^2. Following are examples which he gives:[5]

[5] The author wishes here to correct an error in his 1921 paper which was written without knowledge of FISHER'S results cited above. In this paper it was assumed that the correlation with no dominance needed merely to be multiplied by the squared correlation between genotype and phenotype, the same as FISHER'S $\tau^2/\sigma^2 = (1-\epsilon^2/\sigma^2)$, to obtain that with dominance. This gives correct results (if there is no assortative mating) in the case of offspring with parents, all other ancestors and also in the case of collaterals where one of the individuals is related to the other through only one parent but it is more or less in error in other cases, the fraternal correlation being that most affected. The reasoning followed was not exact because a correlation in the deviations due to dominance in the cases indicated was overlooked.

	Correlation	
	No dominance	*Dominance*
Parent and offspring	$\frac{1}{2}$	$\frac{1}{2}(1-(\epsilon^2/\sigma^2))$
Brothers	$\frac{1}{2}$	$\frac{1}{2}(1-(\epsilon^2/2\sigma^2))$
Uncle and nephew	$\frac{1}{4}$	$\frac{1}{4}(1-(\epsilon^2/\sigma^2))$
Double first cousins	$\frac{1}{4}$	$\frac{1}{4}(1-(3\epsilon^2/4\sigma^2))$

FISHER has shown that the contribution of a single factor to ϵ^2, if there is complete dominance, may be written $\delta^2 = 4q^2(1-q)^2a^2$ where q is the frequency for dominant allelomorphs. Whether a particular dominant gene has a plus or minus effect on the character under consideration is immaterial. The contribution due to genetic segregation he gives as $\beta^2 = 8q(1-q)^3a^2$ thus

$$\frac{\epsilon^2}{\sigma^2} = \frac{\Sigma\delta^2}{\Sigma(\delta^2+\beta^2)} = \frac{\int_0^1 q^2(1-q)^2\phi(q)dq}{2\int_0^1 q(1-q)^3\phi(q)dq + \int_0^1 q^2(1-q)^2\phi(q)dq}.$$

In the present case this reduces to $\frac{1}{4}$ as given by FISHER who also obtains a uniform distribution of factor frequencies for the case of no mutation or selection, although a different rate of decay.

Nonrecurrent mutation

If mutation is occurring, however low the rate, the decline in heterozygosis, following isolation of a relatively small group from a large population, cannot go on indefinitely. There will come a time when the chance elimination of genes will be exactly balanced by new genes arising by mutation. The equation to be solved is obviously as follows:

$$\frac{\phi(q_1)}{2N} = \frac{\lfloor 2N}{\lfloor 2Nq_1 \ \lfloor 2N(1-q_1)} \int_0^1 q^{2Nq_1}(1-q)^{2N(1-q_1)}\phi(q)dq.$$

It may be found by trial that the expression $\phi(q) = C_1q^{-1} + C_2(1-q)^{-1}$ is a solution. The terminal condition, reduction of the class of fixed genes ($q=1$) by an occasional mutation (contributing to the class $q=(2N-1)/2N$ necessarily involves the appearance of new genes (contributing to the class $q=1/2N$) and therefore means that only the symmetrical solution $\phi(q) = Cq^{-1}(1-q)^{-1}$ can be accepted as descriptive of the distribution of the entire array of genes at equilibrium (under the rather arbitrary postulated condition, no selection, no migration, no recurrence of the same mutations). Letting $f = (C/2N)\ q^{-1}(1-q)^{-1}$ and making $\sum f = 1$,

$$C = \frac{1}{2[(0.577 + \log(2N-1)]}$$

or approximately $C = 1/(2 \log 3.6N)$ (compare figure 5).

Before attainment of equilibrium with respect to heterozygosis the distribution will pass through phases of approximately the form $\phi(q) = C_1 q^{-1}(1-q)^{-1} + C_3$ in which the term C_1 gradually displaces C_3 as the number of temporarily fixed genes approaches equilibrium with mutation.

Each particular gene has a probability distribution for the future which spreads in time from the initial frequency in curves which are at first approximately normal in form but later (if the initial q was not too close to 1) become flat, the chances of complete fixation or complete loss each increasing by 1/4N each generation. As the chances of complete fixation increase, the chance of mutation must be taken into account. The distribution passes through phases of the type $C_2(1-q)^{-1} + C_3$, C_2 gradually displacing C_3, relatively, but itself beginning to decline as the chance of complete loss increases. With initial q equal (or close) to 1, equilibrium with mutation, and hence the hyperbolic distribution, is reached directly. The ultimate result in any case is complete loss of the gene in question (still assuming no recurrence of the same mutation and hence mutation *of* the gene but not *to* it). If there is reverse mutation, but at very low rate, a term $C_1 q^{-1}$ must be added to the formula, and an equilibrium will be reached in the form $Cq^{-1}(1-q)^{-1}$. This last formula means that in the long run (assuming no disturbances from selection, migration, etc.) the gene will usually be found either completely fixed or completely absent from the population (with frequencies proportional to the mutation rates to and from the gene respectively) but that occasionally fixation or absence will not be quite complete and that at extremely rare intervals the gene will drift from one state to the other.

The turnover among genes in equilibrium in the distribution $Cq^{-1}(1-q)^{-1}$ can be determined from consideration of the variance of q, and independently by application of the Poisson law.

Let $\sigma_q^2 = \Sigma(q - \frac{1}{2})^2 f / \Sigma f$ be the variance of q, excluding the terminal classes, the summation including $2N-1$ terms. This variance is increased in the following generation by the spreading out of each frequency class as a result of random sampling. The variance from the spreading of a single class is $q(1-q)/2N$ and the average is thus

$$\Delta\sigma_q^2 = \frac{\Sigma q(1-q)f}{2N\Sigma f} = \frac{1}{2N}\left(\frac{1}{4} - \sigma_q^2\right) = \frac{2N-1}{(2N)^2}C.$$

The sum $\sigma_q^2+\Delta\sigma_q^2$ includes the newly fixed factors whose contribution is $\frac{1}{4}K$ where K is the rate of fixation, plus loss, but excludes mutation.

Digressing for a moment to the case of no mutation but equilibrium of form, we have at once

$$\sigma_q^2 + \Delta\sigma_q^2 = K\tfrac{1}{4} + (1 - K)\sigma_q^2$$

$\left(K-\frac{1}{2N}\right)\left(\sigma_q^2-\frac{1}{4}\right) = 0$ giving an independent demonstration that the rate of decay is $1/2N$ in this case.

Returning to the case of equilibrium under mutation, the contribution of new mutations to variance is $K(N-1)^2/(2N)^2$.

$$\sigma_q^2 + \Delta\sigma_q^2 - \tfrac{1}{4}K + K\left(\frac{N-1}{2N}\right)^2 = \sigma_q^2$$

$$K = C = \frac{1}{2\log 3.6N}.$$

The proportion exchanged at each extreme is thus about half the corresponding subterminal class where N is large ($f_1 = f_{2N-1} = 2NC/(2N-1)$ by this method. This compares fairly well with the proportion as determined by the Poisson law, which is 0.46 times the subterminal class instead of 0.50.

The equilibrium frequencies can be worked out algebraically in simple cases. The figures below give the results in the case of a population of 3 monoecious individuals for comparison with the theoretical values deduced above. The rate of exchange at each extreme is actually 10.8 percent in comparison with 11.0 percent as $\frac{5}{12}\left(=\frac{2N-1}{4N}\right)$ the subterminal class, or 11.4 percent from the formula $\frac{1}{4(.577+\log 5)}$. The case of irreversible mutation is also given.

	Reversible Mutation		*Irreversible Mutation*	
Gene frequency	*Exact equilibrium*	$Cq^{-1}(1-q)^{-1}$	*Exact*	$C(1-q)^{-1}$
5A:1a	27.5	26.3	47.7	43.8
4A:2a	15.4	16.4	20.6	21.9
3A:3a	14.1	14.6	14.1	14.6
2A:4a	15.4	16.4	10.2	10.9
1A:5a	27.5	26.3	7.3	8.8
Totals	99.9	100.0	99.9	100.0
Terminal exchange	10.8	11.0	18.0	18.25
Loss			3.6	3.65

The number of unfixed loci (L) which a given mutation rate per individual (μ) will support in a population is easily found, assuming only pairs of allelomorphs. The number of mutations is KL as well as $N\mu$. Therefore $L = N\mu/K = 2N\mu \log 3.6N$. The variance of cumulatively determined characters worked out as in the preceding case comes out $2N\mu a^2$ in the case of no dominance and $10/3\ N\mu a^2$ in the case of dominance, in both cases, directly proportional to the size of population[6] and to the mutation rate. In view of the piling up of new mutations, one might perhaps, expect to find a leptokurtic distribution for characters. This, however, turns out not to be the case: PEARSON'S β_2 comes out exactly 3 in the case of no dominance on substitution in the general formula

$$\beta_2 = 3 + \frac{\int_0^1 q(1-q)[1-6q(1-q)]\phi(q)dq}{\left[\int_0^1 q(1-q)\phi(q)dq\right]^2}$$

FISHER'S dominance ratio comes out 1/5 in this case.

The preceding results differ somewhat from those presented by FISHER (1922). The latter's analysis was based on a transformation of the scale of factor frequencies designed to make the variance due to random sampling uniform at all points. The variance at a given value of q is $q(1-q)/2N$. FISHER assumes that if the ratio of small differences on the q scale to the corresponding differences on a new θ scale be made proportional to the varying standard deviation of q, the standard deviation on the θ scale will be uniform. Letting $dq/d\theta = \sqrt{q(1-q)}$ leads to the transformation $\theta = \cos^{-1}(1-2q)$ with uniform variance of factor frequencies of $1/2N$. Letting $y = F(\theta)$ be the distribution of factor frequencies in one generation, he wrote that in the next as

$$y + \Delta y = \int_0^\pi \frac{1}{\sigma\sqrt{2\pi}} e^{-\delta\theta^2/2\sigma^2}\left(y + y'\delta\theta + \frac{\delta\theta^2}{\underline{|2}}y'' \cdots\right)$$

and measuring time in generations (T) he reached the equation

$$\frac{\partial y}{\partial T} = \frac{1}{4N}\frac{\partial^2 y}{\partial \theta^2}.$$

After noting that the solution for the symmetrical stationary case is

[6] These estimates of number of unfixed loci and of variance depend, of course, on the validity of the conditions on which the formula of the distribution curve is based. How far the mutation rate per locus can be considered negligibly small as size of population increases is discussed later.

$y = L/\pi$, he proceeded to derive the formulae for increasing and decreasing y. Considering the latter, $dy/dT = -Ky$ where K is the rate of decay, giving $1/4N\ d^2y/d\theta^2 = -Ky$ as the equation to be solved. In the symmetrical case this yields $y = C \cos [\sqrt{4NK}(\theta - \pi/2)]$ where $C = \sqrt{4NK}/[2 \sin (\frac{1}{2}\pi\sqrt{4NK})]$ in order to give a total frequency of unity and is to be multiplied further by L_0e^{-KT} to show change from the initial frequency of L_0.

The maximum value which K can take without giving negative frequencies within the range is obviously 1/4N and FISHER found reason for accepting this as the value in the case of no mutation. The formula for the distribution in this case reduces to $y = \frac{1}{2} \sin \theta$. FISHER transformed these equations to the scale $Z = \log [q/(1-q)]$ in which the case of no mutation becomes $y = \frac{1}{4} \operatorname{sech}^2 \frac{1}{2}Z$ and the case of loss balanced by mutation becomes $y = 1/2\pi \operatorname{sech} \frac{1}{2}Z$. This transformation brings the curves into an approach to the form of the normal probability curve. For our present purpose it is preferable to transform to the scale of actual factor frequencies. The case of steady decay becomes $y = 1$ with which my results are in agreement, although in disagreement as to rate of decay. In the case of loss balanced by mutation, FISHER'S formula transforms into $y = 1/[\pi\sqrt{q(1-q)}]$ instead of $1/[2(\log 3.6N)q(1-q)]$ as developed in the present paper. FISHER obtained $\sqrt{\pi/2}N^{3/2}\mu$ for the number of unfixed factors, in contrast with $2N\mu \log 3.6N$; and $\sqrt{\frac{2}{\pi N}}$ for the factor turnover in contrast with $1/[2 \log 3.6N]$. It will be seen that FISHER'S solution leads to a smaller number of unfixed factors with more rapid turnover in very small populations (less than 81) but to a larger number of such factors with slower turnover in larger populations. In a breeding population of one million with one mutation per 1000 individuals, FISHER'S formula gives 1,250,000 unfixed factors with a turnover of 0.08 percent while I get 30,000 unfixed factors with a turnover of 3.3 percent.

The exact harmonizing of the results of the two methods of attack has been a somewhat puzzling matter, but Doctor FISHER, on examination of the manuscript of the present paper, has written to me the following which I quote at his suggestion. ". . . . I have now fully convinced myself that your solution is the right one. It may be of some interest that my original error lay in the differential equation

$$\frac{\partial y}{\partial T} = \frac{1}{4N} \frac{\partial^2 v}{\partial \theta^2}$$

which ought to have been

$$\frac{\partial v}{\partial T} = \frac{1}{4N} \frac{\partial}{\partial \theta}(y \cot \theta) + \frac{1}{4N} \frac{\partial^2 y}{\partial \theta^2}$$

the new term coming in from the fact that the mean value of δp in any generation from a group of factors with gene frequency p is exactly zero,[7] and consequently the mean value of $\delta\theta$ is not exactly zero but involves a minute term $-1/4N \cot \theta$. With this correction, I find myself in entire agreement, with your value 2N, for the time of relaxation and with your corrected distribution for factors in the absence of selection."

Reversible recurrent mutation

It only requires a very moderate mutation rate in a large population for the number of unfixed loci to become enormous. This raises the question as to the effect of a limitation in the number of mutable loci, and recurrence of mutations.

Consider now the case of genes with uniform rates of recurrence of mutation and reverse mutation. Let u be the rate per generation for break down of the gene A and v that for origin from allelomorphs. A class of genes with frequency q (that of all allelomorphs, $1-q$) will be distributed in the following generation under random sampling according to the expansion of the expression

$$\{[(1-q)-v(1-q)+uq]a+[q+v(1-q)-uq]A\}^{2N}f.$$

Equating the total contribution to a given class, to the frequency of this class in the parent generation, reduced by the proportion K, if there is a uniform rate of decay, gives as the equation to be solved:

$$\phi(q_1)\frac{1-K}{2N}=\frac{\underline{|2N}}{\underline{|2Nq_1}\ \underline{|2N(1-q_1)}}\int_0^1[q(1-u-v)+v]^{2Nq_1}$$
$$[1-q(1-u-v)-v]^{2N(1-q_1)}\phi(q)dq.$$

It will be found by trial that the right and left members became identical in certain cases in which $\phi(q)$ is of the form $q^s(1-q)^t$

Let $x=q(1-u-v)+v$

$$q=\frac{x-v}{1-u-v} \qquad dq=\frac{dx}{1-u-v}$$
$$q^s=\frac{x^s-svx^{s-1}\cdots}{(1-u-v)^s} \qquad (1-q)^t=\frac{(1-x)^t-tu(1-x)^{t-1}}{(1-u-v)^t}.$$

[7] p is the q of the present paper. Since the above was written, FISHER has published this revision of his results in *The genetical theory of natural selection*, 1930.

The small amount of spread from a given class will justify retention of the untransformed limits of integration.

