

Pasture mating behaviour of donkeys (*Equus minus*) at natural and induced oestrus

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Summary. The mating behaviour of 2 jacks, each with 21 non-pregnant jennies, was studied when the jennies were in natural oestrus and simultaneously induced oestrus. The main observations were: **efficient** pasture breeding at natural and induced oestrus, a territorial sociosexual structure, prolonged pre-copulatory interaction, gradual increase of mating activity up to 2 days before ovulation, a copulatory sequence similar to that of horses, vocalization of the jack as a conspicuous behaviour initiating pre-copulatory interaction, frequent heterotypical behaviour of jennies and active involvement of jennies in initiating pre-copulatory interaction.

Introduction

Numerous authors have described sociosexual behaviour of domestic horses (Collery, 1969; Pickett *et al.*, 1970; Collery, 1974; Asa *et al.*, 1979; Bristol, 1982; Ginther, 1983; McDonnell, 1986; Bristol, 1987), feral horses (Feist & McCullough, 1975, 1976; Klingel, 1982; Keiper & Houpt, 1984), Przewalski's horse (Kolter & Zimmermann, 1988), and plains, mountain and Grevy's zebras (Klingel, 1975). However, studies on social organization and reproductive behaviour of donkeys are few. A study of wild African and Asiatic asses revealed a territorial, **rather** than harem, type of social organization. These jacks defend their boundaries against **neighbouring** territorial males only under specific **conditions**, such as when a jenny is in oestrus (Klingel, 1977). In a 2-year study of domestic jack semen collection (M. Henry, M.M.F. Oliveira, C. Meira & E.L. Gastal, unpublished observations), the mean time from approach to ejaculation of jacks running free with an oestrous jenny varied from 12-26 min. During this time, prolonged Flehmen episodes, mounts without erection, partial drop of the penis, rapid and sudden withdrawal from the female and grazing were observed. In that study, unexplainable, repeated failure to collect semen from some individual jacks was noted. The rather long pre-copulatory interval in domestic jacks and the **difficulty** in collecting semen, compared with horses was noted also by Nishikawa (1959) and Kreuchauf (1984).

These findings, together with subject observations of slow sexual response of jacks, prompted questions concerning their reproductive management. This experiment was conducted to describe reproductive behaviour of domestic donkeys. To permit comparison with horses, our project design included pasture breeding of donkeys under simultaneously induced oestrous conditions similar to those described for horses by Bristol (1982, 1987).

Materials and Methods

Animals and study sites

The trials were conducted on donkey **breeding** farms near **Carlos** Chagas (Trial 1) and **Colina**, Brazil (Trial 2). In Trial 1, Jack A (12-years-old, 280 kg, **Pega**) was kept at **pasture** for 60 days starting in February 1989, with 21 grade jennies. In Trial 2, Jack B (3.5-years-old, 360 kg, Brazilian breed) was pastured for 39 days during January and February 1990 with 21 **Brazilian** jennies that had been together for at **least** 1.5 years before **the** experiment. Three jennies **from** Trial 1 and 10 **from**

Trial 2 had a foal at foot throughout the study. The jacks and jennies at pasture together were observed for 6 daylight hours on Day 1 and Days 7-10; and 12 daylight hours on Days 2-6 and 11-16 for Trial 1. For Trial 2, they were observed for 12 daylight hours on Days 1-18. For the remaining hours of these days, the jacks were separated from the jennies. On Days 17-60 in Trial 1 and Days 19-39 in Trial 2, the animals remained at pasture continuously.

In each trial, Jennies cycled naturally from Days 1-6. To induce oestrus, prostaglandin $F_{2\alpha}$ (10 mg, intramuscularly (i.m.)) was administered on Day 6 to all females not in oestrus.

The pastures were rectangular and approximately 15 hectares (± 37 acres) each. One was divided longitudinally by a creek (Trial 1) and the other had a pond near the fence (Trial 2). Each pasture was divided into 6 sectors by imaginary lines based on landmarks or easily observed fence marks made for this purpose. During the trials, ambient temperature varied from 26 to 42°C. Rain of less than 30 min duration occurred once in each trial.

