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## The temporal estrous patterns of female alpine musk deer in captivity

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### Abstract

From 1996 to 2000, we collected data about rutting habits of captive Alpine musk deer (*Moschus sifanicus*) in the Breeding Center of Alpine Musk Deer (BCAMD) located in the Xinglongshan National Nature Reserve of China. We examined the temporal rutting patterns to determine the timing and synchrony of estrus. From 1997 to 2000, the temporal estrous patterns remained relatively constant across the years but in 1996, the animals entered estrus later and the estrous season was longer than in other years possibly due to the animal being transported in that year. In total, the estrus period of captive musk deer in BCAMD occurred seasonally during 3 months of the year, but 66% of mating occurred in a month from 21 November to 21 December. The total estrous season (when 75% of mating occurred) was 36 days. When the possible factors affecting the temporal estrous patterns were analyzed, it appeared that the rutting of females was influenced by both social and seasonal factors. There were significant differences among the temporal estrous patterns of animals in different rows of enclosures, probably because of keeper effects and social interactions among females within a row of enclosures. Female age can affect timing of estrus: females older than 5.5 years entered estrus earlier than younger age classes. The reproductive success in the previous year was not related to rutting patterns. The temporal estrous patterns were not significantly different between the captive females of the wild-caught deer and their first-generation offspring. Females with more estrous cycles were less likely to enter estrus earlier than those with one or two cycles.

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**Keywords:** Alpine musk deer (*Moschus sifanicus*); Captive; Estrus; Temporal patterns

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## 1. Introduction

Reproductive synchrony, resulting in the birth of offspring within a short time frame, occurs among numerous ungulates and allows the animals to optimize reproductive success. Breeding synchrony usually occurs by adjustment of the timing of estrus (Berger, 1992), so estrus timing and synchrony is of importance to the successful seasonal reproduction of animals. For wild animals in the seasonal environment, such seasonal factors as temperature, precipitation and food availability can exert influences on the temporal rutting patterns (Bronson, 1989). For domestic ungulates, social interactions among animals, housing and management factors, the physical environment impinging on the individual, nutritional status, age and physiological state and presence of the bull can influence the behavioral manifestation of estrus (Orihuela, 2000). Additionally, the environmental factors related to the season can also exert effects on the estrus of the captive domesticated animals (Galina and Arthur, 1990; Penninton et al., 1985). But for captive undomesticated animals, the temporal rutting patterns and the roles of the physical and social factors in shaping the estrous patterns have received little study.

The captive Alpine musk deer (*Moschus sifanicus*) presents an ideal species for the exploration of possible influences of environmental and social conditions on estrus timing. The Alpine musk deer is a species of the genus *Moschus* and is endemic to the Tibet–Qinghai Plateau of China, mainly scattered on the plateau and in adjacent mountainous regions of western China (Yang and Feng, 1999; Sheng, 1998). The wild populations have been declining for decades owing to excessive hunting for the musk gland and habitat loss or degradation in their range areas. Now the species is endangered and threatened with extinction. In order to preserve the wild musk deer population and excise valuable musk from live musk deer, in 1990 the Breeding Center of Alpine Musk Deer (BCAMD) was established in the Xinglongshan National Nature Reserve of China. Since that time, efforts have been made to expand musk deer farming and many farms has been established, but raising musk deer has been mostly unsuccessful due to the difficulty of breeding under captive conditions and the low rate of estrus (Sheng, 1998).

The reproduction of Alpine musk deer has received little study. Estrus is the key to successful reproduction of female musk deer in captivity (Zhang, 1979; Deng, 1989), but estrus of the wild musk deer has barely been researched due to the secretive behavior of the deer and the habitat at relatively high elevation (Sheng, 1998). Hence most knowledge on the estrus of this animal has been attained from observation of captive animals. Female Alpine musk deer attain full body size earlier than they enter estrus at the age of 1.5 years, and bred first at the age of 2 years (Deng, 1989). Female captive Alpine musk deer manifested estrus with 1–3 estrous cycles (Jiang, 1998; Zhang, 1979). Females that fail to conceive in one estrus will recycle and copulate in next estrus, 10–30 days later (Deng, 1989; Zhang, 1979). The duration of true estrus may range from 48 to 72 h (Jiang, 1998). Females may receive over 6–10 successful matings in one estrus when exposed to males (Zhang, 1979).