Noting that $\Gamma(c+s+1)=\underline{|c}\ c^s\left(1+\frac{s(1+s)}{2c}\right)$ approximately when c is an integer and s is small compared with c, and making use of the following derived relation

$$\frac{2N\underline{|2N}}{\underline{|2Nq_1}\ \underline{|2N(1-q_1)}}\int_0^1 x^{2Nq_1+s}(1-x)^{2N(1-q_1)+t}dx$$
$$=\frac{4N+s(s+1)q_1^{-1}+t(t+1)(1-q_1)^{-1}}{4N+(s+t+1)(s+t+2)}q_1^s(1-q_1)^t$$

the equation may be written as followsf or small values of s and t (compared with N) and values of u and v of a still lower order of size.

$$(1-K)q_1^s(1-q_1)^t$$
$$=\frac{1}{1-(u+v)(s+t+1)}\left[\frac{4N}{4N+(s+t+1)(s+t+2)}q_1^s(1-q_1)^t\right.$$
$$+\left(\frac{s(s+1)}{4N+(s+t+1)(s+t+2)}-\frac{4Nsv}{4N+(s+t)(s+t+1)}\right)q_1^{s-1}(1-q_1)^t$$
$$\left.+\left(\frac{t(t+1)}{4N+(s+t+1)(s+t+2)}-\frac{4Ntu}{4N+(s+t)(s+t+1)}\right)q_1^s(1-q_1)^{t-1}\right]$$

The coefficients of $q_1^{s-1}(1-q_1)^t$ and of $q_1^s(1-q_1)^{t-1}$ must equal 0 either under complete equilibrium or equilibrium merely in form of distribution. Neglecting small quantities:

$$s=0 \quad \text{or} \quad s=4Nv-1$$
$$t=0 \quad \text{or} \quad t=4Nu-1.$$

In the case of complete equilibrium ($K=0$), it turns out that the coefficients of $q_1^s(1-q_1)^t$ *in the left and right members are also satisfied to a* first approximation by $s=4Nv-1$, $t=4Nu-1$. They are also satisfied by letting $s=0$, $t=0$ provided that $u+v=1/2N$. The relation between the fixed terminal and the unfixed subterminal classes, however, requires that $u=v=1/4N$ in this case, which thus becomes merely a special case of the first solution. Similarly, the solutions $s=0$, $t=4Nu-1$ and $s=4Nv-1$, $t=0$ require that $v=1/4N$ and $u=1/4N$ respectively and thus also reduce to special cases of the first solution. It appears then that the distribution of gene frequencies in equilibrium under mutation and reverse mutation may be represented approximately by curves of PEARSON'S Type I,

$$\phi(q) = \frac{\Gamma(4Nu + 4Nv)}{\Gamma(4Nu)\Gamma(4Nv)} q^{4Nv-1}(1 - q)^{4Nu-1}.$$

The terminal conditions are of interest in this and other cases to be considered. The factor turnover at each extreme may be written

$$K_0 = \tfrac{1}{2}f_1 = 2Nvf_0$$
$$K_{2N} = \tfrac{1}{2}f_{2N-1} = 2Nuf_{2N}$$

where the subterminal classes have the frequencies

$$f_1 = \frac{1}{2N}\phi\left(\frac{1}{2N}\right)$$
$$f_{2N-1} = \frac{1}{2N}\phi\left(1 - \frac{1}{2N}\right).$$

In the present case, the terminal classes have the frequencies $f_0 = C/[4Nv(2N)^{4Nv}]$ and $f_{2N} = C/[4Nn(2N)^{4Nu}]$ where C is the coefficient in the expression for $\phi(q)$.

It will be seen that the form of the curve depends not only on the rates of mutation of the genes but also on the size of the breeding population. With small populations or rare recurrence of mutations, the distribution approaches the symmetrical form $y = 1/(2 \log 3.6N)\, q^{-1}(1-q)^{-1}$ already discussed (figure 5). The ratio of the class of temporarily fixed genes (f_{2N}) to the class of complete absence (f_0) must be approximately v:u in this case in order that the number of mutations at each extreme of the symmetrical distribution of unfixed factors may be equal.

With increase in size of the population, the gene frequencies tend in general to be distributed in asymmetrical U— or even I— or J—shaped curves. For example, if the size of population reaches 1/4u and v is much smaller than u, the distribution will be the hyperbola $\phi(q) = Cq^{-1}$ with a piling up of factors with few or no plus representatives.

With sufficient increase in the size of population, the distribution at length takes a form approaching that of the normal probability curve; centered about the point $\bar{q} = v/(u+v)$ which, indeed, is always the mean

$$\left(\bar{q} = \int_0^1 q\phi(q)dq = \frac{v}{u + v}\right).$$

The variance of gene frequencies, $\sigma_q^2 = \int_0^1 (q-\bar{q})^2\phi(q)dq$ is

$$\frac{\bar{q}(1 - \bar{q})}{4N(u + v) + 1}.$$

The amount of genetic variation of cumulative characters may be calculated as before. In the case of no dominance and paired allelomorphs

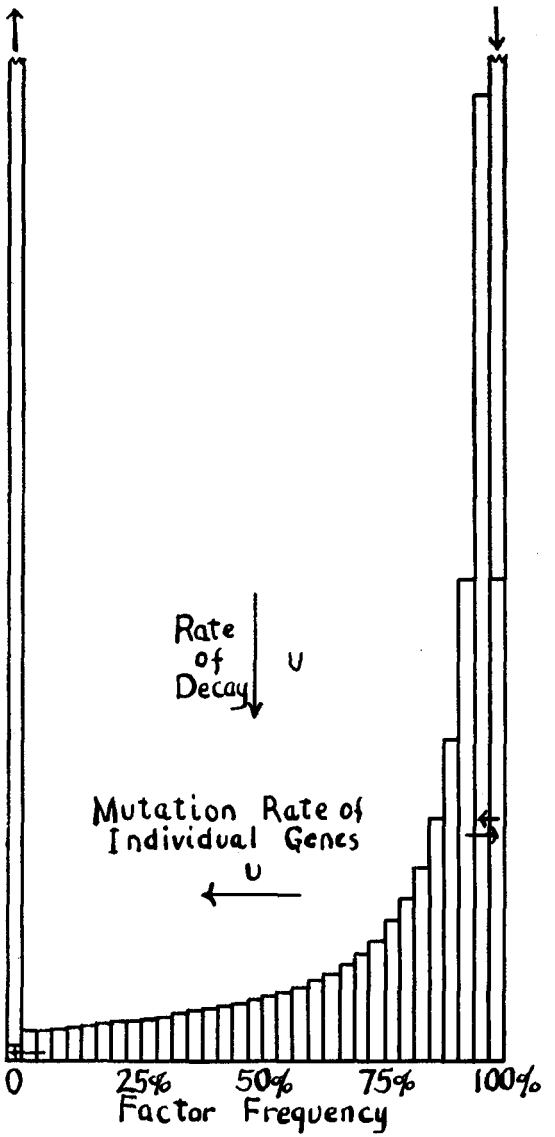


FIGURE 4.—Distribution of type genes in an isolated population in which equilibrium has been reached with destructive mutation but has not been approached with respect to formative mutation. $y=4NuL_0e^{-uT}(1-q)^{4Nu-1}$ with 4Nu much smaller than 1 and the formula approximately $\frac{L_0(1-q)^{-1}}{\log 3.6N}$.

it is $\sigma^2 = 2La^2\ 4Nuq/(4Nu+4Nv+1)$ or $2La^2[\bar{q}(1-q)-\sigma_q{}^2]$. Where u is much greater than v, this can be written approximately $\sigma^2 = 2La^2 4Nv/(4Nu+1)$ approaching $2La^2\bar{q}$ as a limit, as N increases and L comes to include all loci.

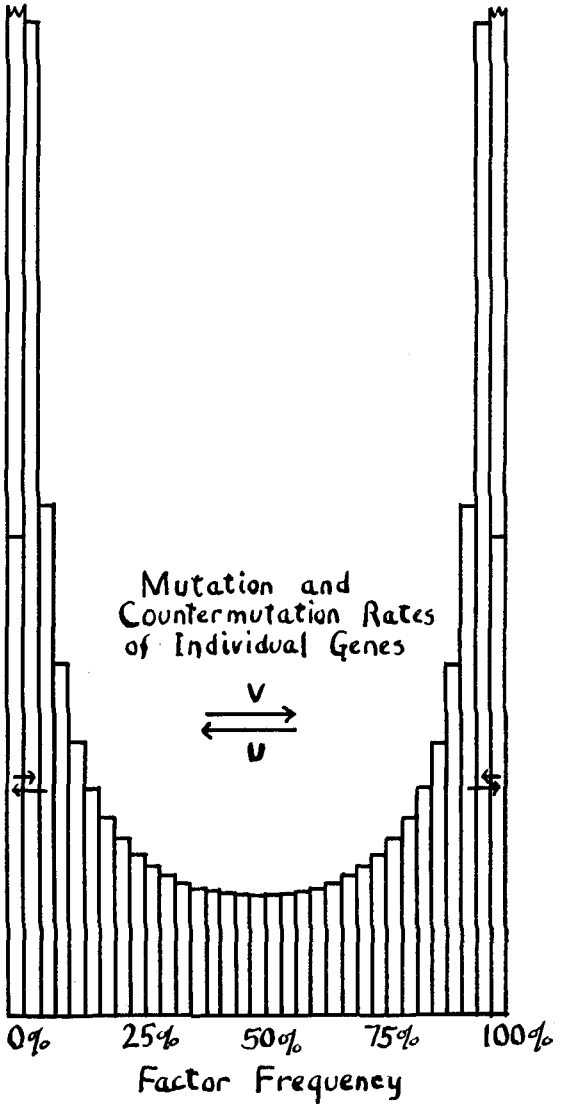


FIGURE 5.—Distribution of gene frequencies (or probability array of gene) where equilibrium with mutation has been attained. Population so small that the terms 4Nu and 4Nv are both much smaller than 1. $y = Cq^{4Nv-1}(1-q)^{4Nu-1}$, approximately $\frac{q^{-1}(1-q)^{-1}}{2 \log 3.6N}$.

As the formula for this case was derived on the assumption of small values of u and v, it is desirable to obtain an independent test of its applicability to large values. This can be done as follows: the increase in variance of q due to random sampling is

$$\frac{1}{2N}\int_0^1 q(1-q)\phi(q)\,dq = \frac{\bar{q}(1-\bar{q})-\sigma^2_q}{2N}.$$

Letting $\Delta q = -uq+v(1-q)$ be, as before, the change in q due to mutation, $q+\Delta q-\bar{q} = (q-\bar{q})(1-u-v)$. Thus the effect of mutation is as if all deviations from the mean were reduced in the proportion $(1-u-v)$. The decrease in the variance of q, due to mutation is therefore $\sigma_q^2[1-(1-u-v)^2]$. At equilibrium the increase in σ_q^2 due to random sampling is exactly balanced by the decrease due to mutation yielding:

$$\sigma^2_q = \frac{\bar{q}(1-q)}{4N(u+v)-2N(u+v)^2+1.}$$

The term $-2N(u+v)^2$ in the denominator is important only when $(u+v)$ has a large absolute value. Omitting this, the formula is identical with that deduced by the first method and thus gives an independent demonstration of its validity. As mutation approaches its maximum value $(u+v=1)$, the variance of q approaches $\bar{q}(1-\bar{q})/2N$, that due to random sampling alone.

Migration

The distribution of gene frequencies in an incompletely isolated subgroup of a large population can be obtained immediately from the preceding results. The change in gene frequency per generation under migration $\Delta q = -m(q-q_m)$ can be written $-m(1-q_m)q+mq_m(1-q)$ which is in the same form as the change of q under mutation, $\Delta q = -uq+v(1-q)$. We may write at once for the distribution under negligible mutation rates:

$$\phi(q) = \frac{\Gamma(4Nm)}{\Gamma[4Nmq_m]\Gamma[4Nm(1-q_m)]}q^{4Nmq_m-1}(1-q)^{4Nm(1-q_m)-1}.$$

The mutation terms 4Nu and 4Nv can be inserted, if mutation rates are not negligible.

Figure 6 shows how the form of the distribution changes with change in m or N. Where m is less than 1/2N there is a tendency toward chance fixation of one or the other allelomorph. Greater migration prevents such fixation. How little interchange would appear necessary to hold a large population together may be seen from the consideration that $m = 1/2N$

means an interchange of only one individual every other generation, regardless of the size of the subgroup. However, this estimate must be much qualified by the consideration that the effective N of the formula is in general much smaller than the actual size of the population or even than the breeding stock, and by the further consideration that q_m of the formula refers to the gene frequency of actual migrants and that a further factor must be included if q_m is to refer to the species as a whole. Taking both of these into account, it would appear that an interchange of the order of thousands of individuals per generation between neighboring subgroups of a widely distributed species might well be insufficient to prevent a considerable random drifting apart in their genetic compositions. Of course,

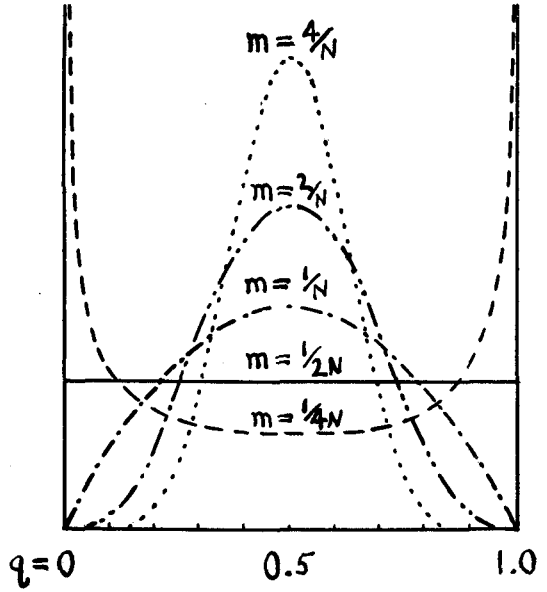


FIGURE 6.—Distribution of frequencies of a gene among subdivisions of a population in which $q_m = 1/2$ (or probability array of gene within a subdivision) under various amounts of intermigration. $y = Cq^{4Nmq_m-1}(1-q)^{4Nm(1-q_m)-1}$.

differences in the condition of selection among the subgroups may greatly increase this divergence. It appears, however, that the actual differences among natural geographical races and subspecies are to a large extent of the nonadaptive sort expected from random drifting apart. An interesting example, apparently nonadaptive, is the racial distribution of the 3 allelomorphs which determine human blood groups (BERNSTEIN 1925).

The variance of distribution of values of q among subgroups (in the ideal

case) is $\sigma_q^2 = q_m(1-q_m)/(4Nm+1)$ by substitution in the formula for the preceding case.

The zygotic distribution $[(1-q)a+qA]^2$ cannot be expected to hold in a population made up of isolated groups among which gene frequency varies. WAHLUND (1928) has shown that the proportions in each homozygous class are increased at the expense of the heterozygotes by the amount of the variance of the gene frequencies among the subgroups,[8] the proportions becoming $[(1-q)^2+\sigma_q^2]aa+[2q(1-q)-2\sigma_q^2]\ Aa+[q^2+\sigma_q^2]\ AA$. By substituting the expression for σ_q^2, given above, in WAHLUND'S formula one might determine empirically the effective value of 4Nm for the population, except that it would be difficult to rule out the possibility that some of the variance of gene frequencies might be due to differences in the selection coefficients among the subgroups instead of merely to random drifting apart.

Irreversible recurrent mutation

The solution $s=0$, $t=4Nu-1$ for the equation reached in the case of recurrent mutation satisfies the conditions for equilibrium of form under irreversible mutation ($v=0$), with decay at rate $K=u$.

$$\phi(q) = 4NuL_0e^{-uT}(1-q)^{4Nu-1}.$$

The proportional frequency of the unfixed subterminal class which is not replenished by mutation is $f_1/(L_0e^{-uT}) = 2u$, twice the rate of decay and thus approximately satisfying the necessary terminal condition.

For values of u as small as $1/(2N \log 3.6N)$ the coefficient in the expression for $\phi(q)$ must be calculated to a closer approximation $\dfrac{4NuL_0e^{-uT}}{1-\left(\dfrac{1}{2N}\right)^{4Nu}}$ which approaches $\dfrac{L}{\log 3.6N}$ as u approaches zero.

The distribution of gene frequencies under irreversible mutation is illustrated in figure 4.