Observations

Two observers were stationed at elevated sites on opposite sides of the pasture (Trial 1) or at 3 different elevated sites within the pasture (Trial 2). Primary focus was on the jack and secondary focus on the jennies. The observers recorded all specific interactive responses between adult donkeys (i.e. olfactory, aggression, vocalization, mounting, insertion, thrusting and ejaculation for the jack, approaching, posturing, jawing, ear and tail position for the jennies), and maintenance behaviours (grazing, resting, drinking, elimination, grooming) on a time base using a microcomputer event recorder, audio recorder and written notation. Ejaculation was identified by tail flagging and seminal reflux. The location of each animal relative to the sectors was recorded at 2-h intervals. At 15-min intervals, the jennies close to the jack were identified as part of the 'proximal group' and were considered to be sexually active if they stayed in the vicinity of the jack and postured to the jack at least once during the 15-min interval.

Observers recorded their subjective appraisal of each jenny's oestrous or dioestrous condition each day based on jawing, ear pinning and posturing to the jack.

Semen collection

Attempts were made to collect semen from the jacks by artificial vagina on the day before the start of the trial and every other day (alternating morning and afternoon) during natural and induced oestrus. Semen was also collected on Day 39 of Trial 2. All semen samples were evaluated as described by Kenney et al. (1983).

Ovarian activity

Ovarian activity was monitored by palpation per rectum every day during oestrus and every third day during dioestrus. For Trial 2, follicular growth was monitored by ultrasound. At least once a week, the jennies were bled by jugular venepuncture. Plasma was stored at -20°C until progesterone analysis was performed by radioimmunoassay (Vermeulen & Verdonck, 1976). The sensitivity of the assay was 0.5 ng/ml and the intra- and inter-assay coefficients of variation were 3.6 and 7.3%, respectively. Palpation per rectum, collection of blood samples and ultrasonography were performed during the evening of each observation day.

Statistical analysis

Proportional and quantitative data were evaluated by Chi squared tests and analysis of variance procedures, respectively. Pierson correlation techniques were used to evaluate associations.

Results

General breeding results

During Trials 1 and 2, 20 (95%) and 21 (100%) of the jennies, respectively, came into oestrus (Fig. 1) and 14 (67%) and 18 (85%) ovulated. The mean (\pm s.d.) length of ovulatory oestrus was 6 days (± 2.1); start of oestrus to ovulation was 5.4 days (± 1.7) and ovulation to end of oestrus was 0.7 (± 0.7) days ($n = 20$). Length of anovulatory oestrus was 2.6 (± 2.2) days ($n = 12$). One split ovulatory oestrus was observed. One of the jennies showing anovulatory oestrus ovulated during a subsequent oestrus that started 8 days later, and 1 was cycling but neither a large follicle nor ovulation was detected. The remaining jennies were not cycling, as indicated by the progesterone assay results (< 1 ng/ml plasma). Plasma progesterone concentrations (> 3 ng/ml) indicated that the detected ovulations resulted in active corpora lutea.

Pregnancy was diagnosed for all jennies by a Single palpation per rectum at least 40 days after ovulation. Of the 14 jennies that ovulated during Trial 1, 11 (78%) were diagnosed as pregnant after the

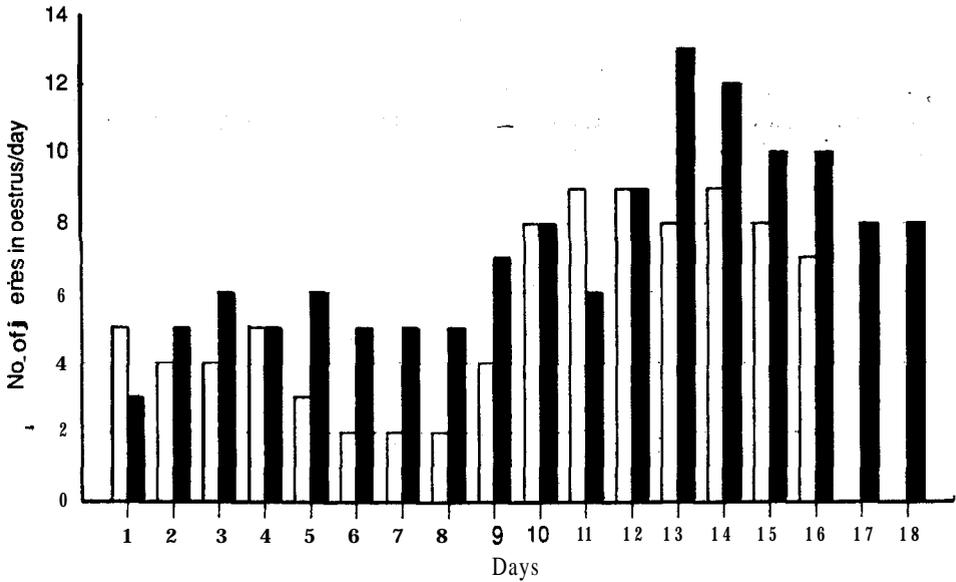


Fig. 1. Number of jennies showing oestrus per day during Trials 1 (□) and 2 (■).

