Behavioural differences between captive male alpine musk deer with and without successful copulation

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Abstract
During June 2004 to February 2005, focal sampling and all occurrence method was used to quantify the behavioural patterns of captive male alpine musk deer (Moschus sifanicus) in Xinglongshan Musk Deer Farm, Gansu Province, China. The objective of this study was to identify the behavioural differences between the males with and without successful copulation in order to find the potential male individuals for reproduction. The results showed that in pre-rut season (between August and October), males with successful copulation demonstrated tail-pasting more frequently than males without successful copulation; during rut season (between November and January next year), the males with successful copulation expressed tail-pasting, locomotor, urinating-defecating and agonistic interaction more frequently while males without successful copulation performed resting and environment sniffing less frequently; the behavioural frequencies of tail-pasting behaviour were significantly different between males with different copulation success during pre-rut and rut season. We thought the behavioural characteristics could be used as reproduction performance indicators of captive male alpine musk deer and concluded that the males with more frequency of tail-pasting could be defined as the potential successfully mating males and should be chosen as the breeding males; the males with low tail-pasting frequency should be excluded from breeding and replaced. The results of this study provided very useful information to increase successful mating and gestation of female musk deer, and reduce the cost of the musk deer farming.

Key words: alpine musk deer (Moschus sifanicus), captive, male, behavioural comparison, copulation

Alpine musk deer (Moschus sifanicus) is endemic to the Tibet Plateau and surrounding areas in China, and now has been endangered due to habitat loss and historic illegal hunting for male musk glands, which secretes musk, a valuable material for both traditional medicine and perfumery (Yang et al., 2003; Meng et al., 2006). For this reason, alpine musk deer has been listed in CITES (Convention on International Trade in Endangered Species) Appendix II, the World Conservation Union IUCN Red List (near-threatened, last assessed in 1996), and as Category I key species in the China Wild Animal Protection Law 1988.
Since 1958, captive farming has been employed in China to conserve and to utilize musk deer resources sustainably (Homes, 1999; Parry-Jones and Wu, 2001). From then on, many musk deer farms have been established, and at present, approximately 2000 individuals are housed in captivity across China (Meng et al., 2006). Since musk deer farming has great significance for the conservation and sustainable use of the existing wild musk deer resources, many attempts have been made to improve the musk deer farming and management. However, many efforts are not successful partially due to difficulties to reproduce baby musk deer in captivity. One of the most important factors in determining the success of musk deer farming is the reproduction rate, which is influenced by a number of factors such as environment, nutrition, genetics, and successful mounting of male musk deer (Meng et al., 2006).

In farming practice, some male musk deer do experience initial difficulty with mating and cannot copulate with exposure to estrus, which decreases the reproduction rate and increases the cost of maintenance (Wu and Wang, 2006).

Behaviour is one of the most important aspects of reproduction, and an understanding of the basics of reproductive behaviour will help improve management practice and thus improve reproductive success. Furthermore, the knowledge of behavioural differences between males with successful and unsuccessful copulation will help build up criteria to choose the male individuals for reproduction (Sheng and Ohtaishi, 1993).

The objectives of this study were to explore the behavioural differences between males with and without successful copulation, and provide a better understanding of behavioural characteristics and activity budget for them, on the basis of which differentiating criteria could be developed, in order to ensure the males with high successful copulation potential enter mating with females to increase the reproductive rate.

Material and methods

Animals, housing and management

This study was conducted from June 2004 to February 2005 at Xinglongshan Musk Deer Farm (XMDF), Xinglongshan National Nature Reserve in Gansu Province of northwest China. Located at an elevation of 2000–2100 m, the reserve has a continental mountain climate with short and cool summers, and long and harsh winters. The average temperatures are coldest in January (9ºC), and warmest in July (14ºC), with annual precipitation of 48–62.2 mm.