This case is of most interest as representing for a long time the distribution of type genes in a small group isolated from a large one in which all type genes are close to fixation. The release of deleterious mutation pressure from equilibrium with selection will result in approximate equi-

[8] The percentage of heterozygotes is $2\int_0^1 q(1-q)\phi(q)dq$ where $\phi(q)$ is the distribution of values of q among the subgroups. As shown above this reduces to $2\bar{q}(1-\bar{q})-2\sigma_q^2$, thus demonstrating WAHLUND'S principle.

librium of the form described above. With decay at the rate u, it may be a very long time before effects of reverse mutation become appreciable and the final equilibrium $y = Cq^{-1}(1-q)^{-1}$ approached. Assuming that type genes are dominant, the dominance ratio in this case is 1/3.

Selection[9]

Using $\Delta q = sq(1-q)$ as the measure of the effect of genic selection, the class of genes with frequency $(1-q)a : qA$ is distributed after one generation according to the expression:

$$\{(1 - q)(1 - sq)a + q[1 + s(1 - q)]A\}^{2N}.$$

The distribution of gene frequencies which is in equilibrium may be obtained from the following equation which represents the total contribution to class q_1 after one generation, as equal to its previous frequency.

$$\frac{\phi(q_1)}{2N} = \frac{\lfloor 2N}{\lfloor 2Nq_1 \ \lfloor 2N(1 - q_1)} \int_0^1 q^{2Nq_1}(1 - q)^{2N(1-q_1)}(1 + s(1 - q))^{2Nq_1}(1 - sq)^{2N(1-q_1)}\phi(q)dq$$

To a first approximation, the selection terms approach the value $e^{2Ns(q_1 - q)}$. The introduction of a factor e^{2Nsq} into the previously reached formula for $\phi(q)$ gives a solution of the equation (for very small values of s) since it cancels the new term e^{-2Nsq} in the integral, and leaves e^{2Nsq_1} as a factor in $\phi(q_1)$. This was the basis for the formula published (WRIGHT 1929a) as $\phi(q) = Ce^{2Nsq}q^{4Nv-1}(1-q)^{4Nu-1}$ intended to exhibit in combination the effects of selection, mutation in both directions and size of population. Further consideration reveals that this solution is the correct one only for the case of irreversible mutation and then only when the selection coefficient is exceedingly small, less than 1/2N in fact. FISHER (1930) in his recently published revision of the results of his method of attack on this problem has given a formula for a special case of selection, equilibrium of flux from an inexhaustible supply of mutating genes. This is given as accurate as long as Ns^2 is small. Assuming one mutation per generation, he writes:

$$y = \frac{2dp(1 - e^{-4anq})}{pq(1 - e^{-4an})}.$$

[9] This and the following section have been rewritten since submission of the manuscript in order to take account of the correction of my formula, suggested by FISHER'S results in *The genetical theory of natural selection*, 1930 as noted herein.

In this formula, $a(=-s)$ is the selection coefficient, $p(=1-q)$ is frequency of mutant genes and dp may be taken as $1/2N$ numerically. This agrees with my previous formula for irreversible mutation, $y=Ce^{2Nsq}(1-q)^{-1}$ only when s is less than $1/2N$, above which value my formula rapidly leads to impossible results. On reexamination of my method, however, I find that the same degree of approximation can be reached by it. The expansion of $[1+s(1-q)]^{2Nq_1}[1-sq]^{2N(1-q_1)}$ yields series of terms which condense into the expression $e^{2Ns(q_1-q)}\{1-Ns^2[q_1(1-q_1)+(q_1-q)^2]\}$ taking into account terms in Ns^2, N^2s^3, N^3s^4, N^4s^5 as well as those in which N and s have the same exponent. Since the random deviations of q have a variance of $q_1(1-q_1)/2N$ the term (q_1-q) is of the order $\sqrt{1/2N}$. A second order approximation should be obtainable by retaining the term $Ns^2q_1(1-q_1)$ while that in $Ns^2(q_1-q)^2$ may be dropped. The equation to be solved can now be written.

$$\phi(q_1) = \frac{2N\,\underline{|2N}}{\underline{|2Nq_1}\;\;\underline{|2N(1-q_1)}}e^{2Nsq_1}[1 - Ns^2q_1(1 - q_1)] \int_0^1 q^{2Nq_1}(1 - q)^{2N(1-q_1)}e^{-2Nsq}\phi(q)dq.$$

Let $\phi(q)=e^{2Nsq}q^{-1}(1-q)^{-1}(a+bq+cq^2+dq^3\cdots)$.

The exponential term in the integral being cancelled, it becomes possible to carry out the integration by means of the approximate formula already used in the case of mutation (page 122).

$$\frac{2N\,\underline{|2N}}{\underline{|2Nq_1}\;\;\underline{|2N(1-q_1)}}\int_0^1 q^{2Nq1+z-1}(1 - q)^{2N(1-q_1)-1}dq = \frac{4N + z(z - 1)q_1^{-1}}{4N + z(z - 1)}q_1^{z-1}(1 - q_1)^{-1}.$$

The resulting coefficients of the powers of q_1 on the right side of the equation may now be equated separately to those of $\phi(q_1)$. To a sufficient approximation it turns out that $c = \frac{(2Ns)^2}{\underline{|2}}a$, $d = \frac{(2Ns)^2}{\underline{|3}}b$, $e = \frac{(2Ns)^4}{\underline{|4}}a$, $f = \frac{(2Ns)^4}{\underline{|5}}b$, $g = \frac{(2Ns)^6}{\underline{|6}}a$, etc.

Letting $C_1 = a/2$ and $C_2 = \frac{b}{4Ns}$

$$\phi(q) = 2e^{2Nsq}q^{-1}(1 - q)^{-1}[C_1 \cosh 2Nsq + C_2 \sinh 2Nsq].$$

From considerations of symmetry, it is obvious that another solution may be obtained by replacing q by $(1-q)$ and s by $-s$. The full solution may be written in the form

$$\phi(q) = q^{-1}(1-q)^{-1}[C_1(e^{4Nsq}+1) + C_2(e^{4Nsq}-1) + C_3(1+e^{-4Ns(1-q)}) + C_4(1-e^{-4Ns(1-q)})].$$

The relative values of the coefficients in the case of equilibrium can be obtained by setting up the equation for the absence of flux. Each group of genes, $f=\phi(q)dq$ tends to be shifted by the amount $\Delta q = sq(1-q)$ in a generation. There is thus a total flux measured by $\int_0^1 \phi(q)\Delta q dq$ unless there is counterbalancing mutation. The amount of mutation in each direction (assuming the rates of recurrence to be very small compared with 1/4N) is approximately half the respective subterminal classes, as demonstrated in the preceding cases.

$$f_1 = 2C_1 + 2sC_2 + (1+e^{-4Ns})C_3 + (1-e^{-4Ns})C_4$$
$$f_{2N-1} = (e^{4Ns}+1)C_1 + (e^{4Ns}-1)C_2 + 2C_3 + 2sC_4.$$

Since mutation moves genes from the fixed classes to the subterminal classes with gene frequencies of 1/2N and $\left(1-\frac{1}{2N}\right)$ respectively, it creates a net flux of $\frac{f_{2N-1}}{4N} - \frac{f_1}{4N}$ which at equilibrium should balance that due to selection

$$\int_0^1 \phi(q)\Delta q dq - \frac{f_{2N-1}}{4N} + \frac{f_1}{4N} = 0.$$

Substitution of the values given above leads to the condition $C_1-C_2+C_3+C_4=0$. Under this condition the formula simplifies greatly, becoming for all values of s $\left(\text{of lower order than } \frac{1}{\sqrt{N}}\right)$

$$\phi(q) = Ce^{4Nsq}q^{-1}(1-q)^{-1}.$$

The effect of selection in this case is perhaps best exhibited in the ratio of the classes of alternative fixed genes in the highly artificial case of equality in the rates of mutation in opposite directions. This ratio is e^{4Ns}. More generally, $f_0 = \frac{C}{4Nv}$ and $f_{2N} = \frac{Ce^{4Ns}}{4Nu}$ where u and v, both assumed to

be very small compared with 1/4N, are the opposing mutation rates. The number of unfixed loci (pairs of allelomorphs) takes the form

$$L = \frac{2N\mu}{f_1 + f_{2N-1}} = \frac{2N\mu}{C(e^{4Ns} + 1)}$$

where μ is the mutation rate per individual and C is chosen so that $\int_0^1 \phi(q)dq = 1$. The effect of selection on the variance of cumulative characters (pairs of allelomorphs) may be seen by comparing the formula

$$\sigma^2 = 2\mu a^2\left(\frac{e^{4Ns} - 1}{2s(e^{4Ns} + 1)}\right)$$

with the previously given form $2N\mu a^2$ which it approaches as s approaches 0.

In the case treated by FISHER, there is assumed to be irreversible mutation at the rate of one per generation from an inexhaustible supply. As each new gene becomes fixed, it may be considered as transferred to the type class, ready to mutate to new allelomorphs in its series. Thus in place of a return flux of $\frac{f_1}{4N}$, due to reversible mutation, we must write $\frac{f_1}{2}$ (if v = 0)

$$\int_0^1 \phi(q)\Delta q dq - \frac{f_{2N-1}}{4N} + \frac{f_1}{2} = 0.$$

This is solved if $C_1 = C_3 = C_4 = 0$ and

$$\phi(q) = C_2(e^{4Nsq} - 1)q^{-1}(1 - q)^{-1}.$$

In case the direction of mutation coincides with that of selection (u = 0), the mutational terms must be written $\frac{f_{2N-1}}{2} - \frac{f_1}{4N}$ giving the solution

$$\phi(q) = C_4(1 - e^{-4Ns(1-q)})q^{-1}(1 - q)^{-1}.$$

These are identical with FISHER's results on proper choice of the coefficient.

An interesting question which FISHER has discussed, is the chance of fixation of a single mutation. This is given by the ratio of the subterminal classes in the formulae just considered. Where selection opposes

mutation, $\frac{f_1}{f_{2N-1}} = \frac{2s}{e^{4Ns} - 1}$, always less than 1/2N. In the case of favorable mutations, $\frac{f_{2N-1}}{f_1} = \frac{2s}{1 - e^{-4Ns}}$, or approximately 2s. FISHER also gives an independent derivation of the last figure.

General formula

It is of especial importance to assemble the effects of all evolutionary factors into a single formula. Unfortunately, the equation of equilibrium of class frequencies becomes rather complicated and has not yet been worked through. Presumably the form is given at least approximately by a formula of the type $Ce^{aq}q^{4Nv-1}(1-q)^{4Nu-1}$ in the case of reversible mutation. In order that there may be no flux, $\int_0^1 \phi(q)\Delta q dq = 0$. It is not necessary to consider the terminal classes in this case. Thus

$$C\int_0^1 e^{aq}q^{4Nv-1}(1-q)^{4Nu-1}[-uq + v(1-q) + sq(1-q)]dq = 0.$$

Integration of the first term (that in $-uq$) by parts gives an expression which is immediately solved by letting $a = 4N$. Thus the selection term appears to be e^{4Nsq} regardless of the rates of mutation provided there is reversibility. It is approximately of this value in the case of irreversible mutation, discussed above, provided that s is considerably larger than 1/4N. The conclusions based on the previously presented value e^{2Nsq} still hold,[10] except that they should be applied to selection intensities just half as great.

The position of the mode of the I-shaped distribution curve given when u and s are greater than 1/4N can be found by equating the differential coefficient of the logarithm of the formula to zero.

$$4Ns + \frac{4Nv - 1}{q} - \frac{4Nu - 1}{1 - q} = 0.$$

When v is small but u and s are both large, q approaches the value $1 - \frac{u}{s}$ already given as the equilibrium point. The mean would be somewhat below this point, as expected from the curvilinear relation of selection

[10] These conclusions were presented at the meeting of the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE for 1929 and were summarized in the abstracts (WRIGHT 1929b).

pressure to gene frequency and in contrast with the case of equilibrium between opposing mutation pressures (but no selection) in which the mean is always the equilibrium point $\frac{v}{u+v}$ and the mode is more extreme than this figure, $\frac{4Nv-1}{4N(u+v)-2}$.

Migration pressure introduces no other complications. Combining all factors:

$$\phi(q) = Ce^{4Nsq}q^{4N(mq_m+v)-1}(1-q)^{4N[m(1-q_m)+u]-1}.$$

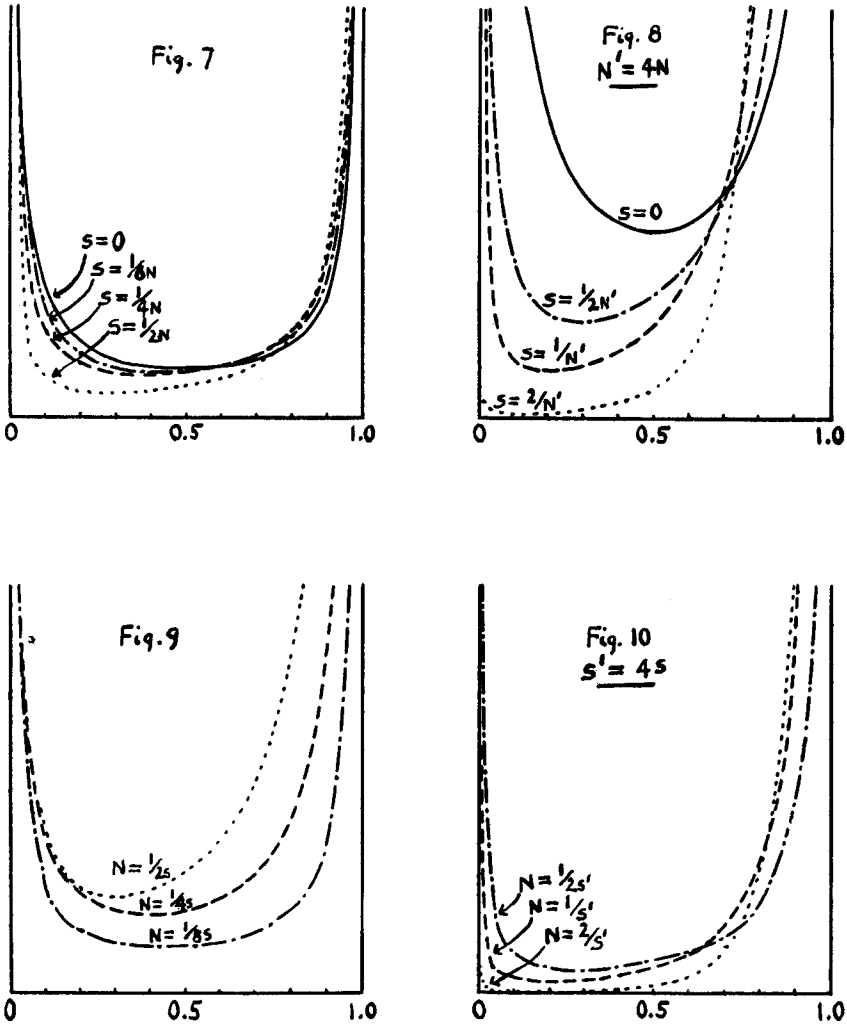
The selection coefficient refers here to the difference between the selection in the group under consideration and that in the species as a whole, the effect of the latter being taken account of in the mean gene frequency of the species q_m.