first cycle and 3 (22%) after the second cycle. For Trial 2, ultrasonography of the uterus began on Day 10 after ovulation. An embryo was detected by Day 12 after ovulation in 11/16 (69%) jennies mated during the first cycle. Two jennies were not mated during the first ovulatory oestrus. All but 1 jenny became pregnant within 2 cycles. Early embryonic death was detected in 2 jennies during the first cycle and in 1 in the second cycle.

Sexual behaviour

Pre-copulatory interaction. In both trials, arrival of the jack at the paddock each morning evoked vocalization and vigorous general interaction with all jennies, followed by a more thorough individual teasing of one of the jennies. Teasing behaviour included naso-nasal contact, nibbling the head, neck, knee, and flank and sniffing parts of the body, mostly the perineal area. Teasing generally concluded with 1 or more mounts without erection. As the jack interacted with an individual jenny, other jennies (predominately those in oestrus) gathered in the vicinity. After each mount, the jacks grazed or rested for 22 (\pm 14.2) min before the next vocalization or period of teasing. During the rest period, the sexually active jennies remained nearby, but if they approached the male they were generally rebuffed by threatening or kicking behaviour.

Spontaneous vocalization by the male, and less frequently by a female in oestrus, was the signal for a period of pre-copulatory interaction. Occasionally, the approach of a jenny in oestrus was the stimulus for the jack to vocalize after his rest. Immediately after the jack's vocalization, the jennies in oestrus approached the male. Intense teasing usually followed. Vocalization by jennies was more frequent when they were in induced, as opposed to spontaneous, oestrus. During Trial 2, 12 of 21 jennies vocalized at least once; 78% of the total number of vocalizations came from jennies in oestrus.

The pattern of vocalization, general and individual teasing and retiring of the male was repeated several times. Vocalization appeared to attract oestrous jennies to the vicinity. Occasionally, intense teasing of an individual occurred, usually not culminating in mating with that particular individual. The long, intermittent periods of sexual stimulation ended with the jack achieving an erection and mating with a jenny, not necessarily the closest one. Erection and copulation occurred suddenly at the end of an intermittent 'rest' period.

Proximal group and sexually active jennies. A group of jennies stayed **close** to the jack **and** expressed both heterotypical and homotypical sexual behaviour. Heterotypical behaviour included mounting, herding/chasing, teasing and Flehmen response, which are infrequently observed in mares. At least one incident of heterotypical behaviour was exhibited by 37 of the 42 (88%) jennies. For Trials 1 and 2 combined, 169 mounts, 286 herding/chasing episodes, 62 teasing episodes and 99 **Flehmen** responses were observed. Mounting by jennies was more frequent during the second part of each trial when more jennies were showing **oestrus**. All mounts during Trial 1 and 52/63 (82%) in Trial 2 involved one jenny mounting another. During **Trial 2**, 1 jenny mounted the jack 10 times and another jenny mounted the jack once. Also during Trial 2, 46 of the 52 (88%) mounts involved jennies that were both in **oestrus**. On 3 occasions, only the jenny that mounted was in oestrus, on 2 occasions only the mounted jenny was in **oestrus**, and on 1 occasion, neither was in oestrus. Most heterotypical **herding/chasing** episodes occurred during intense mutual teasing, increasing in frequency when the jack was about to mate a jenny. Occasionally one jenny prevented the jack from mating another by kicking, biting, striking and pushing the jack off the jenny.

The proximal group was comprised mostly of jennies in oestrus. During natural oestrus in both **trials**, there was a clear separation of the proximal group from the remainder. During induced oestrus, the division was less distinct. The remaining jennies continued to graze independently in all sectors of the pasture. All pasture sectors were used by the jennies in a frequency varying from 14% for 1 sector to 75% for another in Trial 1 and from 7–49% in Trial 2.