There is limited knowledge on the estrus timing of Alpine musk deer, as related above, and most of it was attained through descriptive observation of a small number of captive animals or through an analogy to other species of musk deer (Zhang, 1979). More detailed temporal patterns of estrus and the influencing factors remain poorly understood, and

studies of influences of other possible factors are sparse. To develop farming of this species of musk deer, experiment with artificial fertilization, explore the ex situ conservation strategies for wild populations and attain sustainable utilization, a comparative study on the temporal estrus patterns and the influencing factors is of importance. We collected data in BCAMD from 1996 to 2000 and here describe the timing and synchrony of estrus, and discuss potential factors affecting estrus, in Alpine musk deer.

## 2. Materials and methods

### 2.1. *Animals and management*

The study was conducted at the Breeding Center of Alpine Musk Deer (BCAMD) located in the Xinglongshan National Nature Reserve, a region of western China (35°N, 104°E), situated 2000–2100 m above sea level with an annual rainfall of 480–622 mm and averaging temperature of 2.5–6.4 °C.

Our subjects were either wild-caught captive deer (W) or first-generation offspring (F<sub>1</sub>) from captive individuals. Five to seven female musk deer were kept in an enclosure which consisted of an outdoor yard measuring 10 m × 10 m square and lined seven brick cells, every of which with space measuring 2 m × 2 m square and with the ceiling 2 m above the floor. Five to eight enclosures were linked in a row and an iron-mesh fence separated the enclosures to prevent subjects from contacting deer in neighboring enclosures, although they can hear and smell them. Between the rows of enclosures, there was a building and an area wide enough to prevent communication among individuals in the two adjoining rows. Animals in one row of enclosures were maintained by one deer-keeper and were fed twice a day, at dawn and dusk, mainly with fresh leaves (in summer and autumn) or dried leaves (in winter and spring) which were collected from the natural habitats of wild musk deer. Supplementary artificial food (mainly consisting of flour, wheat bran and some vegetables in season) and water ad libitum were also provided. Females were artificially separated from males from February to late September, but in early October, one sexually experienced male musk deer was introduced into one enclosure to mate with the females until February when the females went out of estrus (Jiang, 1998). Fawns were artificially weaned on 1 October, prior to the male being introduced to the female enclosures (Jiang, 1998).

### 2.2. *Data collection and statistical analyses*

From 1996 to 2000, true estrus was evaluated by direct observation of copulation (Jiang, 1998; Zhang, 1979). In all our collected data, the earliest mating occurred on 21 November, so we call this date the standard date (zero value), and calculated the days from 21 November to each rutting date. The length of the mating season was defined as the minimum period in which over 75% of mating occurred. The data consisted of information on 140 copulations.

For statistical analyses, the data was classified by the following characteristics of females: origins (wild-caught captive deer, W; first-generation from the captive, F<sub>1</sub>); age (females aged, 1.5; 2.5; 3.5; 4.5; ≥5.5 years); reproductive experience (sexually

experienced, SE; sexually inexperienced, SI); reproductive success in the previous year (barren the previous year, BA; bred the previous year, BR); estrus cycles (1; 2; 3;  $\geq 4$  estrous cycles); rows of enclosures (Rows 1–2 and 5–6).

Before statistical analysis, the data was normalized by square-root transformation (Shackle et al., 1984; Festa-Bianchet, 1988). To compare mating dates between females classified by origins, reproductive experience and reproductive success the previous year, the Student's *t*-test was applied. ANOVA was employed to determine whether mating dates of females were different among the years (1996–2000), rows of enclosures, age classes and with different estrous cycles. If the differences were significant among groups, the test of least significant difference (LSD) or Games–Howell was used to make pairwise comparisons among the categorized females dependent on the significance of Levene's variance test.

Analyses were conducted with square-root transformed data, but the data listed in the following tables was original. Statistic analyses were completed using the SPSS8.0 (SPSS Inc., Chicago, Illinois) and all reported statistical probability are two-tailed at  $P = 0.05$ .