The distribution curves

Some of the forms taken by the probability array of gene frequencies, in cases involving selection, are illustrated in figures 7 to 14. Figures 7 to 10 deal with the case in which the rates of mutation are negligibly small compared with 1/4N. The curves are thus all variants of the form $y = Ce^{4Nsq}q^{-1}(1-q)^{-1}$. Figure 7 illustrates the relatively slight effect of selection below a certain relation to size of population. All conditions are the same in figure 8 except that the populations are four times as large as in figure 7. Thus while the absolute intensities of the selection coefficients are the same, the relations to size of population are altered.[11] The curves bring out the great effect of selection beyond the critical point, $s = \frac{1}{2N}$ (where mutation rates are low). Figures 9 and 10 are intended to show the effects of change in size of population where the intensity of selection remains constant (low in figure 9, four times as severe in figure 10). Up to a certain point $\left(N = \frac{1}{2s}, \text{ figure } 9\right)$ increase in population raises the middle portion of the curve. Above this point (figure 10) increase in population depresses the middle portion. In the former case, the increase in unfixed factors brings increased variability of cumulative characters, in the latter there is little change of variability in relation to population size, the depression among middle frequencies being balanced by the accumulation of nearly but not quite fixed factors. All of these fig-

[11] The probability that increase in the number of unfixed genes would react on the individual gene selection coefficients, reducing them, is here ignored.

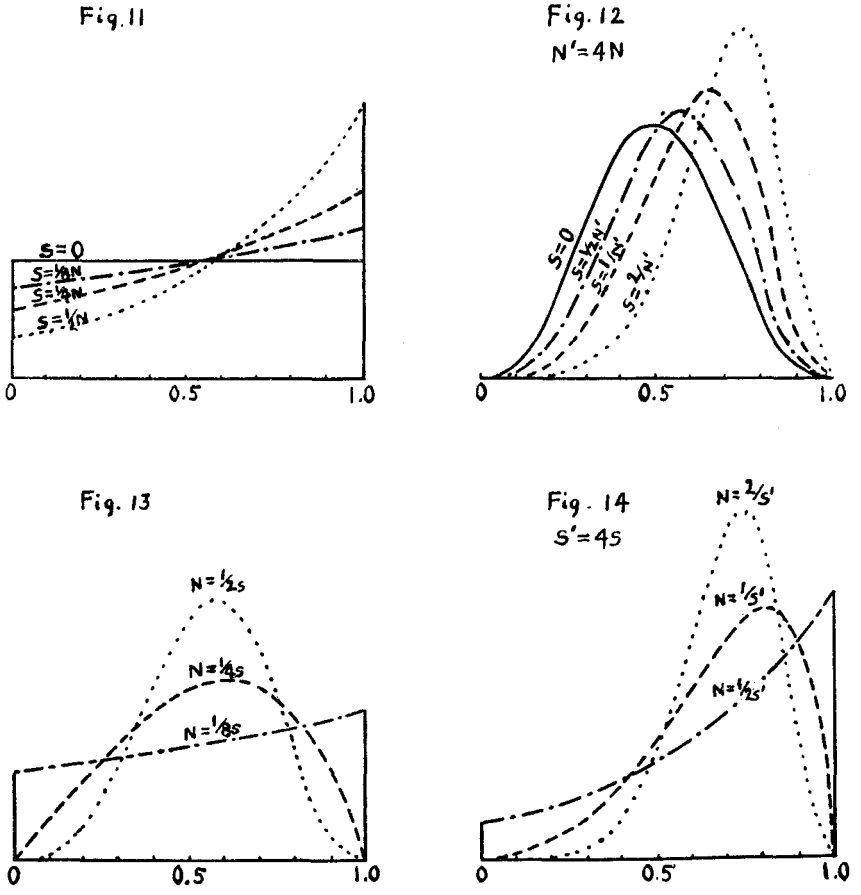
ures (7 to 10) may be taken as representative of conditions in small inbred populations which have been isolated sufficiently long to reach equilibrium in relation to mutation. It will be recalled that figures 3 and 4 represent successive stages preceding the attainment of such equilibrium.



FIGURES 7, 8, 9, and 10.—Distribution of gene frequencies in relation to size of population and intensity of selection where rates of mutation and migration are small compared with 1/4N. Formulae all of type $y = Cy^{4Nsq}q^{-1}(1-q)^{-1}$.

Figure 7. Small population, four degrees of selection. Figure 8. Population four times as large as in figure 7 under the same four (absolute) degrees of selection. Figure 9. Three sizes of population under given weak selection. Figure 10. Same three sizes of population as in figure 9, under selection four times as severe.

Figures 11 to 14 present exactly the same series of comparisons as figures 7 to 10, for small populations that are not completely isolated from the main body of a species.[12] In all cases the gene frequency (q_m) of the



FIGURES 11 to 14.—Distribution of gene frequencies in subgroups of large population (mean frequency $q_m = 1/2$) in relation to size of population and intensity of selection. Formulae all of the type $y = Ce^{4Nsq}q^{4Nmq_m-1}(1-q)^{4Nm(1-q_m)-1}$ Same comparisons as in figures 7 to 10.

Figure 11. Small subgroups ($2Nm = 1$), four degrees of selection. Figure 12. Subgroups four times as large as in figure 11, under same four (absolute) degrees of selection. Figure 13. Subgroups of three sizes under given weak selection. Figure 14. Same three sizes as in figure 13 under selection four times as severe.

The figures may also be used to illustrate cases of equal mutation to and from a gene ($u = v$). $y = Ce^{4Nsq}q^{4Nv-1}(1-q)^{4Nu-1}$

[12] These figures also represent the distribution of gene frequencies in population in which mutation and reverse mutation are equally frequent, but this seems to be so exceptional a case especially under multiple allelomorphism, as to be of little importance.

whole species is assumed to be 1/2. The relations of migration to size of population are such that there is very little complete fixation of genes. In figure 11, $m = 1/2N$ and the purely exponential curves show how increasing intensity of genic selection shifts a uniform distribution in the direction favored by the selection. The fourfold greater population of figure 12 brings about concentration, in curves approaching the normal in form. Figure 13 brings out the concentrating effect of increase in population in the case of weak selection while figure 14 does the same for the case of selection four times as severe.

The important case in which mutation is balanced by selection in a moderately large population (both s and u large compared with 1/4N) is illustrated in figure 19. The four curves represent four degrees of selection, rising by doubling of severity at each step from a case in which mutation pressure practically overwhelms the effect of selection to the reverse situation. The limiting condition in populations so large that 1/4N is very small compared with both s and u is that of concentration of factor frequency almost at a single value (figure 20, page 148).

Dominance ratio

The form of the distribution of the frequencies of the dominant genes affecting a character is of interest in connection with the dominance ratio. Since different genes have different mutation rates and selection coefficients, this distribution is a composite of curves of the types discussed. In small populations which have reached equilibrium, all of these arrays and hence their composite are of the type $Cq^{-1}(1-q)^{-1}$. The dominance ratio is 1/5 in so far as dominance is complete. FISHER gives the value as $3/13 = 0.23$ for the case "when in the absence of selection, sufficient mutation takes place to counteract the effect of random survival." The difference from the value 0.20 given above is due solely to the difference in the formula for the curve, discussed earlier.

In the case of the isolation of a small part of a large population, the dominance ratio takes the value 1/3 in so far as dependent on dominant type genes in equilibrium with recessive mutation but not with reverse mutation ($y = C/(1-q)$). Where following isolation both fixation and loss are substantially irreversible ($y = 1$) the dominance ratio is 1/4 in agreement with FISHER'S result. In both of these cases, of course, the dominance ratio falls to 1/5 when equilibrium is finally attained.

The foregoing discussion applies practically only to very small completely isolated populations. In large populations where the distribution of gene frequencies, even in partially isolated subgroups, tends to approach

the normal type, the dominance ratio comes to depend mainly on the mean gene frequency which depends on the relation of selection to mutation, or on selection against both homozygotes in favor of heterozygotes. In the extreme case in which the gene frequency is reduced to a single value, the dominance ratio is $q/(2-q)$. Values close to unity should not be uncommon, especially where gene frequency is controlled by the balance of selection and mutation. Such a dominance ratio has rather surprising effects on the correlation between relatives. The correlation between parent and offspring approaches 0 although that between brothers may remain as high as 0.25. However, the occurrence of an appreciable number of genes at lower frequencies, for example, held in equilibrium by selection favoring the heterozygote against both homozygotes would greatly lower the dominance ratio.

All of these figures are on the assumption that dominance is complete. Dominance, however, is frequently not complete. Among 22 heterozygotes in the guinea pig which have been studied with some care, at least 9 or about 40 percent are to some extent intermediate. Most of these have to do with color characters. It is not unlikely that incomplete dominance will be found to be even more frequent on careful study of size characters.

FISHER (1922) comes rather definitely to the conclusion that the dominance ratio is typically in the neighborhood of 1/3. This was based primarily on a distribution of factor frequencies which he reached for the case of selection against a recessive,[13] with which the results of the present study are not at all in agreement. He also finds, however, that the differences between fraternal and parent-offspring correlations in data which he analyzes indicate the same figure. The analysis of a large number of correlations of these sorts would undoubtedly furnish valuable information with regard to the statistical situation in populations. It is to be noted, however, that similarity in the environment of brothers as compared with parent and offspring may also contribute to a higher fraternal correlation and that in any case one cannot reason from the dominance ratio deduced from correlations to the distribution of factor frequencies without making some assumption as to the prevalence of dominance.

About all that seems justified by the present analysis, is the statement that for permanently small populations under low selection the value should

[13] This was given as $df \propto \frac{d\theta}{\sin \frac{1}{2}\theta \cos^3 \frac{1}{2}\theta}$ or $\phi(q) = Cq^{-1}(1-q)^{-2}$ on transformation of scale.

FISHER does not discuss dominance ratio in connection with his recent revision of his results in *The genetical theory of natural selection*, 1930.

be less and probably considerably less than 0.20 but that this figure may be raised by severe selection (favoring dominants) and especially by increase in size of population. It may even approach unity in very large populations under severe selection, if complete dominance is the rule.

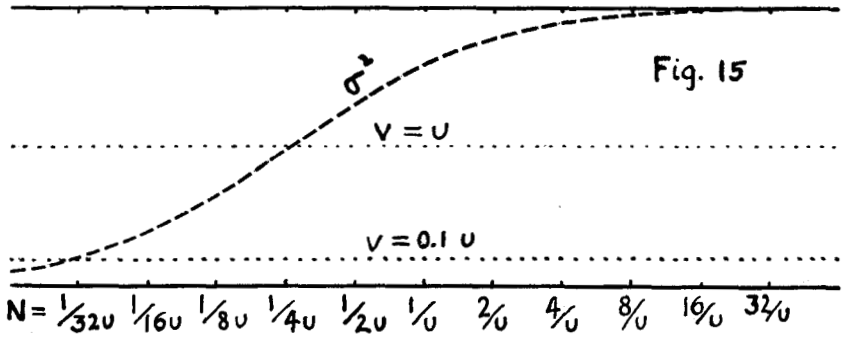
Mean and variability of characters

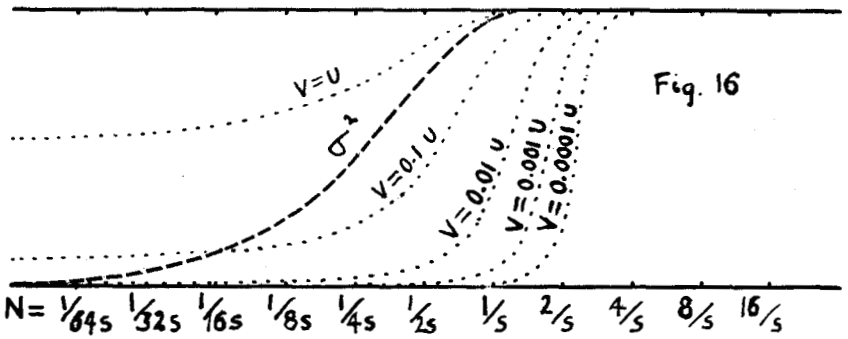
In the case of genes which are indifferent to selection (s less than u), the mean frequency $\bar{q} = v/(u+v)$ remains unchanged through all transformations from a U-shaped distribution in small populations to an I-shaped one in large populations. The variance, due to such genes, is small in small populations, rises in nearly direct proportion to size of population up to a certain critical point (about $N = 1/4u$) and then approaches a limiting value. For the case in which mutations in one direction (u) occur at a much greater rate than in the other (v), the general formula reduces to $\sigma^2 = \sigma_\infty^2\left(\frac{4Nu}{1+4Nu}\right)$, in which $\sigma_\infty^2 (= 2La^2v/u)$ is the limiting value. This case is illustrated in figure 15. The dotted lines represent mean gene frequencies and the line of dashes the variance.

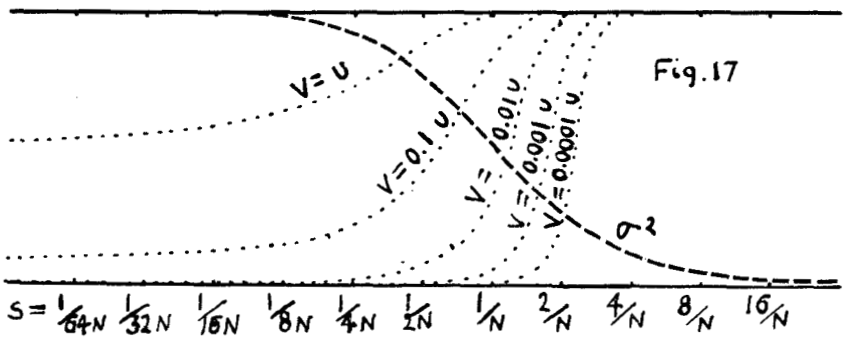
Actual changes in the size of a given population are not of course accompanied by instant adjustment of the distribution of gene frequencies. A decrease in size to a point well below the critical value is followed by decrease in heterozygosis and variance at a rate between 1/4N and 1/2N per generation depending on the number of allelomorphs. This may be a fairly rapid process in terms of geologic time but the recovery of heterozygosis through growth of the population to its original size occurs more slowly, since this depends on mutation pressure. On the other hand, the intercrossing of a number of isolated strains, in each of which the reduction of variance has occurred, is followed by immediate recovery of the original statistical situation (except with respect to factor combinations in which there is some delay).

In the opposite case of genes under vigorous selection (s much greater than u) mean frequency as well as variance is affected by size of population and by severity of selection. As in the preceding case, variance is small in small populations, rises in nearly direct proportion to growth of population[14] until a critical point is approached (here about $N = 1/4s$) and then rapidly approaches a limiting value $\sigma^2 = \sigma_\infty^2\left(\frac{e^{4Ns}-1}{e^{4Ns}+1}\right)$ where

[14] As before, the probability that the increase in variance, due to growth of population, would react on the selection coefficients of the individual genes, reducing them, requires some qualification of this statement in application to actual populations.







FIGURES 15 to 17.—The variance (σ^2) and mean gene frequencies (dotted lines) at equilibrium under various conditions of mutation, selection and size of population. Figure 15. Effects of increasing population where selection is negligible relative to mutation. Figure 16. Effects of increasing population where mutation rates are small compared with 1/4N. Figure 17. Effects of increasing selection where mutation rates are small compared with 1/4N.

$\sigma_\infty^2 = \mu a^2/s$. The mean factor frequency in large populations ($\bar{q} = 1 - u/s$) is close to 1, nearly complete fixation of the favorable gene. In small populations, on the other hand, the equilibrium point approaches that of the opposing mutation pressures ($\bar{q} = v/(u+v)$) and hence practically 0, with complete loss ($v = 0$) as the inevitable ultimate fate in an extended multiple allelomorphic series. Up to the point at which mutation pressure seriously disturbs the form of the distribution curve, the mean gene frequencies are simply the ratios of the chances of fixation at each extreme, namely, $ve^{4Ns}/(u+ve^{4Ns})$.

The relations of mean frequency and variance to size of population in this case are both shown in figure 16, the former for various relative values of u and v. Inspection of figures 9 and 10 may also be of assistance in understanding this situation.