From Day -8 to Day -3 before ovulation, individual jennies showed an increase in the time spent in the proximal and sexually active groups, as well as an increase in the number of approaches to the jack (Fig. 2). There was a significant difference among days during the periovulatory period ($P < 0.001$) in the number of approaches to the jack and in the sexually active group. Mean number of approaches by the jennies and by the jack from Day -8 to +1 were, respectively, 24.7 (3 1.7) and 6.3 (9.3) ($P < 0.001$) **per day** and per jenny.

During induced oestrus in both trials, strong relationships were formed between certain jennies who often approached the jack in pairs even if 1 of them was not in oestrus. Three such pairs were identified in Trial 1, and 6 in Trial 2; possibly because jennies in Trial 2 were pastured together before the experiment. With the increased number of oestrous jennies in the sexually active group during induced **oestrus**, the number of jennies in the proximal group also increased.

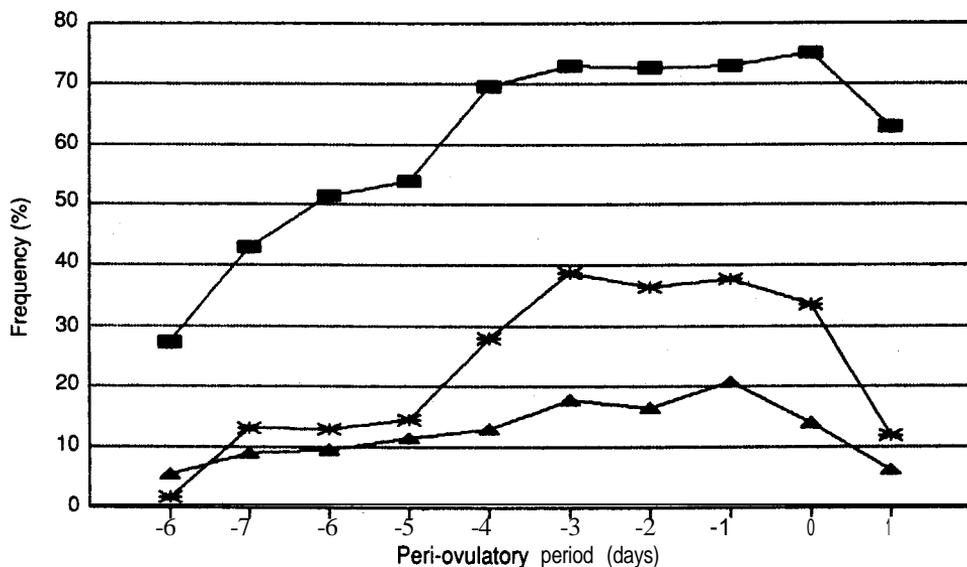


Fig. 2. Frequency (%) in the proximal (■) and sexually active (*) groups and percentage of the total number of approaches to the jack (▲) during the **peri-ovulatory** period. Day 0 = day of ovulation.

There was no correlation ($P > 0.05$) between number of approaches and number of matings per day of oestrus per jenny; or between the presence of a jenny in the proximal group per day of oestrus and number of matings. The number of matings per jenny was correlated ($r = 0.43$, $P < 0.01$) with the frequency of her presence in the sexually active group.

Compared with horse mares, jennies played an active role in mating. The first sign indicative of impending sexual receptivity was increased time in the proximal group: some joined this group just a few hours before showing the first overt signs of oestrus. Some jennies displayed prolonged periods of alternating sexual receptivity and rejection of the jack. During early oestrus, jennies frequently kicked at the jacks approach. Kicking gradually decreased during the following days.

Copulatory frequencies and intervals. The mean interval from introduction of the jack to **first** mount with ejaculation was $39.9 (\pm 30.4)$ min for Jack A and $25.9 (\pm 17.8)$ min for Jack B ($P < 0.05$). Mean inter-ejaculatory interval was $88.4 (\pm 71.5)$ min for Jack A and $93.3 (\pm 54.5)$ min for Jack B ($P < 0.05$). Details of the activity of the 2 jacks during natural and induced oestrus are given in Table 1.