Males without successful copulation (WC) were defined as those male musk deer which experienced initial difficulty with mating and could not successfully copulate with exposure to estrus, and males with successful copulation (SC) were defined as those male musk deer which could normally mount and copulate with estrous females. The WC and SC males were decided on the basis of the successful copulation, in which the series of chasing and mounting occurred between male and female, and moreover, the male was observed to perform subsequent lying down and to show obvious exhaustion (Zhang, 1979; Wu and Wang, 2006).
Twenty-nine adult male alpine musk deer (14 WC males and 15 SC males), which were all born and raised in captivity at XMDF, were observed from June 2005 to January 2006. Groups of up to seven individuals were housed in outdoor exercise area (100 m²), with unrestricted access provided to six adjoining indoor brick cells (4 m²). Neighbouring enclosures were separated by wire mesh, enabling olfactory and auditory communication between individuals, but preventing physical contact. Animals were fed twice daily, at dawn and dusk, on a diet of fresh leaves (May to November) or dried leaves (December to April). Leaves of the preferred forage species (*Crataegus kansuensis* and *Acer tetramerum*) were collected from the Xinglongshan National Nature Reserve, a habitat for wild musk deer. The diet was supplemented with artificial feed containing approximately 40% corn, 25% wheat, and 25% beans, which was mixed onsite. Seasonal vegetables were also provided occasionally and water was provided *ad libitum*. Diet manipulation was not possible in this study because all experiments were conducted at a commercially operating musk deer farm. However, feeding was consistent throughout the study.

Male and female musk deer were housed separately from March to October, during non-mating season. In line with commercial breeding practices, at the commencement of rut season (November to March), one male was put into each of the female enclosures, and taken back to its original enclosure after the female ended estrus. All animals were individually identified by a numbered plastic ear tag.

**Ethogram behaviour definition**

Based on previous behavioural studies (Zhang, 1979; Sheng and Ohtaishi, 1993; Green, 1987) and preliminary observations from June 2005 to July 2006, the following ethogram was established for captive musk deer:

- **Resting (RE):** Animal lies on the ground and is in an inactive and relaxed state.

- **Vigilance (SA):** Animal is still, alert and gazes at stimuli.

- **Locomotion (LO):** Animal moves around without any accompanying behaviours.

- **Feeding (FD):** Animal ingests leaves, artificial feed or drinks water.

- **Ruminating (RU):** Animal shows typical behavioural series of rumination such as chewing, swallowing and regurgitating, etc.

- **Tail-pasting (TP):** Animal shows scent mark by rubbing the base of the tail against the surface of a wall, trough or doorframe.

- **Urinating-defecating (UD):** Animal fully or partially exhibits activities such as squatting on hind legs, earth-scratching, urinating, defecating and covering pellets by scratching behaviour observed both in association and isolated from latrines.

- **Environmental sniffing (ES):** Animal explores the wall or ground with its nose.

- **Ano-genital sniffing (AS):** Animal sniffs or licks the ano-genital region of another musk deer.

- **Self-directed behaviour (SD):** Animal shows activities directed to itself, including self-grooming with mouth, self-scratching, and other self-directed behaviours.

- **Affinitive interaction (AI):** Direct physical contact between adult animals without obvious aggression, i.e., mutual grooming, sniffing, licking.

- **Agonistic interaction (CI):** Aggressive behaviours with or without direct body contact, including chasing, striking with forelegs, or canines (males).

- **Miscellaneous behaviour (MB):** All other behaviours with infrequency, such as stereotypical behaviour, copulation and homosexual behaviour.
Data collection and statistical analysis

At XMDF, alpine musk deer fawning occurs from June to July; mating occurs from November to February, and weaning of calves is conducted in October (Meng et al., 2003). Henceforth, during this study, the observation period was defined as ‘pre-rut season’ (August to October) and ‘rut season’ (November to January). Due to lighting restrictions, behavioural observations were recorded during daylight hours with the assistance of binoculars (10 × 42°) to confirm individual ear tag numbers. The focal sampling and occurrence was utilized to observe and record behaviour (Altman, 1974). To measure behavioural patterns, we randomly selected a musk deer as the focal musk deer and recorded its behaviours for five minutes and then recorded the behaviours of next deer until all musk deer were observed. The observations were conducted four times a day, three times a week, over a period of six months for each animal. The total time of observations was 216 hours.