### 3. Results

#### 3.1. Temporal occurrence of estrus

The total temporal rutting distribution of estrus (Fig. 1) was not normal (Kolmogorov–Smirnov test:  $Z = 1.484$ ,  $P = 0.024$ ) and with skewness of  $0.596 \pm 0.205$ , but the distribution of square-root transformed data appeared to be more normal ( $Z = 0.967$ ,  $P = 0.307$ ). In BCAMD over 5 years from 1996 to 2000, most rutting of females (65.7%) occurred in the month from 21 November to 21 December, but the last copulation may occur up to 63 days after the first one, namely on 22 January over the 5 years, the mean rutting date was on 18 December ( $\pm 1.06$  days). The total estrous season was 36 days.

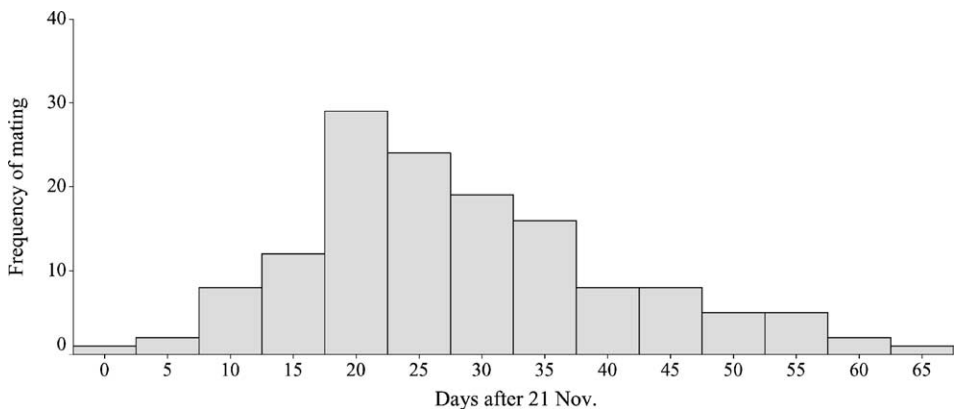


Fig. 1. The distribution of mating in captive Alpine musk deer.

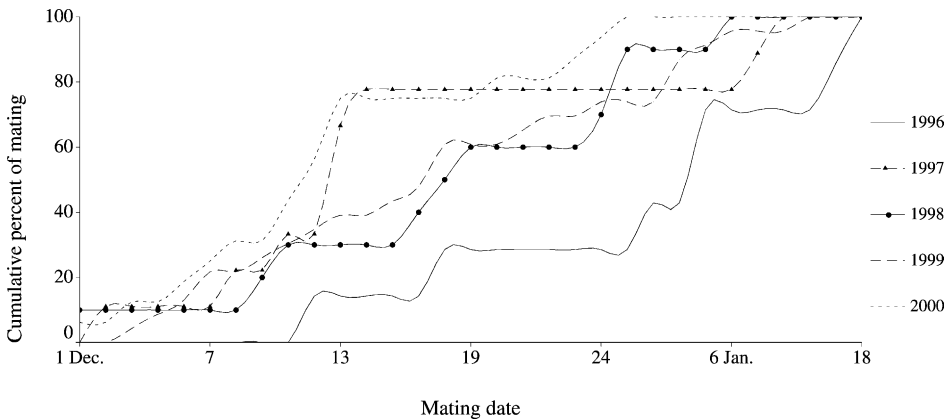


Fig. 2. The distribution of female rutting in Row 5 (1996–2000).

### 3.2. The differences of rutting patterns over the 5 years

The data of estrus in the 5 years from 1996 to 2000 was accessible only in Row 5, so the analysis was limited to this row (Fig. 2). The differences of mating dates over the 5 years was significant ( $F_{4,60} = 2.913$ ,  $P = 0.029$ ). The pair-wise comparisons of the LSD test showed that the averaging mating date in 1996 (30 December  $\pm$  5.21) was markedly later, the rutting season (57 days) was longer than those of other years, and the temporal rutting patterns in 1997–2000 were not significantly different, which indicated that the time of the rut was seasonal and relatively constant except in 1996.