Total ejaculatory and non-ejaculatory mounts were, respectively, 107 (33%) and 217 (67%) for Jack A, and 133 (32%) and 286 (68%) for Jack B. The numbers of mounts with and without ejaculation per day and jenny for Trials 1 and 2 are shown in Fig. 3. Mating frequency was not related to time of day ($P > 0.05$) for either trial. Matings per jenny per ovulatory oestrus ranged from 0-18 in Trials 1 and 2 together. individual jennies did not **affect** the number of mounts without ejaculation or matings per day of ovulatory oestrus ($P > 0.05$). Of 10 jennies in anovulatory oestrus, 3 were mated, compared to 30 of 32 in ovulatory oestrus. Also, there were fewer ($P < 0.05$) matings per day among jennies in anovulatory oestrus (5/31) than those in ovulatory oestrus (165/212, Jack A + Jack B). Jack A exhibited fewer mounts without ejaculation per day of anovulatory oestrus ($P < 0.01$). Significant correlations were observed between number of jennies in oestrus and number of matings per day for each jack individually and for both

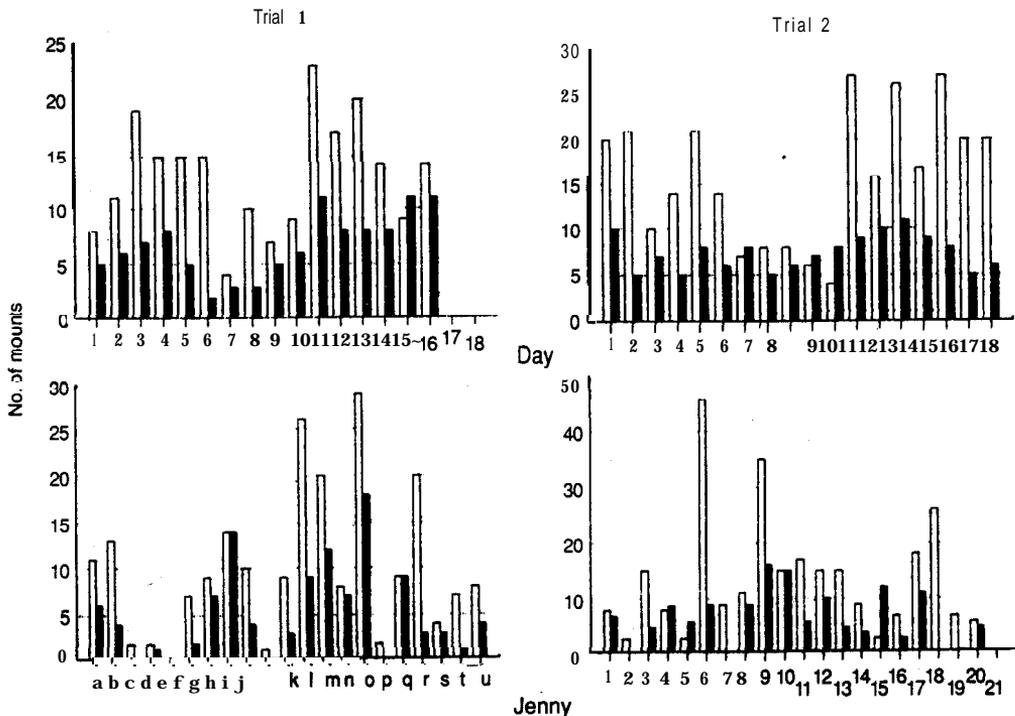


Fig. 3. Number of mounts with (■) and without (0) ejaculation by Day and Jenny in Trials 1 and 2.

Table 1. Activity of the jacks and mean number of jennies in natural and induced oestrus

Mean number per day	Natural oestrus		Induced oestrus	
	Jack A mean \pm s.d.	Jack B mean \pm s.d.	Jack A mean \pm s.d.	Jack B mean \pm s.d.
Jennies in oestrus	3.8 \pm 0.8	5.4 \pm 0.5	8.5 \pm 0.8	9.5 \pm 2.3
Mount without erection	14.8 \pm 2.8	11.4 \pm 2.8	16.2 \pm 4.9	16.8 \pm 7.9
Ejaculatory mounts	5.6 \pm 2.3	6.2 \pm 1.3	9.5 \pm 1.6	9.1 \pm 1.2
Jennies bred	3.0 \pm 1.4	3.0 \pm 1.2	4.8 \pm 1.8	4.6 \pm 0.8
Masturbations	1.4 \pm 1.1	3.2 \pm 1.3	2.0 \pm 0.6	3.8 \pm 1.3

Natural oestrus = Days 2-6; induced oestrus = Days 11-16. There was no significant difference in these measures ($P > 0.05$) between natural and induced oestrus for both jacks and between jacks for natural and induced oestrus.