The frequency of each behaviour was calculated in occurrence of every sample (5 min) with seasonal averages (± standard error) compared for each animal and between SC and WC females. Behaviours were standardized by individual and number of samples, respectively. Due to the low frequency and large variation, miscellaneous behaviours (MB) were excluded from the analysis. Since WC and SC males were housed together during the study period and thus behavioural data were not independent, the Wilcoxon Signed Rank Test was utilized to explore behavioural differences between SC and UC females. Statistical analysis was conducted with SPSS11.0 (SPSS Inc., Chicago, Illinois).

Results

The comparison of general behavioural modes between SC and WC male musk deer

As shown in Table 1, there existed no significant behavioural difference between SC and UC male musk deer if the season factor was not taken into account (P>0.05).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>WC (N = 14)</th>
<th>SC (N = 15)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting, RE</td>
<td>0.29±0.05</td>
<td>0.98±0.71</td>
<td>ns</td>
</tr>
<tr>
<td>Vigilance, SA</td>
<td>1.83±0.44</td>
<td>2.44±0.42</td>
<td>ns</td>
</tr>
<tr>
<td>Locomotor, LO</td>
<td>0.92±0.31</td>
<td>1.76±0.36</td>
<td>ns</td>
</tr>
<tr>
<td>Feeding, FD</td>
<td>0.62±0.14</td>
<td>0.82±0.25</td>
<td>ns</td>
</tr>
<tr>
<td>Ruminating, RU</td>
<td>0.16±0.04</td>
<td>0.51±0.21</td>
<td>ns</td>
</tr>
<tr>
<td>Tail-pasting, TP</td>
<td>0.06±0.02</td>
<td>0.35±0.14</td>
<td>ns</td>
</tr>
<tr>
<td>Urinating-defecating, UD</td>
<td>0.07±0.03</td>
<td>0.13±0.06</td>
<td>ns</td>
</tr>
<tr>
<td>Self-directed behaviour, SD</td>
<td>0.03±0.02</td>
<td>0.06±0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Environmental sniffing, ES</td>
<td>0.93±0.38</td>
<td>0.78±0.15</td>
<td>ns</td>
</tr>
<tr>
<td>Ano-genital sniffing, AS</td>
<td>0.01±0.01</td>
<td>0.05±0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Affinitive interaction, AI</td>
<td>0.02±0.01</td>
<td>0.03±0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Agonistic interaction, CI</td>
<td>0.16±0.06</td>
<td>0.59±0.24</td>
<td>ns</td>
</tr>
</tbody>
</table>

Note: Data shown as Mean ± S.E.; ns: non significant difference (P>0.05).
The behavioural comparison between SC and WC male musk deer during pre-rut season

In pre-rut season (Table 2), SC males showed significantly more tail-pasting behaviour than WC males (P<0.05), whereas all other behavioural differences were not significant (P>0.05).

Table 2. Behavioural comparison between male musk deer with and without successful copulation during pre-rut season

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>WC (N = 10)</th>
<th>SC (N = 9)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting, RE</td>
<td>0.30±0.06</td>
<td>1.63±1.23</td>
<td>ns</td>
</tr>
<tr>
<td>Vigilance, SA</td>
<td>1.19±0.21</td>
<td>1.39±0.28</td>
<td>ns</td>
</tr>
<tr>
<td>Locomotor, LO</td>
<td>0.58±0.12</td>
<td>0.82±0.27</td>
<td>ns</td>
</tr>
<tr>
<td>Feeding, FD</td>
<td>0.73±0.19</td>
<td>0.85±0.33</td>
<td>ns</td>
</tr>
<tr>
<td>Ruminating, RU</td>
<td>0.23±0.05</td>
<td>0.58±0.32</td>
<td>ns</td>
</tr>
<tr>
<td>Tail-pasting, TP</td>
<td>0.08±0.03</td>
<td>0.13±0.05</td>
<td>*</td>
</tr>
<tr>
<td>Urinating-defecating, UD</td>
<td>0.06±0.04</td>
<td>0.07±0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Self-directed behaviour, SD</td>
<td>0.04±0.03</td>
<td>0.05±0.02</td>
<td>ns</td>
</tr>
<tr>
<td>Environmental sniffing, ES</td>
<td>0.51±0.14</td>
<td>0.41±0.13</td>
<td>ns</td>
</tr>
<tr>
<td>Ano-genital sniffing, AS</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Affinitive interaction, AI</td>
<td>0.02±0.01</td>
<td>0.05±0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Agonistic interaction, CI</td>
<td>0.06±0.03</td>
<td>0.03±0.01</td>
<td>ns</td>
</tr>
</tbody>
</table>