As in the other case, actual change in size of population is not accompanied by immediate attainment of the new equilibrium. Decrease in population to a number well below the critical point is followed by decrease in heterozygosis at the rate described, bringing with it at the same rate the well known inbreeding effects, loss of variance and, in general, decline in vigor toward a new level. This immediate decline in vigor is not due to change in mean gene frequency, but merely to the greater proportion of recessive phenotypes as homozygosis increases, and thus comes to an end when the degree of homozygosis has reached equilibrium. The change in mean gene frequency proceeds more slowly since it depends on mutation pressure. Long continued isolation should thus involve two distinct degeneration processes, a rapid but soon completed process of fixation and a very slow process of accumulation of injurious genes. The recovery on increase in size of population is slow in both cases, depending on mutation pressure. The intercrossing of isolated lines, on the other hand, is followed by immediate return to the original status of the population if only the immediate inbreeding effect has occurred, but must wait on favorable mutations if there has been time for the slower process.

The effects of different intensities of selection on mean gene frequency and variance (population size constant) are illustrated in figure 17, still assuming that the selection coefficient is of higher order than mutation rate. Figures 7 and 8 showing the distribution of gene frequencies in this case may also be of assistance here. Selection has little effect on variability until it reaches about the value 1/8N, about half the variance is eliminated when selection reaches 1/N and most of it at $s = 4/N$. The formula is $\sigma^2 = \sigma_0^2\left(\frac{e^{4Ns}-1}{2Ns(e^{4Ns}+1)}\right)$. Selection, of course, affects the mean gene

frequency, the formula being the same as that given above under the effect of size of population. On actual increase in the intensity of selection, the rate of change toward the new equilibrium both in mean and variance is controlled by selection pressure and may thus be fairly rapid in terms of geologic time in a large population. This is the case in which HALDANE'S formulae for progress under selection are most applicable. The increase in variance and in the proportion of unfavorable genes following relaxation of selection, on the other hand, are controlled by mutation pressure and thus approach equilibrium relatively slowly. A shift in gene frequency at rate uq may well mean no more than 0.000,001 per generation.

The type of result where the selection coefficient is of the same order of magnitude as mutation rate can be inferred, qualitatively, at least from the preceding extreme cases. Inspection of figure 19 may also be of assistance here.

THE EVOLUTION OF MENDELIAN SYSTEMS

Classification of the factors of evolution

In attempting to draw conclusions with respect to evolution one is apt, perhaps, to assume that factors which make for great variation are necessarily favorable while those which reduce variation are unfavorable. Evolution, however, is not merely change, it is a process of *cumulative* change: fixation in some respects is as important as variation in others. Live stock breeders like to compare their work to that of a modeller in clay. They speak of moulding the type toward the ideal which they have in mind. The analogy is a good one in suggesting that in both cases it is a certain intermediate degree of plasticity that is required.

The basic cumulative factor in evolution is the extraordinary persistence of gene specificity. This doubtless rests on a tendency to precise duplication of gene structure in the proper environment. The basic change factor is gene mutation, the occasional failure of precise duplication. Since the time of LAMARCK, a school of biologists have held that the primary changes in hereditary constitution must be adaptive in direction in order to account for evolutionary advance. Unfortunately, the results of experimental study have given no support to this view. Instead, the characteristics of actually observed gene mutations seem about as unfavorable as could be imagined for adaptive evolution. In the first place, is their fortuitous occurrence. No correlation has been found between external conditions and direction of mutation, and those few agents which have been found to affect the rate (X-ray, radium, and to a relatively unimportant extent, temperature) merely speed up the rate of random mutation. The great

majority of mutations are either definitely injurious to the organism or produce such small effects as to be seemingly negligible. MULLER has graphically compared the range of mutations to a spectrum in which the nonlethal conspicuous mutations form a narrow field between broad regions of individually inconspicuous mutations on the one hand and of sublethal and lethal mutations on the other. In addition, the great majority of mutations are more or less completely recessive to the type genes from which they arise. These effects are easily understood if mutation is an accidental process. Random changes in a complex organization are more likely to injure than to improve it, and with respect to the immediate products of the gene, random changes are more likely to be of the nature inactivation (and hence probably recessive) than of increased activation. Finally is to be mentioned the extreme rarity of gene mutation. Even in Drosophila, mutation rates as high as $u = 10^{-5}$ per locus seem to be exceptional and 10^{-6} or less more characteristic. This infrequency seems unfavorable to rapid evolution, yet it is a necessary corollary of the usually injurious effect, if life is to persist at all. Moreover, the more advanced the evolution, the slower must be the time rate of mutation. In one-celled organisms, dividing several times a day, a rapid time rate of mutation will not prevent the production of sufficient normal offspring to maintain the species. The same time rate in Drosophila with an interval of some two weeks between generations would mean such an accumulation of lethals in every gamete that the species would come to an abrupt end. The time rate of lethal mutation in Drosophila (7 per 1000 chromosomes per month under ordinary conditions according to MULLER (1928)) would be quite impossible in the human species. The problem is to determine how an adaptive evolutionary process may be derived from such unfavorable raw material as the infrequent, fortuitous and usually injurious gene mutations.

It will be convenient here to classify factors of evolution according as they tend toward genetic homogeneity or heterogeneity of the species. They are grouped below in more or less definitely opposing pairs.

Factors of Genetic Homogeneity	*Factors of Genetic Heterogeneity*
Gene duplication	Gene mutation (u, v)
Gene aggregation	Random division of aggregate
Mitosis	Chromosome aberration
Conjugation	Reduction (meiosis)
Linkage	Crossing over
Restriction of population size (1/2N)	Hybridization (m)
Environmental pressure (s)	Individual adaptability
Crossbreeding among subgroups (m_1)	Subdivision of group ($1/2N_1$)
Individual adaptability	Local environments of subgroups (s_1)

The first pair have been discussed above. They enter into the formulae through the mutation rates u and v. MULLER has pointed out the necessary similarity, in order of size, of genes and of filterable viruses and has suggested the possibility that the latter may consist of single genes. If so, their evolution rests wholly on a not too high rate of mutation, and selection, which seems possible enough in organisms as simple as these presumably are, especially as in this case where the gene is the organism, the mutation of the gene need not be expected to be as fortuitously related to the activities of the organism as in more complex cases.

Presumably the first step toward higher organisms is the aggregation of such genes with multiplication of the aggregate by random division. Given occasional gene mutation, this leads to a new kind of variation, that in proportional abundance of the different kinds of genic material. The larger the aggregate, the less violent the variation. Large aggregates present a labile system capable of quantitative variation in response (perhaps physiologically as well as through selection) to changing conditions. As far as observation goes, the bacteria, and blue green algae have no mechanism of division beyond a random division of the protoplasmic constituents. Such apportionment of more or less autonomous materials may also be important in the differentiating cell lineages of multicellular organisms, but, except for a few plastid characters, seems to play no important role in heredity from generation to generation, as far as has been determined by experiment. There seems here an adequate basis for an evolutionary process in organisms so simple that the handing on of a few different protoplasmic constituents can determine all of the characteristics of the species but the conditions are not favorable for an extensive cumulative process.

Mitosis provides the mechanism by which an indefinitely large number of qualitatively different elements may be maintained in the same proportions. But it provides so perfectly for the persistence of complex organization that further change is difficult. Irregularities in mitosis provide a source of variation but of so violent a nature for the most part as to be of infrequent evolutionary importance, although the differences in chromosome numbers of related species demonstrate that they play a genuine rôle. Complete duplication (tetraploidy) is important in doubling the possible number of different kinds of genes. Other aberrations, especially translocations, are probably more important in isolating types, than for the character changes which they bring. Gene mutation remains the principal factor of variation, but seems inadequate as the basis of an evolutionary process under exclusively mitotic (asexual) reproduction.

The most important factor in transcending the evolutionary difficulties

inherent in the characteristics of gene mutation is undoubtedly the attainment of biparental reproduction (EAST 1918). This involves two phases, conjugation, a factor which makes the entire interbreeding group a physiological unit in evolution, and meiosis, with its consequence, Mendelian recombination which enormously increases the amount of variability within the limits of the species. Each additional viable mutation in an asexually reproducing form merely adds one to the number of types subject to natural selection. The chance that two or more indifferent or injurious mutations may combine in one line to produce a possibly favorable change is of the second or higher order. Under biparental reproduction, each new mutation doubles the number of potential variations which may be tried out. The contrast is between $n+1$ and 2^n types from n viable mutations.

Biparental reproduction solves the evolutionary requirement of a rich field of variation. But by itself it provides rather too much plasticity. It makes a highly adaptable species, capable of producing types fitted to each of a variety of conditions, but a successful combination of characteristics is attained in individuals only to be broken up in the next generation by the mechanism of meiosis itself.

An excellent illustration of the principle that a balance between factors of homogeneity and of heterogeneity may provide a more favorable condition for evolution than iether factor by itself may be found in the effects of an alternation of a series of asexual generations with an occasional sexual generation. Evolution is restrained under exclusive asexual reproduction by the absence of sufficient variation, and under exclusive sexual reproduction by the noncumulative character of the variation, but, on alternating with each other, any variety in the wide range of combinations provided by a cross may be multiplied indefinitely by asexual reproduction. The selection of individuals is replaced by the much more effective selection of clones and leads to rapid statistical advance which, however, comes to an end with reduction to a single successful clone. On the other hand a new cross (before reduction to a single clone) may provide a new field of variation making possible a repetition of the process at a higher level. This method has been a favorite of the plant breeder and is perhaps the most successful yet devised for human control of evolution in those cases to which it can be applied at all. Under natural conditions, alternation of asexual and sexual reproduction is characteristic of many organisms and doubtless has played an important rôle in their evolution.

The demonstration of the evolutionary advantages of an alternation of the two modes of reproduction seems to prove too much. Asexual re-

production is practically absent in the most complex group of animals, the vertebrates, and is rather sporadic in its occurrence elsewhere. The purpose of the present paper has been to investigate the statistical situation in a population under exclusive sexual reproduction in order to obtain a clear idea of the conditions for a degree of plasticity in a species which may make the evolutionary process an intelligible one.

First may be mentioned briefly a modification of the meiotic mechanism which has been introduced only qualitatively into the investigation where at all. This is the aggregation of genes into more or less persistent systems, the chromosomes. Complete linkage cuts down variability by preventing recombination. Wholly random assortment gives maximum recombination but does not allow any important degree of persistence of combinations once reached. An intermediate condition permits every combination to be formed sooner or later and gives sufficient persistence of such combinations to give a little more scope to selection than in the case of random assortment. Close linkage, moreover, brings about a condition in which selection tends to favor the heterozygote against both homozygotes and so helps in maintaining a store of unfixed factors in the population.

Resrtiction of size of population, measured by $1/2N$, is a factor of homogeneity and conversely with increase of size. The effects of restricted size may also be balanced by those of occasional external hybridization, measured by m.

Environmental pressure on the species as a whole is a factor of homogeneity. It has been urged by some that because natural selection is a factor which reduces variability, and most conspicuously by eliminating extreme types, it cannot be the guiding principle in adaptive evolution. From the viewpoint of evolution as a moving equilibrium, however, the guiding principle may be found on the conservative as well as on the radical side. The selection coefficient, s, depends on the balance between environmental pressure and individual adaptability. High development of the latter permits the survival of genetically diverse types in the face of severe pressure.

Subdivision of a population into almost completely isolated groups, whether by prevailing self fertilization, close inbreeding, assortative mating, by habitat or by geographic barriers is a factor of heterogeneity with effects measured by $1/2N_1$, N_1 being here the size of the subgroup. This factor may be balanced by crossbreeding between such groups, measured by m_1.

It is interesting to note that restriction of population size is a factor of homogeneity or of heterogeneity for the species, depending on whether it relates to the species as a whole or to subgroups and conversely with the crossbreeding coefficient. Similarly the selection pressures of varied environments within the range of the species (s_1) constitute factors of heterogeneity, restrained from excessive genetic effect by the same individual adaptability which appears in the opposite column in relation to the general environment of the species. Individual adaptability is, in fact, distinctly a factor of evolutionary poise. It is not only of the greatest significance as a factor of evolution in damping the effects of selection and keeping these down to an order not too great in comparison with 1/4N and u, but is itself perhaps the chief object of selection. The evolution of complex organisms rests on the attainment of gene combinations which determine a varied repertoire of adaptive cell responses in relation to external conditions. The older writers on evolution were often staggered by the seeming necessity of accounting for the evolution of fine details of an adaptive nature, for example, the fine structure of all of the bones. From the view that structure is never inherited as such, but merely types of adaptive cell behavior which lead to particular structures under particular conditions, the difficulty to a considerable extent disappears. The present difficulty is rather in tracing the inheritance of highly localized structural details to a more immediate inheritance of certain types of cell behavior.

Lability as the condition for evolution

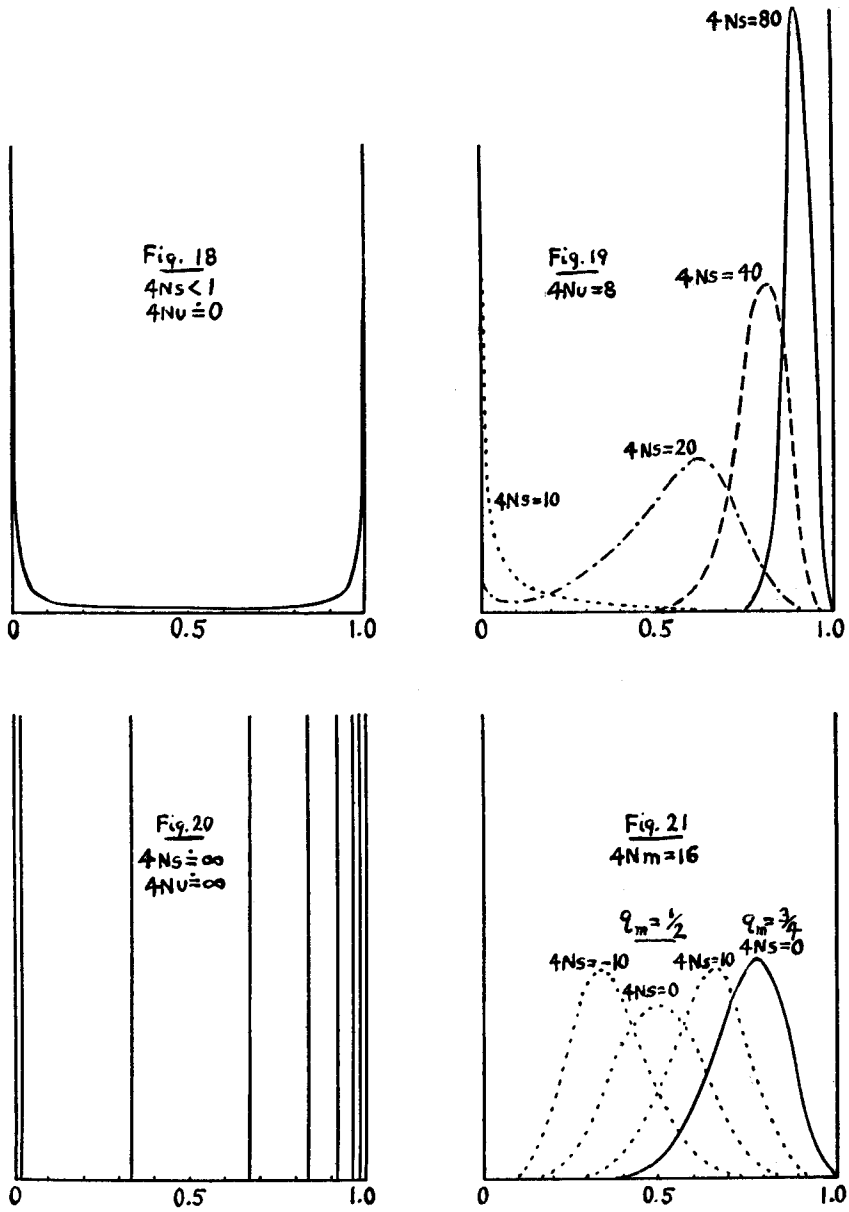
The statistical effects of the more important of these factors in a freely interbreeding population are brought together in the formula

$$y = Ce^{4Nsq}q^{-1}(1 - q)^{4Nu-1}.$$

The term 4Nv in the exponent of q is here assumed to be negligible and the terms applicable in case of external hybridization are also omitted.