Table 2. Copulatory sequence of 2 jacks mating 21 jennies each in a free-range breeding management system

Measures (sec)	Jack 'A'			Jack 'B'		
	Mean	s.d.	n	Mean	s.d.	n
Approach-erection*	0.5	1.7	98	4	46	101
Approach-mount*	13	19	98	32	49	104
Approach-ejaculation*	32	20	98	51	50	83
Mount-ejaculation	19	5.5	98	19	5	99
Mount-dismount*	25	5	98	30	12	121
Number of thrusts	5.5	1.4	98	4.9	1.5	110

n = number of observations.

*Significant difference between jacks, $P < 0.001$.

together ($P < 0.01$; $r = 0.61$; Jack A and Jack B) and between number of jennies showing oestrus and different number of jennies mated. ($P < 0.01$; $r = 0.71$; Jack A and Jack B).

A gradual increase in frequency of matings and number of jennies mated per day and per number of jennies showing oestrus per day, from Days -8 to -2 (of ovulation) was observed ($P < 0.05$; Figs 4 & 5). For statistical analysis, the data of both trials were combined. Frequency of matings and proportion of jennies mated among days were compared using combined data for Days -8 to -5, -4 to -3, -2 to -1 and 0 to Day +1.

Copulatory sequences for both jacks are shown in Table 2. For Jack A, there was a difference among jennies in the interval from approach to mount and from approach to ejaculation ($P < 0.05$). For Jack B, there were differences among jennies for all measures except the interval from approach to erection and number of thrusts ($P < 0.05$). Except for the mean number of thrusts, Jack A demonstrated no significant differences related to day. Jack B showed an effect related to day for all copulatory measures except number of thrusts ($P < 0.05$). Throughout the trials, there was no trend toward increasing or decreasing copulatory performance. There was no effect of time of day on any copulatory measures for either jack ($P > 0.05$) except for mean number of thrusts for Jack A (5.5 in the morning, **5.9** in the afternoon; $P < 0.05$).

Masturbation and spontaneous erection. This behaviour was observed during both trials. Incidences of masturbation and spontaneous erection, respectively, were 24 and 8 (Jack A) and 56 and 24 (Jack B)

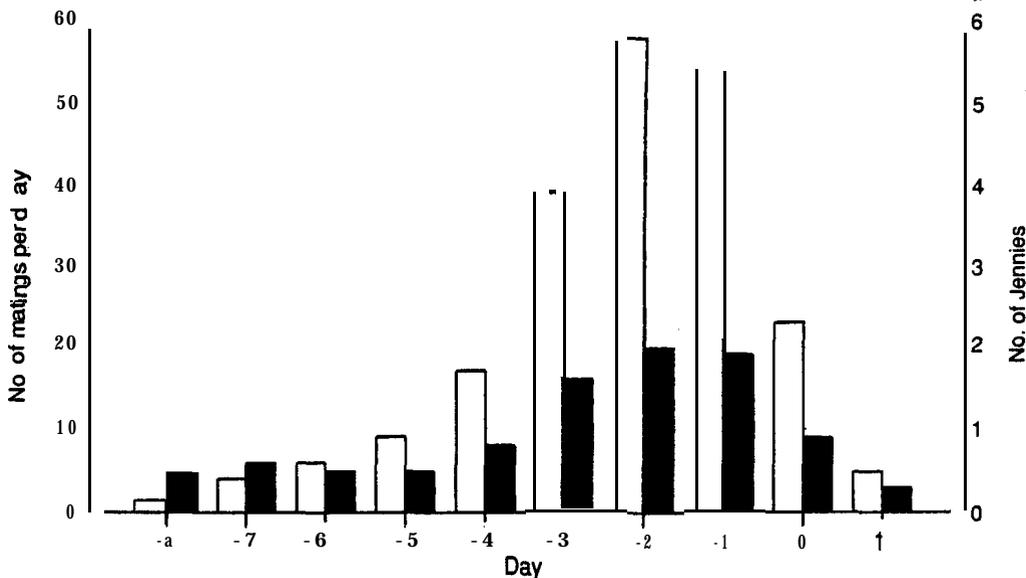


Fig. 4. Number of matings per day (□) and per jenny in oestrus (× 10) (■) during the peri-ovulatory period. Day 0 = day of ovulation.