Note: Data shown as Mean ±S.E.; * – significantly different (P<0.05); ns: non significant difference (P>0.05).

The behavioural comparison between SC and WC male musk deer during rut season

As shown in Table 3, in rut season, SC males expressed locomotor behaviour, tail-pasting, urinating-defecating and agonistic interaction significantly more frequently than WC male musk deer (P<0.05). In contrast, the WC males demonstrated resting and environmental sniffing significantly more frequently than SC males (P<0.05). The other behavioural differences were not significant (P>0.05).

Table 3. Behavioural comparison between successfully and unsuccessfully mounting males during rut season

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>WC (N = 4)</th>
<th>SC (N = 6)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting, RE</td>
<td>0.26±0.13</td>
<td>0.13±0.06</td>
<td>*</td>
</tr>
<tr>
<td>Vigilance, SA</td>
<td>3.09±1.17</td>
<td>3.83±0.80</td>
<td>ns</td>
</tr>
<tr>
<td>Locomotor, LO</td>
<td>1.60±0.88</td>
<td>3.02±0.65</td>
<td>*</td>
</tr>
<tr>
<td>Feeding, FD</td>
<td>0.38±0.19</td>
<td>0.77±0.39</td>
<td>ns</td>
</tr>
<tr>
<td>Ruminating, RU</td>
<td>0.08±0.01</td>
<td>0.42±0.27</td>
<td>ns</td>
</tr>
<tr>
<td>Tail-pasting, TP</td>
<td>0.02±0.01</td>
<td>0.64±0.28</td>
<td>*</td>
</tr>
<tr>
<td>Urinating-defecating, UD</td>
<td>0.08±0.07</td>
<td>0.21±0.13</td>
<td>*</td>
</tr>
<tr>
<td>Self-directed behaviour, SD</td>
<td>0.02±0.02</td>
<td>0.09±0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Environmental sniffing, ES</td>
<td>1.78±1.09</td>
<td>1.28±0.24</td>
<td>*</td>
</tr>
<tr>
<td>Ano-genital sniffing, AS</td>
<td>0.01±0.01</td>
<td>0.08±0.07</td>
<td>*</td>
</tr>
<tr>
<td>Affinitive interaction, AI</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Agonistic interaction, CI</td>
<td>0.36±0.15</td>
<td>1.33±0.52</td>
<td>*</td>
</tr>
</tbody>
</table>

Note: Data shown as Mean ± S.E.; * – significantly different (P<0.05); ns: non significant difference (P>0.05).
Discussion

Generally, an animal in rut has increased activities and movements in open areas, which is associated with higher reproductive efforts (Cushing, 1985). Zhang (1979) and Meng et al. (2003) reported that, in mating season, both male and female captive musk deer may become more active in general, and males may actively seek out and attempt to stay in the vicinity of the estrous female, which was supported by this study. We found that WC males showed more resting behaviour and less locomotor activity than SC males in rut season, which suggested that WC males were more sedentary and the activity level of SC males was higher in rut season, and SC males more often intermitted other behaviours to initiate locomotion. This study thereby suggested that SC males demonstrated more natural active pattern of normal males, in other words, SC is more active in rut season while WC male was more sedentary.