Consider first the situation in a small population in which 1/4N is much greater than u and than s (figure 18). Nearly all genes are fixed in one phase or another. Even rather severe selection is without effect. There is no equilibrium for individual genes. They drift from one state of fixation to another in time regardless of selection, but the rate of transfer is extremely slow. Such evolution as there is, is random in direction and tends toward extinction of the group.

Consider next the opposite extreme, a very large undivided population under severe selection. Assume that s is in general much greater than u and that the latter is much greater than 1/4N. There is almost complete



FIGURES 18 to 21.—Distributions of gene frequencies in relation to size of population, selection, mutation and state of subdivision. Figure 18. Small population, random fixation or loss of genes ($y = Cq^{-1}(1-q)^{-1}$. Figure 19. Intermediate size of population, random variaton of gene frequencies about modal values due to opposing mutation and selection ($y = Ce^{4Nsqq-1}(1-q)^{4Nu-1}$. Figure 20. Large population, gene frequencies in equilibrium between mutation and selection ($q = 1-u/s$, etc.). Figure 21. Subdivisions of large population, random variation of gene frequencies about modal values due to immigration and selection. ($y = Ce^{4Nsqq_4Nmq_m-1}(1-q)^{4Nm(1-q_m)-1}$.

fixation of the favored gene for each locus. Here also there is little possibility of evolution. There would be complete equilibrium under uniform conditions if the number of allelomorphs at each locus were limited. With an unlimited chain of possible gene transformations, new favorable mutations should arise from time to time and gradually displace the hitherto more favored genes but with the most extreme slowness even in terms of geologic time.[15]

Even if selection is relaxed to such a point that the selection coefficients of many of the genes are not much greater than mutation rates, the conditions are not favorable for a rapid evolution (figure 20). The amount of variability in the population may be great, maximum in fact, but if the distributions of gene frequencies are closely concentrated about single values, the situation approaches one of complete equilibrium and hence of complete cessation of evolution. At best an extremely slow, adaptive, and hence probably orthogenetic advance is to be expected from new mutations and from the effects of shifting conditions.

It should be added that a relatively rapid shift of gene frequencies can be brought about in this case by vigorous increase in the intensity of selection. The effects of unopposed selection of various sorts and in various relations of the genes has been studied exhaustively by HALDANE, with regard to the time required to bring about a shift of gene frequency of any required amount. The end result, however, is the situation previously discussed. The rapid advance has been at the expense of the store of variability of the species and ultimately puts the latter in a condition in which any further change must be exceedingly slow. Moreover, the advance is of an essentially reversible type. There has been a parallel movement of all of the equilibria affected and on cessation of the drastic selection, mutation pressure should (with extreme slowness) carry all equilibria back to their original positions. Practically, complete reversibility is not to be expected, and especially under changes in selection which are more complicated than can be described as alternately severe and relaxed. Nevertheless, the situation is distinctly unfavorable for a continuing evolutionary process.

Thus conditions are unfavorable for evolution both in very small and in very large, freely interbreeding, populations, and largely irrespective of severity of selection. We have next to consider the intermediate situa-

[15] This, nevertheless, seems to be the case which FISHER (1930) considers most favorable to evolution. The greatest difference between our conclusions seems to lie here. His theory is one of complete and direct control by natural selection while I attribute greatest immediate importance to the effects of incomplete isolation.

tion in which s is not much greater than u for many genes and the latter is not much greater than 1/4N. Such a case is illustrated in figure 19. The size of population is sufficient to prevent random fixation of genes, but insufficient to prevent random drifting of gene frequencies about their mean values, as determined by selection and mutation. It is to be supposed that the relations of the selection and mutation coefficients vary from factor to factor. The more indifferent ones drift about through a wide range of frequencies in the course of geologic time while those under more severe selection oscillate about positions close to complete fixation. In any case, all gene frequencies are continually changing even under uniform environmental conditions. But the selection coefficients themselves are in general to be considered functions of the entire array of gene frequencies and will therefore also be continually changing. The probability arrays of some genes will travel to the right and close up as their selection coefficients stiffen, while some of the genes which have been nearly fixed will come to be less severely selected and their probability arrays will shift to the left and open out or even move to the extreme left under displacement by another allelomorph. A continuous and essentially irreversible evolutionary process thus seems inevitable even under completely uniform conditions. The direction is largely random over short periods but adaptive in the long run. The less the variation of gene frequency about its mean value, the closer the approach to an adaptive orthogenesis. Complete separation of the species into large subspecies should be followed by rather slow more or less closely parallel evolutions, if the conditions are similar, or by adaptive radiation, under diverse conditions, while isolation of smaller groups would be followed by a relatively rapid but more largely nonadaptive radiation.

As to rate, since the process depends mainly on the value of 1/4N, assumed to be somewhat less than u (and s) the process cannot be as rapid as one due temporarily to either unopposed selection or unopposed mutation pressure. Hundreds of thousands of generations seem to be required at best for important nonadaptive evolutionary changes of the species as a whole; while adaptive advance, depending on the chance attainment of favorable combinations would be much slower. Even so the process is much the most rapid non-self-terminating one yet considered.

In reaching the tentative conclusion that the situation is most favorable for evolution in a population of a certain intermediate size, one important consideration has been omitted. This is the tendency toward subdivision into more or less completely isolated subgroups in widely distributed populations. Within each subgroup there is a distribution of gene fre-

quencies dependent largely on its own size (N_1), amount of crossing with the rest of the species (m_1), selection due to local conditions (s_1) and the mean gene frequency of migrants from the rest of the species (q_m). It may be assumed that $1/4N_1$ is so much larger than u that mutation pressure (and also the average selection coefficient, s, for the whole species) can be ignored.

$$y = Ce^{4N_1s_1q}q^{4N_1m_1q_m-1}(1 - q)^{4N_1m_1(1-q_m)-1}.$$

Gene frequency in each subgroup oscillates about a mean value, which is that of the whole species only if conditions of selection are uniform. Figure 21 represents various cases. The random variations of gene frequency have effects similar to those described above within each group. The result is a partly nonadaptive, partly adaptive radiation among the subgroups. Those in which the most successful types are reached presumably flourish and tend to overflow their boundaries while others decline, leading to changes in the mean gene frequency of the species as a whole. In this case, the rate of evolution should be much greater than in the previous cases. The coefficients $1/4N_1$ and s_1 may be relatively large and bring about rapid differentiation of subgroups, while the competition between subgroups will bring about rapid changes in the gene frequencies of the species as a whole. The direction of evolution of the species as a whole will be closely responsive to the prevailing conditions, orthogenetic as long as these are constant, but changing with sufficiently long continued environmental change.

A question which requires consideration is the effect of alternation of conditions, large and small size of population, severe and low selection. The effects of changes in the conditions of selection have already been touched upon. Persistence of small numbers or of severe selection for such periods of time as to bring about extensive fixation of factors compromises evolution for a long time following, there being no escape from fixation except by mutation pressure. Many thousands of generations may be required after restoration to large size and not too severe selection, before evolutionary plasticity is restored. Short time oscillations in population number or severity of selection, on the other hand, probably tend to speed up evolutionary change by causing minor changes in gene frequency.

Control of evolution

With regard to control of the process, it is evident that little is possible either within a small stock or a freely interbreeding large one. Even drastic

selection is of little effect in the former, and in the latter, while it may bring about a rapid immediate change in the particular respect selected, this must be at the expense of other characters, and in any case, soon leads to a condition in which further advance must wait on the occurrence of mutations more favorable than those fixed by the selection. The limitations in this case have been well brought out in a recent discussion by KEMP (1929). Maximum continuous progress in a homogeneous population requires an intensity of selection for each of the more indifferent genes not much greater than its mutation rate and also a certain size of population. Even so, the direction of advance is somewhat uncertain and the rate to be measured in geologic time.

If infrequency of mutation is the limiting factor here, it would seem that a considerable increase in the rate of evolution should be made possible by a speeding up of mutation, as by X-rays. There is a limit, however, imposed by the prevailingly injurious character of mutations. Even the most rigorous culling of individuals means in general, only a low selection coefficient (in absolute terms) for each of the presumably numerous unfixed genes, which are not in themselves lethal or sublethal in effect. Such culling would become insufficient to hold mutation pressure in check when the latter had increased beyond a certain point ($u > s$). Moreover, as the number of unfixed genes becomes greater under an increased mutation rate, the smaller becomes the separate gene selection coefficients, making it certain that mutation rate could not increase very much before the possibility of effective selection (in all respects at once) rather than infrequency of mutation would become the limiting factor. With respect to lethal mutations, it has already been noted that the observed natural time rate in Drosophila is such as would mean immediate extinction, if transferred to the human species. It is clear that an evolution in the direction of increased gene stability, rather than mutability, has been a necessary phase, in the evolution of the longer lived higher animals. This makes it unlikely that a general increase in mutation rate would increase the rate of evolutionary advance along adaptive lines.

The only practicable method of bringing about a rapid and non-self-terminating advance seems to be through subdivision of the population into isolated and hence differentiating small groups, among which selection may be practiced, but not to the extent of reduction to only one or two types (WRIGHT 1922a). The crossing of the superior types followed by another period of isolation, then by further crossing and so on *ad infinitum* presents a system by means of which an evolutionary advance through the field of possible combinations of the genes present in the original stock, and

arising by occasional mutation, should be relatively rapid and practically unlimited. The occasional use of means for increasing mutation rate within limited portions of the population should add further to the possibilities of this system.

Agreement with data of evolution

We come finally to the question as to how far the characteristics of evolution in nature can be accounted for on a Mendelian basis. A review of the data of evolution would go far beyond the scope of the present paper. It may be suggested, however, that the type of moving equilibrium to be expected, according to the present analysis, in a population comparable to natural species in numbers, state of subdivision, conditions of selection, individual adaptability, etc. agrees well with the apparent course of evolution in the majority of cases, even though heredity depend wholly on genes with properties like those observed in the laboratory. Adaptive orthogenetic advances for moderate periods of geologic time, a winding course in the long run, nonadaptive branching following isolation as the usual mode of origin of subspecies, species and perhaps even genera, adaptive branching giving rise occasionally to species which may originate new families, orders, etc.; apparent continuity as the rule, discontinuity the rare exception, are all in harmony with this interpretation.

The most serious difficulties are perhaps in apparent cases of nonadaptive orthogenesis on the one hand and extreme perfection of complicated adaptations on the other. In so far as extreme degeneration of organs is concerned, there is little difficulty—this is to be expected as a by-product of other evolutionary changes. Because of their multiple effects, there can be no really indifferent genes, whatever may be true of organs which have been reduced beyond a certain size. Zero as the value of a selection coefficient is merely a mathematical point between positive and negative values. It is common observation that mutations are more likely to reduce the development of an organ than to stimulate it. It follows that evolutionary change in general will have as a by product the gradual elimination of indifferent organs. Nonadaptive orthogenesis of a positive sort, increase of size of organs to a point which threatens the species, constitutes a more difficult problem, if a real phenomenon. Probably many of the cases cited are cases in which the line of evolution represents the most favorable immediately open to a species doomed by competition with a form of of radically different type or else cases in which selection based on individual advantage leads the species into a cul-de-sac. The nonadaptive differentiation of small subgroups and the great effectiveness of subsequent

selection between such groups as compared with that between individuals seem important factors in the origin of peculiar adaptations and the attainment of extreme perfection. It is recognized that there are specific cases which seem to offer great difficulty. This should not obscure the fact that the bulk of the data indicate a process of just the sort which must be occurring in any case to some extent as a statistical consequence of the known mechanism of heredity. The conclusion seems warranted that the enormous recent additions to knowledge of heredity have merely strengthened the general conception of the evolutionary process reached by DARWIN in his exhaustive analysis of the data available 70 years ago.

"Creative" and "emergent" evolution

The present discussion has dealt with the problem of evolution as one depending wholly on mechanism and chance. In recent years, there has been some tendency to revert to more or less mystical conceptions revolving about such phrases as "emergent evolution" and "creative evolution." The writer must confess to a certain sympathy with such viewpoints philosophically but feels that they can have no place in an attempt at scientific analysis of the problem. One may recognize that the only reality directly experienced is that of mind, including choice, that mechanism is merely a term for regular behavior, and that there can be no ultimate explanation in terms of mechanism—merely an analytic description. Such a description, however, is the essential task of science and because of these very considerations, objective and subjective terms cannot be used in the same description without danger of something like 100 percent duplication. Whatever incompleteness is involved in scientific analysis applies to the simplest problems of mechanics as well as to evolution. It is present in most aggravated form, perhaps, in the development and behavior of individual organisms, but even here there seems to be no necessary limit (short of quantum phenomena) to the extent to which mechanistic analysis may be carried. An organism appears to be a system, linked up in such a way, through chains of trigger mechanisms, that a high degree of freedom of behavior as a whole merely requires departures from regularity of behavior among the ultimate parts, of the order of infinitesimals raised to powers as high as the lengths of the above chains. This view implies considerable limitations in the synthetic phases of science, but in any case it seems to have reached the point of demonstration in the field of quantum physics that prediction can be expressed only in terms of probabilities, decreasing with the period of time. As to evolution, its entities, species and ecologic systems, are much less closely knit than individual organisms.

One may conceive of the process as involving freedom, most readily traceable in the factor called here individual adaptability. This, however, is a subjective interpretation and can have no place in the objective scientific analysis of the problem.

SUMMARY

The frequency of a given gene in a population may be modified by a number of conditions including recurrent mutation to and from it, migration, selection of various sorts and, far from least in importance, mere chance variation. Using q for gene frequency, v and u for mutation rates to and from the gene respectively, m for the exchange of population with neighboring groups with gene frequency q_m, s for the selective advantage of the gene over its combined allelomorphs and N for the effective number in the breeding stock (much smaller as a rule than the actual number of adult individuals) the most probable change in gene frequency per generation may be written:

$$\Delta q = v(1 - q) - uq - m(q - q_m) + sq(1 - q)$$

and the array of probabilities for the next generation as $[(1-q-\Delta q)a + (q+\Delta q)A]^{2N}$. The contribution of zygotic selection (reproductive rates of aa, Aa and AA as $1-s^1:1-hs^1:1$) is $\Delta q = s^1q(1-q)[1-q+h(2q-1)]$. In interpreting results it is necessary to recognize that the above coefficients are continually changing in value and especially that the selection coefficient of a particular gene is really a function not only of the relative frequencies and momentary selection coefficients of its different allelomorphs but also of the entire system of frequencies and selection coefficients of non-allelomorphs. Selection relates to the organism as a whole and its environment and not to genes as such. The mutation rate to a gene (v) can usually be treated as of negligible magnitude assuming the prevalence of multiple allelomorphs.

In a population so large that chance variation is negligible, gene frequency reaches equilibrium when $\Delta q = 0$. Among special cases is that of opposing mutation rates $\left(q = \frac{v}{u+v}\right)$, of selection against both homozygotes $\left(q = \frac{1-h}{1-2h}\right)$, of mutation against genic selection $\left(q = 1 - \frac{u}{s}\right)$, of mutation against zygotic selection $\left(q = 1 - \frac{u}{hs^1}\right.$ unless h approaches 0, when $\left. q = 1 - \sqrt{\frac{u}{s}}\right)$, of selection and migration $\left(q = 1 - \frac{m}{s}(1 - q_m)\right)$ or

$-\frac{mq_m}{s}$ if s is much greater than m, $q = q_m\left(1+\frac{s}{m}(1-q_m)\right)$ if s is much smaller than m, while the values $q = \sqrt{q_m}$ or $1-\sqrt{1-q_m}$ when $s = \pm m$ illustrate the intermediate case).

Gene frequency fluctuates about the equilibrium point in a distribution curve, the form of which depends on the relations between population number and the various pressures. The general formula in the case of a freely interbreeding group, assuming genic selection, is

$$y = Ce^{4Nsq}q^{4N[mq_m+v]-1}(1-q)^{4N[m(1-q_m)+u]-1}.$$

The correlation between relatives is affected by the form of the distribution of gene frequencies through FISHER's "dominance ratio." It appears that this is less than 0.20 in small populations under low selection but may even approach 1 in large populations under severe selection against recessives.