### 3.3. The differences of rutting patterns between females classified by origins, reproductive experience and reproductive success the previous year

For the females categorized by the variables of origins, reproductive experience and reproductive success the previous year, the time of the rut and estrous season are shown in Table 1. Between the classes of W and F<sub>1</sub>, the difference of rutting was not significant (d.f. = 138,  $t = 1.230$ ,  $P = 0.221$ ). Although the mean mating date of females which were barren the previous year (BA) appeared later and whose estrous season was longer than that of females which were reproductive successfully (BE), the difference was not statistically significant (d.f. = 69,  $t = 0.882$ ,  $P = 0.381$ ). The sexual experience of two female was not clear, so the data of them were removed from testing. The estrous patterns of SI and SE were significantly different (d.f. = 136,  $t = 2.057$ ,  $P = 0.042 < 0.05$ ), and the SI musk deer mated later and their estrus season was longer than SE (Table 1).

### 3.4. The differences of rutting patterns among females with different estrous cycles

In total, the animals manifested 1–5 estrus cycles, with a mean of  $1.78 \pm 0.08$  cycles ( $n = 140$ ), but most females showed only one (50%) or two cycles (32%). Owing to small sample sizes, the females with 4 ( $n = 4$ ) and 5 ( $n = 5$ ) estrous cycles were pooled as

Table 1

Temporal estrus patterns of females classified by the origins, reproductive success the previous year and reproductive experience

Grouping	Sample size	Mean mating date (mean $\pm$ S.E.)	Median mating date	Mating		Estrous season
				First	Latest	
Origins						
W	120	19 December $\pm$ 1.12 days	16 December	26 November	22 January	36
F <sub>1</sub>	20	17 December $\pm$ 3.23 days	17 December	21 November	14 January	37
Success the previous year						
BA	9	21 December $\pm$ 3.94 days	18 December	2 December	8 January	42
BE	62	17 December $\pm$ 1.66 days	13 December	21 November	22 January	35
Experience						
SI	51	21 December $\pm$ 1.57 days	18 December	1 December	19 January	37
SE	87	17 December $\pm$ 1.37 days	14 December	21 November	22 January	34

Table 2

Temporal estrus patterns of females with different estrous cycles

Estrous cycle	Sample size	Mean mating date (mean $\pm$ S.E.)	Median mating date	Mating		Estrous season
				First	Latest	
1	70	18 December $\pm$ 1.28 days	14 December	1 December	19 January	34
2	46	19 December $\pm$ 1.95 days	18 December	26 November	16 January	39
3	15	21 December $\pm$ 3.35 days	17 December	3 December	14 January	38
$\geq 4$	9	16 December $\pm$ 7.03 days	10 December	21 November	22 January	44

female-class with  $\geq 4$  estrous cycles. There was not a positive relationship between the mating dates and the estrous cycles (ANOVA,  $F_{3,136} = 1.158$ ,  $P = 0.328$ ) (Table 2).

### 3.5. The differences of rutting patterns among rows

The temporal rutting patterns of females were significantly different among four rows of enclosures ( $F_{3,136} = 4.479$ ,  $P = 0.005$ ) (Fig. 3), but according to the LSD ( $P = 0.171$ ) test, the significant pair-wise differences were only between Row 6 and the other three rows. Of the four rows of enclosures, the mean mating date in Row 6 was earliest (9 December  $\pm$  1.80 days), and the estrous season was shortest (24.75 days).

### 3.6. The differences of rutting among female age classes

The rutting temporal patterns of different female age classes showed in Table 3. There was significant difference of mating dates between the five age classes ( $F_{4,135} = 2.487$ ,  $P = 0.026$ ). The LSD test showed that the mating date (12 December  $\pm$  1.51 days) of females older than 5.5 years was earlier than those of all other age classes, and highly significantly earlier than those aged 2.5 and 4.5 years (19 December  $\pm$  2.54 days and 18 December  $\pm$  2.41 days, respectively).