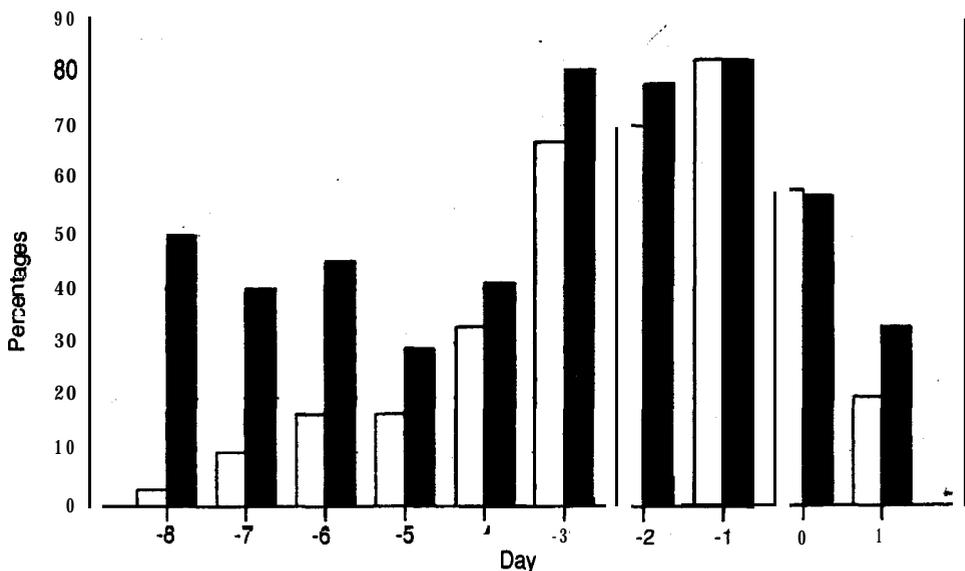


Fig. 5. Percentage of jennies mated per day (□) and per number of jennies showing oestrus per day (■). Day 0 = day of ovulation.

distributed over the duration of the trials with no discernible pattern.

Territorial social structure

assistance during trial 2 and M. Armstrong for assistance in the collection of the material. **Harem formation and maintenance behaviours, (herding and chasing to maintain a cohesive group), as observed in horses (McDonnell, 1986); were not observed in the present study. Infrequently, prolonged chasing of an individual jenny (almost to exhaustion) was observed.**

Jack A and B appeared to have a preferred area of pasture where they rolled; grazed, rested and

Table 3. Seminal characteristics of 2 jacks, before, during and at the end of a free-range breeding management system

Day	Period of collection	Sperm-motility total/progressive (%)	Sperm vigour (1-5)	Total no. of spermatozoa (x10 ⁹)
Jack 'A'				
1	morning	95/90	5	12.5
2, 3, 15*	afternoon	65/45	4	2.9 (2.2)
7	morning	85/70	4	6.2
Jack 'B'				
1	morning	90/85	5	19.3
7, 10, 18*	afternoon	80/70	3.5	1.7 (0.9)
6, 13, 15, 17*	morning	85/70	2.5	3.1 (2.1)
39	morning	85/80	5	4.8

* The results are expressed as the mean for the days indicated (\pm s.d.).

groomed, and where most matings occurred. Jacks A and B were within the range of 2 bordering sectors of the pasture for, respectively, 73 and 65% of the 2-h interval recording times. Notwithstanding the absence of harem behaviour, the jacks were alone in a given sector only 10% (Jack A) and 1% (Jack B) of the time.

Quality of semen

Semen was collected from Jack A in the morning on Days 1 and 7 and in the afternoon on Days 2, 4, 7 and 15; and from Jack B in the morning on Days 1, 6, 13, 15, 17 and 39 and in the afternoon on Days 7, 10 and 18. As shown in Table 3, all ejaculates yielded at least 1×10^9 spermatozoa. Morphologies were evaluated on 4 samples from Jack A and 9 samples from Jack B. The mean (\pm s.d.) percentage of abnormal heads, middle pieces and tails were, respectively, 2.6 (\pm 1.3), 5.1 (\pm 1.8) and 1.9 (\pm 0.9) for Jack A and 12.9 (\pm 8.3), 12.1 (\pm 7.4), 2.3 (\pm 2.3) for Jack B.