In a large population in which gene frequencies are always close to their equilibrium points, any change in conditions other than population number is followed by an approach toward the new equilibria at rates given by the Δq's. Great reduction in population number is followed by fixation and loss of genes, each at the rate 1/4N per generation, where N refers to the new population number. This applies either in a group of monoecious individuals with random fertilization or, approximately, in one equally divided between males and females (9.6 percent instead of 12.5 percent, however, under brother-sister mating, N = 2). More generally with an effective breeding stock of N_m males and N_f females, the rates of fixation and of loss are each approximately $(1/16N_m+1/16N_f)$ until mutation pressure at length brings equilibrium in a distribution approaching first the form $y = C(1-q)^{-1}$ with decay at rate u and ultimately $Cq^{-1}(1-q)^{-1}$. The converse process, great increase in the size of a long inbred population, is followed by a slow approach toward the new equilibrium at a rate dependent in the early stages on mutation pressure.

With respect to genes which are indifferent to selection, the mean frequency is always $q = v/(u+v)$. The variance of characters, dependent on such genes, is proportional (at equilibrium) to population number up to about $N = 1/4u$. Beyond this, there is approach of variance to a limiting value.

In the presence of selection (s considerably greater than 2u) the mean frequency at equilibrium varies between approximate fixation of the favored genes ($q = 1 - u/s$) in large populations and approximate, if not complete, fixation of mutant allelomorphs ($q = v/(u+v)$) in small popula-

tions, the rate of change from one state to the other being the mutation rate (u). A consequence is a slow but increasing tendency to decline in vigor in inbred stocks, to be distinguished from the relatively rapid but soon completed fixation process, described above as occurring at rate 1/2N. The variance of characters in this as in the preceding case, is approximately proportional to population number up to a certain point (N less than 1/4s) and above this rapidly approaches a limiting value. Variance is inversely proportional to the severity of selection in large populations unless the selection is very slight but in small populations is little affected by selection unless the latter is very severe (s greater than 1/4N).

Evolution as a process of cumulative change depends on a proper balance of the conditions, which, at each level of organization—gene, chromosome, cell, individual, local race—make for genetic homogeneity or genetic heterogeneity of the species. While the basic factor of change—the infrequent, fortuitous, usually more or less injurious gene mutations, in themselves, appear to furnish an inadequate basis for evolution, the mechanism of cell division, with its occasional aberrations, and of nuclear fusion (at fertilization) followed at some time by reduction make it possible for a relat vely small number of not too injurious mutations to provide an extensive field of actual variations. The type and rate of evolution in such a system depend on the balance among the evolutionary pressures considered here. In too small a population (1/4N much greater than u and s) there is nearly complete fixation, little variation, little effect of selection and thus a static condition modified occasionally by chance fixation of new mutations leading inevitably to degeneration and extinction. In too large a freely interbreeding population (1/4N much less than u and s) there is great variability but such a close approach to complete equilibrium of all gene frequencies that there is no evolution under static conditions. Change in conditions such as more severe selection, merely shifts all gene frequencies and for the most part reversibly, to new equilibrium points in which the population remains static as long as the new conditions persist. Such evolutionary change as occurs is an extremely slow adaptive process. In a population of intermediate size (1/4N of the order of u) there is continual random shifting of gene frequencies and a consequent shifting of selection coefficients which leads to a relatively rapid, continuing, irreversible, and largely fortuitous, but not degenerative series of changes, even under static conditions. The rate is rapid only in comparison with the preceding cases, however, being limited by mutation pressure and thus requiring periods of the order of 100,000 generations for important changes.

Finally in a large population, divided and subdivided into partially isolated local races of small size, there is a continually shifting differentiation among the latter (intensified by local differences in selection but occurring under uniform and static conditions) which inevitably brings about an indefinitely continuing, irreversible, adaptive, and much more rapid evolution of the species. Complete isolation in this case, and more slowly in the preceding, originates new species differing for the most part in nonadaptive respects but is capable of initiating an adaptive radiation as well as of parallel orthogenetic lines, in accordance with the conditions. It is suggested, in conclusion, that the differing statistical situations to be expected among natural species are adequate to account for the different sorts of evolutionary processes which have been described, and that, in particular, conditions in nature are often such as to bring about the state of poise among opposing tendencies on which an indefinitely continuing evolutionary process depends.

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Short- to Mid-Term Effects of Ovariectomy on Bone Turnover, Bone Mass and Bone Strength in Rats

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To determine the short- to mid-term effects of ovariectomy on bone turnover, bone mass and bone strength in rats. SD rats aged 12 weeks were randomly divided into No-treatment, Sham and OVX groups. The rats were sacrificed for sample collection at week 0, week 4 and week 18 after surgical operation. Chemistries in serum and urine were measured by standard colorimetric methods and bone turnover markers were measured by ELISA kits. Bone mass and bone strength were determined using pQCT system and three-point bending tests, respectively. At week 4, OVX rats showed drastic increase in the level of urine Ca, P and DPD. At week 18, in OVX rats the levels of serum ALP, urine DPD and Ca were much higher and the level of serum Ca was much lower when comparing with Sham rats. Ovariectomy produced significant reduction in cancellous BMD, total BMD and SSI of proximal tibial metaphysis rapidly at week 4 and continuously at week 18 after surgical operation. However, no marked changes of bone mass and bone strength were found in the diaphysis of tibia and femur, respectively. The current study concluded that ovariectomy induced the uncoupling of bone turnover, and the proximal metaphysis of long bone was the sensitive site for the short- to mid-term effect of ovariectomy, demonstrated as the markers of bone mass and stress strain index.

Key words ovariectomy; osteoporosis; bone turnover; bone mass; bone strength

Osteoporosis (OP) that is associated with ovarian hormone deficiency following menopause is by far the most common cause of age-related bone loss. Postmenopausal osteoporosis (POP) has become a major problem with significant morbidity and mortality.[1)] Bone loss induced by ovariectomy in rats has been widely used as a model of POP and has been validated as a clinically relevant model of this condition in human.

Peripheral quantitative computed tomography (pQCT) and dual energy X-ray absorptiometry (DEXA) are non-invasive bone densitometry methods used in clinical settings for assessment of bone density as well as the efficacy of treatment for osteoporosis.[2,3)] pQCT provides measurement of volumetric bone densities (vBMD, total and cortical bone mineral density) as well as a set of geometric variables including cross-sectional area; while DEXA provides measurements of bone mineral content (BMC) and the area of the scanned region and areal bone mineral density. Although DEXA is one of the most commonly used clinical methods for measurement of bone mass, it is not ideal for use in animal studies and clinical trials due to its inability to distinguish between cancellous and cortical bone[4)] and provide limited information regarding bone architecture.[5)] In contrast, pQCT is now the method of choice as it can provide vBMD, differentiate cortical and trabecular bone compartments and perform *in vivo* measurement of histomorphometric parameters like endosteal and periosteal circumferences, total and cross-sectional bone areas.[6)]

As an alternative to four-point bending testing, three-point bending testing has become more widely used for the evaluation of whole bone strength. Although the two testing modalities are complementary to some extent, when describing the mechanical characteristics of bone, it appears that four-point bending testing has some disadvantages, which requires that the force at each loading point be equal. This requirement is simple to achieve in regularly shaped specimens but difficult to achieve in whole bone tests.[7)] Therefore, three-point bending is used more often for measuring the mechanical properties of bones from rodents.

The differential responses of bone properties to the mid- to long-term effects of ovariectomy in rats has been widely studied,[8—13)] and simultaneously efficacy testing of potential chemicals for anti-POP in ovariectomized (OVX) rats was also conducted in a duration of mid- to long time. Previous studies showed that the earliest statistically significant time of cancellous bone loss of rats in the proximal tibial metaphysis (PTM), lumbar vertebral body (LVB) and femoral neck (FN) occurring at 14, 60 and 30 d post ovariectomy, respectively, and the time of 50% bone loss for the PTM was 30—60 d,[8,9)] and for LVB and FN was 180—270 d.[10,11)] While the earliest changes in cortical bone width and medullary cavity size to be between 90—120 d and to reach a steady state often 180 d or more.[12,13)] These suggested that influence of ovariectomy on bones showed site- and time-specific development.

The research purpose and content of previous studies are very different from the present study. Previous studies investigated the long-term effects of ovariectomy or ageing on animal skeletons, furthermore, bone parameters studied were limited to bone mass and peak load in bone strength, neglecting bone morphological parameters, predicted bone strength parameters and other structural mechanical strength (including break load, deformation, energy). Thus, few systemic reports about the short- to mid-term effects of ovariectomy in appendicular bones of rats are available. Against this background, we investigated short- to mid-term effects of ovariectomy on bone turnover markers, bone mass, bone morphologic and predicted bone strength using a pQCT system, and

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invasive bone strength using three-point bending test in rats from 4 to 18 weeks post operation.

MATERIALS AND METHODS

Animals and Experimental Protocol Forty-eight virgin female Sprague-Dawley rats aged 12 weeks (225±6.9 g) were purchased from GuangZhou University of Traditional Chinese Medicine (GuangZhou, China). The rats were housed in a room which provided alternating 12 h periods of light and dark with the room temperature at 23±1 °C and humidity 55±5%. All animals were allowed free access to distilled water and fed on a commercial diet. Husbandry of the animals was based on the NIH Guide for Care and Use of Laboratory Animals.[14] After a week of acclimatization, the rats were divided at random into three groups as followed: No-treatment control (NT, n=20); Sham-operated (Sham, n=14) and ovariectomized (OVX, n=14). The animals in each group were randomly assigned to two subgroups based on the duration of ovariectomy, either 4 weeks (NT, n=6; Sham, n=6; OVX, n=6) or 18 weeks (NT, n=8; Sham, n=8; OVX, n=8). The residual normal rats (n=6) were as baseline control at week 0. Urine and serum was collected after 0, 4, 18 weeks of operation, and the tibias and femurs were dissected out and cleaned of all soft tissue, then wrapped in saline-soaked towels, sealed in plastic bags and stored −20 °C for further analysis.

Blood Test Serum calcium, phosphorus concentrations and alkaline phosphatase level were measured by standard colorimetric methods using an automatic analyzer type ALCYON 300i (Abbott Laboratories Ltd., U.S.A.). The kits were purchased from Zhongsheng Beikong Bio-technology and Science Inc. (Beijing, China). Serum osteocalcin level was determined using sandwich ELISA kit (Biomedical Technologies Inc., Stoughton, U.S.A.) for rat osteocalcin only.

Urinary Test The concentration of calcium, phosphorus and creatinine in the urine was measured by the same method as that of the serum samples. Urine deoxypyridinoline was assayed by competitive enzyme immunoassay using METRA™ DPD EIA kit (Quidel Corporation, San Diego, U.S.A.). Results of calcium, phosphorus and DPD were corrected for urinary concentration by creatinine.

Peripheral Quantitative Computerized Tomography (pQCT) Analysis Right tibias were thawed at room temperature before testing, and the bone mineral density of intact tibias was measured by peripheral quantitative computerized tomography (pQCT) with an XCT2000 machine (Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany) aided by specially designed software for small animals. Cone Phantom (Slice 1: 787 mm^2 Slice 2: 0.539 1/cm Slice 3: 0.333 1/cm Slice 4: 0.436 1/cm) and Standard Phantom (0.436 1/cm) were scanned to calibrate the instrument. The exact position of the tibia within the gantry core of the XCT machine was visualized with a fan beam from a 58 kV X-ray source with a current of 0.178 mA. Two sites of each tibia were scanned; the first site for trabecular bone was measured at 2.5 mm distal to the proximal end, and the second site for cortical bone was measured at 15 mm distal to the same position (Fig. 1). Humidity was maintained at 50 to 60% to ensure that the machine ran normally. Cancellous BMD (Cn.BMD), total BMD (To.BMD), BMC, cancellous bone area (Cn.Ar), total bone area (To.Ar) and stress strain index (SSI) in the first site and cortical BMD, cortical cross-sectional area and SSI in the second site were recorded as given by the pQCT software. SSI was calculated using the following formula: $SSI=\sum_{i=1,n} r_i^2 \times aCD/ND/r_{max}$ where r is the distance of a voxel to the center of gravity, r_{max} is the maximum distance of a voxel to the center of gravity (mm), a is the area of a voxel (mm^2), CD is the measured volumetric cortical density (mg/cm^2), and ND is the normal physiological density (1200 mg/cm^2).

Biomechanical Tests Rat femurs were removed from freezer and allowed to submerge in physiological saline solution to thaw and held at room temperature on the day of test. Bone strength was measured on intact femurs using a three-point bending test. Each specimen was placed on two supports spaced 15 mm apart and load was applied to the bone midway between the supports at a deformation rate of 2 mm/min until fracture occurred. The press head and the two support points were rounded to avoid shear load and cutting. The femurs were positioned such that bending occurred about the medial-lateral axis. Load–deformation curves were recorded during bending process using Hounsfield material testing machine (Model H10KM, Hounsfield Test Equipment Limited, U.K.). The following parameters shown in Fig. 2 reflecting structural properties of bones were calculated by automated computation: peak load (a measure of the maximum force that the bone withstood before fracture); peak deformation (distance traveled by the press head until peak point is reached); fracture load (a measure of the force that the bone fracture occurred); fracture deformation (distance traveled by

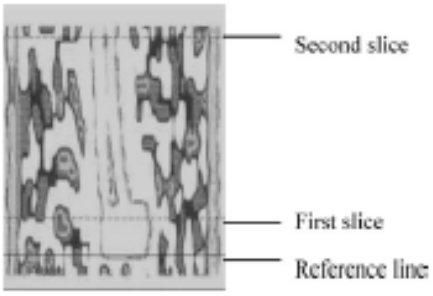


Fig. 1. The Analysis of Bone Mineral Density in Tibia
The reference line is selected at the proximal end of the tibia. The first slice (containing a high percentage of trabecular structure) is 2.5 mm from the reference line. The second slice (mainly cortical structure) is 15 mm from the reference line.

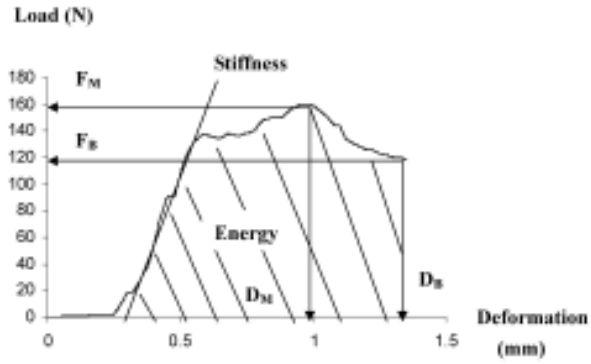


Fig. 2. Load–Deformation Curve in Three-Point Bending Tests and the Related Parameters for Structural Property

Table 1. Body Weight, Uterus Index and Chemistries in Serum and Urine

Groups	Body weight (g)	Uterus index (mg/g)	Serum Ca (mg/dl)	Serum P (mg/dl)	Urine Ca/Cr (mg/mg)	Urine P/Cr (mg/mg)
W0						
NT (n=6)	225.7±1.2	1.79±0.19	11.2±0.6	6.1±0.3	0.112±0.030	3.05±0.30
W4						
NT (n=6)	261.4±6.3	1.67±0.20	11.3±0.1	5.3±0.2	0.057±0.010	2.00±0.33
Sham (n=6)	255.6±6.3	1.70±0.11	11.4±0.3	4.9±0.3	0.037±0.010	2.11±0.13
OVX (n=6)	285.3±4.1[a)]	0.31±0.01[c)]	10.6±0.4	5.1±0.4	0.092±0.013[b)]	3.07±0.12[c)]
W18						
NT (n=8)	297.6±8.9	1.84±0.14	11.1±0.2	5.8±0.4	0.026±0.003	1.45±0.13
Sham (n=8)	303.1±12.6	1.87±0.13	10.9±0.2	5.7±0.2	0.035±0.004	1.62±0.06
OVX (n=8)	337.9±7.0[d)]	0.32±0.01[f)]	10.3±0.1[d)]	5.4±0.1	0.068±0.007[e)]	1.92±0.16

Data are expressed as mean±S.E.M. Means in columns with superscript are significantly different from Sham group. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ *vs.* Sham group (W4). *d*) $p<0.05$, *e*) $p<0.01$, *f*) $p<0.001$ *vs.* Sham group (W18). Ca, calcium; P, phosphorus; Cr, creatinine.

the press head until fracture point is reached); stiffness (slope of the linear part of the curve, a measure of the extrinsic rigidity of the femur) and energy (area under the curve, energy required to fracture the bone).