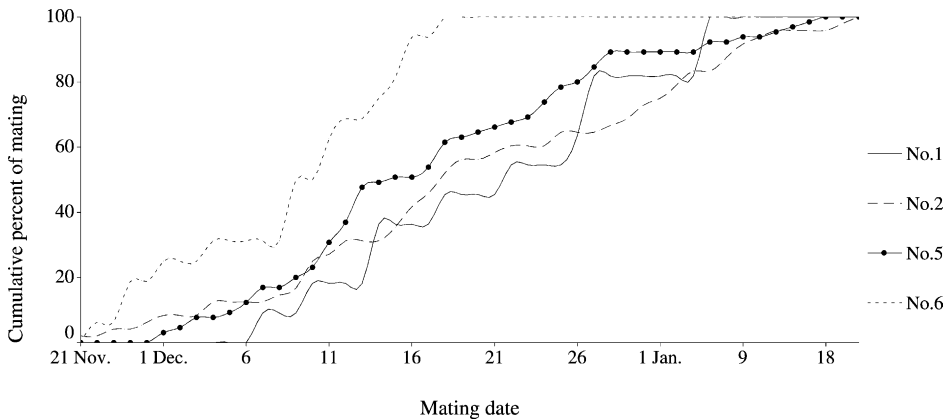


Fig. 3. The distribution of female rutting in rows of enclosures.

Table 3  
Temporal estrus patterns of females in different age classes

Age classes	Sample size	Mean mating date (mean $\pm$ S.E.)	Median mating date	Mating		Estrous season
				First	Latest	
1.5	30	20 December $\pm$ 1.87 days	20 December	1 December	2 January	37
2.5	32	19 December $\pm$ 2.54 days	16 December	21 November	19 January	37
3.5	33	22 December $\pm$ 2.62 days	19 December	30 November	22 January	47
4.5	17	18 December $\pm$ 2.41 days	18 December	2 December	8 January	35
$\geq 5.5$	28	12 December $\pm$ 1.51 days	12 December	26 November	27 December	26

#### 4. Discussion

Although the Alpine musk deer in BCAMD were maintained in captivity, animals did not become thoroughly accustomed to artificial maintenance and remained undomesticated, so the estrous patterns of captive females musk deer was not the same as those of the wild animals or of other domesticated ungulates, but had some of the characteristics of both.

In some captive domestic animals living the seasonal environments, environmental factors related to the season can influence the temporal estrous patterns: for instance, ambient temperature can exert influence on the sexual receptivity and reproductive efficiency of cattle (Zakari et al., 1981; Tucker, 1982; Penninton et al., 1985).

Wild musk deer live in a seasonal environment and enter estrus seasonally (Sheng, 1998). In our study, BCAMD is located in the natural habitat of wild Alpine musk deer and undergoes seasonal climatic patterns also, which remain relatively constant interannually (Wang, 1996). Although the animals were maintained in captivity, the deer can move freely in their yard of enclosures and were less likely to stay in the cells. In addition, no apparatus was fixed in the enclosures to control the temperature, so the environment in BCAMD was seasonal and coincided with the natural patterns. Additionally, captive deer were mainly

fed on leaves picked from the surrounding habitat as well as some seasonal supplemental vegetables, so the food availability also showed a seasonal pattern harmonized with natural seasonality. Lastly, the captive environment in BCAMD was full of seasonal cues by which the captive musk deer can modulate estrus timing. Hence, as seasonal factors influenced estrus of the wild Alpine musk deer (Wang, 1996), the seasonal environment in BSAMD also exerted an effect on the temporal rutting patterns of captive musk deer. In BCAMD, the distribution of rutting in captive musk deer appeared to be seasonal and constant interannually from 1997 to 2000. But given the effect of the enclosure and supplemental food, which reduce fluctuations in temperature and food availability etc. and reduce reproductive seasonality in captive animals (Orihuela, 2000; Hurnik et al., 1975), the environmental seasonality of captive musk deer should not be as strong as that of wild deer, and it may be that the rutting patterns of captive females are less synchronous than those of wild musk deer.

In our study, the estrus pattern in 1996 was markedly later than the other 4 years (1997–2000) and appeared to be more skewed, probably as a result of transport in 1996. In this year, all animals were transported from elsewhere to the present site, and it is known that transport may stress musk deer, inhibiting estrus of farming animals (Allrich, 1993; Hafez, 1962). Furthermore, the adaptation of the transported musk deer to the new environment can play an important role in estrous manifestation (Deng, 1989). Our study supported the findings on other farmed ungulates (Vaca et al., 1985).