Discussion

Although the experiment was not designed to evaluate the breeding potential of donkeys, the findings indicate that donkeys mate efficiently at pasture. All but 2 jennies showing ovulatory oestrus were mated at least once. Of the 2 that were not mated, one showed overt oestrous behaviour for only 2 days and did not approach the jack frequently, and the other had foaled recently and kicked the jack vigorously at the beginning of her period of sexual receptivity. The jack rejected her later when she was in oestrus. First-cycle pregnancy rate was 78% for Jack A (more than 40 days after ovulation) and 69% for Jack B (Day 12 after ovulation), which decreased to 56% with 2 early pregnancy losses. The final pregnancy rates at 40 days after ovulation were 100% and 89% for Jack A and B, respectively. This indicates, as semen quality suggested at each collection, that high frequency of mating was not detrimental to fertility. Similar findings were reported for mares mated at pasture under similar conditions (Bristol, 1982, 1987) and under farm and feral conditions (Colley, 1974).

The observations of this study indicate that donkeys have a territorial, non-harem type of sociosexual organization. The jacks spent most time and did most mating in two contiguous sectors. Jennies moved freely around all sectors without any apparent concern or interference by the male. On one occasion, during Trial 1, a second jack approached the fence; the original jack confronted the intruding male but did not attempt to herd the jennies together.

Another prominent **feature** was the sexually active group, which showed intense heterotypical and **homotypical** behaviour. **Oestrous** responses appeared similar to those described by Clayton *et al.* (1981) for domestic jennies, and by Trumler (1958) for zebras and donkeys. jawing, ears depressed **against** extended neck, tail **slightly raised, urination and posturing for the jack.** A proximal group **has been** observed in pasture-bred **mares** (Asa *et al.*, 1979; Bristol, 1982), but mare&how much less heterotypical behaviour. In contrast to Klingel's (1969, 1975) studies of wild horses, during which mares assumed the oestrous stance only when the stallion approached and mounted, jennies in this study exhibited spontaneous external signs of oestrus and approached the jack frequently. There was a significant difference between jennies in the number of approaches to the jack and frequency in the proximal and sexually active **groups**. Cows in oestrus form a sexually active group, differing from jennies in that they were extremely mobile and not restricted to the proximity of the male (Blockey, 1978). Another similarity between cows and jennies was the high frequency of heterotypical behaviour, particularly mounting, a behaviour rarely observed in mares. Heterotypically active jennies interfered with mating and, as described in mares (Ginther *et al.*, 1983), their efforts **were directed** toward the jennies before mounting and toward the jack during mating.

Vocalization was more pronounced in donkeys than has been reported for horses and appeared to play a role in initiating pm-copulatory interaction. The jacks **appeared** to disregard vocalization between dam and foal. Vocalization was not noted as a prominent aspect of reproductive behaviour among wild African and Asiatic asses (Klingel, 1977). In wild plains zebras (*Equus quagga*), a contact call that serves to keep the herd members together was noted (Klingel, 1967). Feist & McCullough (1976) suggest that whinnying in feral horses may be a prelude to aggressive interactions rather than cohesion.

Pasture breeding performance and copulatory behaviour were very efficient for both jacks, although their performance during attempts to collect semen was slow and inconsistent, as is observed in **hand-**mated or intensely managed jacks. Mounts without erection, as seen in feral horses (Feist & McCullough, 1976), **preceded** ejaculatory **mounts**.

Although the number of different jennies mated per day increased with the number of jennies in **oestrus**, the jacks still continued to mate individuals up to 6 times per day when the number of jennies in **oestrus** per day was at its highest. Preference to mate certain individuals has been suggested in other studies (Bristol, 1982; Asa *et al.*, 1979) and was observed during Trial 2, when the jack persisted in attempting to mate a particular jenny that did not promptly accept mounting. A high number of matings of individual jennies (up to 18) did not appear to be due to preference of the male exclusively: jennies also appeared to influence the number of times they were mated. There was a positive correlation between participation in the sexually active group and number of matings per day per jenny. The number of matings per day of anovulatory oestrus was less than for ovulatory oestrus and mating activity increased **gradually** up to the 2 days before the ovulation. Independent of the participation of the female, the jack seemed to determine the exact time of mating and the target jenny. This had been reported in stallions (Klingel, 1969; Bristol, 1982).

The copulatory behaviour sequence appeared similar to that of stallions. The mean number of thrusts for stallions was 7 (Asa *et al.*, 1979) compared with 5 in these jacks. Copulation time was about 30 **sec** for a stallion breeding at pasture (S.M. McDonnell & F. Bristol, unpublished observations) and varied from 25 to 30 **sec** for stallions in a semen collection programme (McDonnell, 1986; Pickett *et al.*, 1970). compared with 25 and 30 **sec** found for the jacks in this study.

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