Statistical Analysis Data were expressed as the mean values±S.E.M. Inter-group differences were analyzed by one-way analysis of variance (ANOVA) and Turkey's test as post test after ANOVA was performed to compare the group means by the statistical software of GraphPad PRISM (Version 4.0). These parametric statistical tests could be used as the data were normally distributed. A p value less than 0.05 was considered statistically significant.

RESULTS

Body Weight and Uterus Weight Rats in all experimental groups had similar initial body weights. The body weight of the OVX rats were significantly increased at 4 weeks post operation ($p<0.05$ *vs.* Sham, Table 1), and it increased continuously in OVX group during the experimental duration. As expected, ovariectomy induced uterus atrophy in rats and the uterus index was decreased significantly ($p<0.001$ *vs.* Sham, Table 1).

Ca and P Level in Serum and Urine Chemistries in serum and urine for all groups studied were shown in Table 1. Serum Ca level in OVX rats was reduced slightly at 4 weeks, and significantly ($p<0.05$) at 18 weeks following ovariectomy, while serum P level showed no difference among groups in experimental period. At week 4, urinary excretion of Ca ($p<0.01$) and P ($p<0.001$) in OVX rats was much higher than that in Sham rats. At week 18, high urinary Ca excretion induced by ovariectomy was still observed, while, the OVX rats did not show a significant change in urinary P level.

Bone Turnover Markers Serum alkaline phosphatase (ALP) is the biomarker for bone formation, and urinary deoxypyridinoline (DPD, a product of the post-translational modification of collagen during bone resorption) is the biomarker for bone resorption. Ovariectomy resulted in a slightly reduction in the level of serum ALP in short time after ovary extraction and significantly elevation ($p<0.01$ *vs.* Sham, Fig. 3A) at week 18 after ovariectomy. For urine DPD/Cr, its level was significantly higher in OVX rats than that in Sham rats either at week 4 ($p<0.001$ *vs.* Sham, Fig. 3B) or at week 18 ($p<0.05$ *vs.* Sham, Fig. 3B). Moreover, the

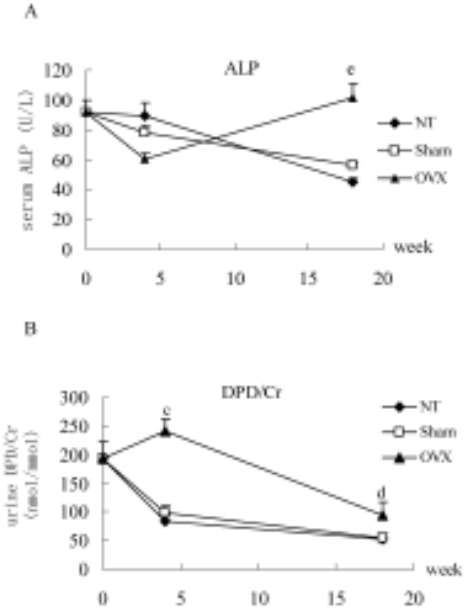


Fig. 3. Effect of Short- (4 Weeks) to Mid-Term (18 Weeks) of Ovariectomy in Rats on Bone Turnover Markers, Serum ALP (A) and Urine DPD/Cr (B)

Data are expressed as mean±S.E.M. n=6—8. [c]$p<0.001$ *vs.* Sham group at week 4. [d]$p<0.05$, [e]$p<0.01$ *vs.* Sham group at week 18.

level of serum ALP and urine DPD/Cr in NT and Sham rats were both reduced during the experimental period.

Bone Properties in Tibia Metaphysis and Diaphysis From the images of cancellous bone in the proximal tibial metaphysis scanned by pQCT (Fig. 4), it was clearly seen that ovariectomy resulted in the reduction of cancellous BMD, as demonstrated by a significant decrease in cancellous BMD (34.0% at week 4 and 28.1% at week 18, Table 2) and total BMD (20.9% at week 4 and 21.2% at week 18, Table 2) in OVX rats with regard to their corresponding Sham rats. Simultaneously cancellous BMC in tibial metaphysis was reduced ($p<0.05$) in OVX rats at week 18. SSI-p and SSI-x, index that estimates bone strength in torsion and bending, respectively, were declined ($p<0.05$) significantly 4 weeks after ovariectomy and remained the marked reduction ($p<0.05$) at week 18 compared to Sham rats. During the study period, bone cross-sectional area in tibial metaphysis was not altered by ovariectomy. Furthermore, bone mass, bone area and predicted bone strength in tibial diaphysis did

not achieve sufficient statistical power by the effects of short- to mid-term of ovariectomy (Table 3).

Bone Mechanical Property Ovariectomy produced no change in maximal force, break force or the deformation in femoral diaphysis compared to Sham rats (Table 4). Furthermore, neither energy absorption, required for breaking the bone, nor stiffness, represented the bone rigidity, reached statistical significance between OVX rats and Sham rats over the duration of the study.

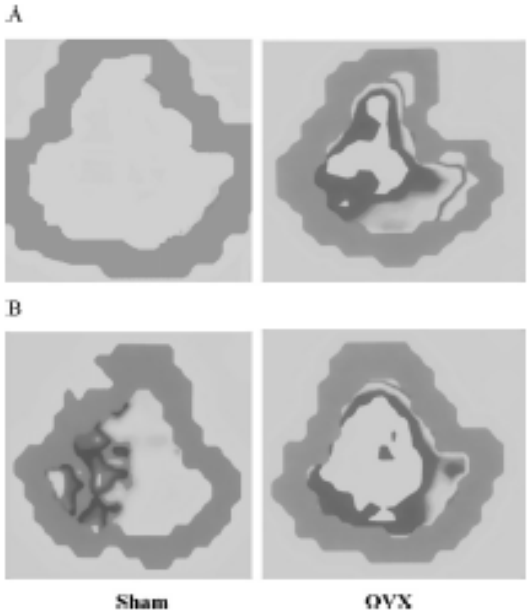


Fig. 4. Cross-Sectional Scanning of the Proximal Tibial Metaphysis in Sham Rats and OVX Rats at Week 4 (A) and Week 18(B)
Cancellous bone loss was observed in the OVX rats compared with Sham.

DISCUSSION

In the current study, we used 3-month-old rats based on the results of previous studies.[15,16] They compared the histomorphometric and chemical parameters and found that significant differences were observed only in the OVX rats at a young or young-mature age, *i.e.*, mainly at 1 to 3 months. Furthermore, the characteristics of bone loss in young rats were mostly similar to those of the aged rat model. Therefore, we selected 3-month-old rats having ovariectomy for

Table 3. Bone Properties in Tibial Diaphysis

Groups	Ct.BMD (mg/ccm)	Ct.Ar (mm^2)	SSI-p (mm^3)	SSI-x (mm^3)
W0				
NT (n=6)	955.4±10.9	3.11±0.12	2.30±0.11	1.40±0.08
W4				
NT (n=6)	1087.0±21.0	4.41±0.14	3.86±0.22	2.14±0.14
Sham (n=6)	1077.1±12.6	4.16±0.20	3.45±0.20	1.97±0.12
OVX (n=6)	1065.1±11.8	4.47±0.12	3.75±0.12	2.12±0.11
W18				
NT (n=8)	1125.7±15.8	5.03±0.16	4.13±0.22	2.24±0.12
Sham (n=8)	1149.6±8.9	5.06±0.17	4.20±0.20	2.19±0.11
OVX (n=8)	1111.5±12.8	5.13±0.11	4.14±0.16	2.15±0.08

Data are expressed as mean±S.E.M. Ct, cortical; BMD, bone mineral density; Ar, area; SSI-p, stress strain index-polar.

Table 2. Bone Properties in Tibial Metaphysis

Groups	Cn.BMD (mg/ccm)	To.BMD (mg/ccm)	BMC (mg/mm)	Cn.Ar (mm^2)	To.Ar (mm^2)	SSI-p (mm^3)	SSI-x (mm^3)
W0							
NT (n=6)	300.6±9.6	417.3±4.6	7.2±0.2	7.72±0.26	17.23±0.59	4.44±0.26	2.64±0.22
W4							
NT (n=6)	475.6±34.2	593.3±29.3	12.0±1.5	8.08±0.24	20.09±2.07	8.65±1.13	6.10±1.34
Sham (n=6)	484.9±28.0	582.7±19.5	10.7±0.5	8.19±0.27	18.27±0.60	8.83±0.55	5.70±0.30
OVX (n=6)	320.2±30.9[b]	460.8±12.1[c]	9.5±0.9	8.32±0.32	20.05±1.56	6.73±0.42[a]	4.40±0.38[a]
W18							
NT (n=8)	672.5±23.5	704.3±24.2	19.6±1.1	12.66±0.87	28.26±1.93	20.57±1.65	12.64±1.18
Sham (n=8)	655.9±16.6	682.3±14.0	20.3±0.9	12.53±1.00	27.99±2.22	20.60±2.31	12.80±1.31
OVX (n=8)	471.7±19.9[f]	537.9±17.1[f]	16.4±1.0[d]	13.86±0.95	30.93±2.11	13.02±1.32[d]	8.56±0.87[d]

Data are expressed as mean±S.E.M. Means in columns with superscript are significantly different from Sham group. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ *vs.* Sham group (W4). *d*) $p<0.05$, *e*) $p<0.01$, *f*) $p<0.001$ *vs.* Sham group (W18). Cn, cancellous; BMD, bone mineral density; BMC, bone mineral content; Ar, area; To, total; SSI-p, stress strain index-polar.

Table 4. Bone Mechanical Property in Femoral Diaphysis

Groups	F_{max} (N)	D_{max} (mm)	F_{bre} (N)	D_{bre} (mm)	E (N·mm)	S (N/mm)
W0						
NT (n=6)	92.8±3.6	0.62±0.02	64.9±5.6	1.26±0.06	87.6±4.8	289.7±22.4
W4						
NT (n=6)	136.4±3.6	0.60±0.03	111.9±9.0	0.88±0.12	88.3±13.7	412.3±59.1
Sham (n=6)	127.8±3.9	0.57±0.02	100.6±10.4	0.91±0.11	89.1±7.8	397.0±21.2
OVX (n=6)	133.9±3.1	0.61±0.02	99.4±7.7	1.02±0.19	78.7±6.8	388.9±6.1
W18						
NT (n=8)	159.6±6.7	0.59±0.03	144.8±10.4	0.70±0.04	75.7±4.7	449.5±16.9
Sham (n=8)	157.9±3.5	0.62±0.01	138.8±4.5	0.86±0.05	87.9±7.5	453.7±36.1
OVX (n=8)	146.3±5.4	0.57±0.02	138.3±6.2	0.69±0.05	69.5±8.4	412.8±22.4

Data are expressed as mean±S.E.M. F, load; D, deformation; E, energy; S, stiffness; max, maxium; bre, break.

the present investigation.

The present study demonstrated that rapid bone loss in rats following ovariectomy occurred in a site-specific fashion that cancellous osteopenia developed, while skeletal properties in the site of cortical-rich bone had no responses to ovariectomy, in our experimental period. In addition, short- and mid-term effects of ovariectomy resulted in different changes on bone turnover markers with serum ALP level decreased 4 weeks and increased 18 weeks following ovariectomy compared to sham rats, while urine DPD/Cr level was higher than sham rats in this study period.

In our study, bone loss manifested by BMD and BMC in the proximal tibial metaphysis, an area that represents cancellous bone, was observed 4 weeks after ovariectomy and lasted to 18 weeks post operation. Several studies have described similar findings related to ovariectomy lasting a few days to several weeks.[17,18] In the present study, we attempt to employ pQCT-derived parameters as non-invasive indicator for biomechanical properties of rat tibia in response to ovariectomy. Stress strain index (SSI, a variable that takes into account the amount of bone mass, its density, and its distribution around the neutral axis),[19] generated by pQCT analysis were being confirmed as a good estimate for assessment on bone strength.[19—21] The torsional and bending strength predicted by SSI-p and SSI-x, respectively, were also decreased rapidly in proximal tibial metaphysis after ovariectomy. Such change was also observed in a clinical study in which the torsional strength at the 4% proximal point to the distal end of human radius was reduced in German postmenopausal women above 52 years of age.[4] Nevertheless our study revealed that bone mass, predicted bone strength and bone morphology in the tibial diaphysis were not altered by the effect of short- to mid-term of ovariectomy in accordance with the previous reports that long time (at least 6 months) of ovariectomy induced bone loss at the mid-shaft of long bone.[17,22] Therefore, the changes of bone mass and bone strength in the proximal metaphysis in long bone could be applied in discovering new drugs and inspecting the efficacy of drugs for the prevention in a short- to mid-term for POP.

It is now generally believed that the occurrence of fracture is dependent not only on bone mass and density but also on bone structure and architecture.[23,24] Previous studies on the femurs of rats and mouse [20,25] has verified that there was a strong correlation between bone strength and BSI (bone strength index), which was the combination of cross-sectional moment of inertia (geometrical property) and BMD. No obvious influence of ovariectomy in short- to mid-term on bone strength in femoral diaphysis could be explained by the following two respects. Firstly, in this experimental period, cortical BMD in OVX rats was almost the same level as that in sham rats. Secondly, altered bone cross-sectional geometry for the adaptation to higher weight gain in OVX rats counteracted the affection of ovariectomy on bone mechanical property. However, this feedback regulation for load-bearing capacity will be overrun in long time by ovariectomy.

Instead of describing postmenopause as only a state of bone loss, it might be more appropriate to describe it as a state of rapid bone metabolism in which bone remodeling is high.[26,27] High bone remodeling was shown by the significant elevation of the level of bone turnover markers in serum and urine 18 weeks after ovariectomy in this study. Our study also showed that an increase in bone resorption occurred (at week 4) earlier than an increase in bone formation (at week 18). This suggested that the rate of bone resorption exceeded bone formation and bone remolding uncoupled in a short time after ovariectomy, which could account for the rapid cancellous bone loss in OVX rats in the present study and negative BMU (basic multicellular units) balance, a consequence of the delay in bone formation within each of the many more and new remodeling sites reported in previous study.[27] Since the bone resorption marker, urine DPD, is quite susceptible immediate to ovariectomy, it is thus conceivable that it is able to provide an early indication of an effect of candidate drugs on bone, while the bone formation marker, serum ALP, is useful in providing supporting evidence in evaluations on bone health in a mid- to long-time due to its responding more slowly to ovariectomy. In addition, an interesting result in our study was observed that serum ALP level showed an obvious trend of reduction by the short-term of ovariectomy, which corrects the classical opinion that the level of bone formation marker elevates due to high bone turnover after ovariectomy. In fact, previous results were from the studies conducted in a longer time after ovariectomy, which is consistent with our present result in the mid-term effect of ovariectomy on serum ALP level.

In conclusion, our study reveals that the proximal metaphysis, not the mid-shaft, of long bone is the sensitive site for the short- to mid-term effect of ovariectomy in rats. In addition, ovariectomy induces the uncoupling of bone turnover, demonstrated as the rapid increase of bone resorption and slow increase of bone formation by the short-term and mid-term effect of ovariectomy, respectively.

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