For the captive animals, many social interactions took place among individuals, which may have played an important role in the manifestation of estrous behaviors through behavioral modulation (Hafez and Lindsay, 1965; Galina et al., 1996). The time of the rut and synchrony may be modulated by olfactory behavior (McClintock, 1971): for instance, in *Bison bison*, unmated females use olfactory cues to explore the status of other females prior to their own estrus, but not after (Berger, 1992). In addition, the estrous animals of a captive herd can exert a certain influence over the behavior of their herdmates by causing imitation of estrous behaviors in others (Gutierrez et al., 1993). Furthermore, social cues through mating with an attendant male can influence reproductive timing also (Porter and Wilkinson, 2001). As found in bighorn sheep, late lambing may be the result of delayed socially-induced estrus (Festa-Bianchet, 1988c). Evidence from domestic sheep and feral goats suggests that the younger females enter estrus later than older ewes because the onset of their estrus required additional stimulation from attendant males (Coblentz, 1980).

Because of its solitary behavior and need for cover, wild musk deer rely chiefly on olfaction and chemical communication (Shrestha, 1998; Sheng and Ohtaishi, 1993) through abundant glands such as the caudal gland and interdigital gland (Ohtaishi and Sheng, 1993). In BCAMD, five to seven females were fenced in an enclosure, and behavioral interaction frequently occurred among individuals, even among animals in the adjacent enclosures within a row because the partitions permit sensory contact (Zhang, 1979). Hence, among individual musk deer in a row of enclosures, there was much social modulation through frequent and intense behavioral interaction and volatile chemicals which can exert effects on the temporal estrous patterns of females, making them more synchronous within than between rows of enclosures. In this research, the temporal clustering of rutting date is more obvious within rows than between maybe, or possibly because of social modulation as found in other captive herds (Kilgour et al., 1977; Hurnik



and King, 1987; Mohammed et al., 1991; Medrano et al., 1995). In BCAMD, besides the social interaction among animals, keeper may affect temporal rutting patterns since these different effects of rows were maintained by different keepers.

Social modulation and the keeper may explain why the distribution of rutting dates in females was differed among rows of enclosures, and showed more clustering within rows than between.

The rutting patterns were not significantly different between females of W and F<sub>1</sub>, perhaps indicating that the estrus patterns of captive musk deer does not have strong relation with the origin of females in BCAMD.

Our data showed that the mean rutting dates did not differ significantly among females with different estrous cycles, and the estrus timing of females is not associated with the estrus cycles, which may be because the females with more estrus cycles were more likely to come into estrus earlier than those with one or two cycles.

Studies on some farmed ungulates have suggested that the timing of the rut may be influenced by female age (Lamonthe et al., 1995), and it is believed to be more synchronous among younger animals (Hurnik et al., 1975); however, our data of mating in musk deer suggested the contrary because the younger females entered estrus less synchronously than the older ones and their estrous season was longer. This may have resulted from the age-dependent dominance in the herd of captive musk deer as Castellanos et al. (1997) found that the behavioral manifestations of estrus in the socially dominant members of the herd had a strong influence on the number of cows in estrus.

In BCAMD, the temporal mating patterns significantly differed between SI and SE females. SI females entered estrus later than SE females, and the averaging mating date of SI was 21 December ( $\pm 1.57$  days), 4 days later than that of SE, and the estrous season of the SI was 37 days, 3 days longer than the SE. The SI musk deer, which were usually younger in the herd, may have lost mating opportunities partially because of lower age-dependent dominance. Their inexperience with behavioral and volatile chemical cues may also have affected their estrus pattern.

Females that reproduced the previous year did not enter estrus later, as occurs in some ungulates (Clutton-Brock et al., 1989, 1996; Guinness et al., 1978; Mitchell et al., 1976), but this is probably because the animals in BCAMD were provided with supplemental artificial food, and fawns born later were artificially lactated with milk from sheep and cows, so their mothers were allowed to recover body reserves adequate for estrus.

Conclusively, in BCAMD, the temporal estrous patterns of the captive females remained relatively constant across the years, and the rutting of females was influenced by both social and seasonal factors, furthermore, the female age and the keeper can affect timing of estrus, but the origins of the females and the reproductive success in the previous year was not related to rutting patterns.

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