Male musk deer may move around and deposit scent to advertise their reproductive status and to attract a mate as observed in alpine musk deer and forest musk deer (*Moschus berezovskii*) (Sheng and Ohtaishi, 1993; Zhang, 1979). Musk deer is solitary and territorial, and inhabits shrub-covered slopes in the sub-alpine zones of mountain regions, and thus the olfactory signalling between individuals is highly developed, the scent is musk deer’s key means of communication, and the droppings and urine are used as important scent marks (Green, 1987). Musk deer repeatedly defecate at special sites or latrines in captivity. Musk deer cover their pellets with leaf litter, soil and other adjacent debris by fore hooves. Moreover, males may scent their urine with musk (Sokolov, 1984). Therefore, defecation sites are communication centres providing information on the identification, whereabouts and perhaps even the reproductive condition of the individual. Furthermore, the defecating and urinating mark is seasonal and the peaks are distributed at the height of the mating season (Wu and Wang, 2006; Green, 1987; Zhang, 1979). In captivity, male alpine musk deer remain territorial and build up their own preferred location in enclosure, which does not overlap with each other, and they defecate at special sites accompanied by pellets-covering behaviour (Meng, 2002). The current study showed that male musk deer increased the defecation and urination in rut season, and SC males defecated and urinated more frequently than WC males during rut season, which suggested that SC males increase the scent mark in rut season through increasing of defecation and urination frequency.

Tail-pasting is another scent marking behaviour of musk deer and has been defined as the male specific behaviour (Green, 1987; Homes, 1999). The caudal gland of the male occurs as a thickening at the base of the short tail, which exudes a viscous yellow secretion with an offensive odour. Typically, wild male musk deer rub the base of their tails throughout their home ranges against the stems of bushes or dried herbs and grasses (Sokolov, 1984; Green, 1987). In captivity, when male musk deer elicits tail-pasting, it pastes its tail and ano-genital region against the projected objects such as the wall and doorframe of enclosure, and the obvious movements of up-down and left-right could be recognized clearly. In this study, the tail-pasting in SC males was more frequent than WC males in rut season, which was found among
other musk deer such as forest musk deer (*Moschus berezovskii*) (Sheng and Ohtaiishi, 1993), and indicated that SC males increased the scent marking intensity in rut season through increasing of tail-pasting frequency, which could increase its chances of finding and mating with a receptive female.

In musk deer, the information collecting behaviours such as environment sniffing and ano-genital sniffing were common, especially during rut season. In rut season, captive male musk deer constantly moves from doe to doe to detect the ones in estrus, during which it demonstrates high intensity of sniffing, licking and nuzzling, and the doe even fans or wriggles its tail to attract male’s copulation at the peak of estrus (Wu and Wang, 2006; Zhang, 1979). Sheng and Ohtaiishi (1993) reported that captive forest musk deer increases the information collection in rut season, which was supported by the present results. This study showed that male musk deer increased the environment sniffing and ano-genital sniffing behaviour to strengthen the information collection; however, compared to WC males, SC males more frequently elicited ano-genital sniffing to collect information which was related to the physical status of females whereas WC males were inclined to demonstrate the environment sniffing to collect environment information.

Wild musk deer are essentially sedentary and territorial. Accordingly, musk deer occupying adjacent territories may come into contact with each other from time to time, and often engage in border disputes (Green, 1987). In the artificial captive environment of XMDF, musk deer would interact and fight more frequently because of the relatively narrow enclosure, the lack of environmental richness and shelter, especially during rut, when individuals fight for the potential mate. In this study, SC male musk deer elicited more agonistic behaviour during rut season than WC males. It was reported that there exist different aggressiveness levels among male individuals and the placid males should be chosen to breed as mating males in order to get the more genetically placid offspring (Zhang, 1979; Wu and Wang, 2006), which was not supported by actual musk deer farming practice because such placid males more often fail in mating with estrous females. Our data indicated that the more pugnacious males should be chosen to copulate with estrous females, which would benefit the increase of reproduction rate of captive musk deer.

In general, this study demonstrated that some behaviours were different between WC and SC males during rut and pre-rut seasons, respectively. If the season factor was not considered, however, there was no significant difference in general behavioural patterns between SC and WC males, perhaps owing to the counteracts of behavioural frequencies between two seasons. Therefore, this study suggested that when the comparison is conducted to explore the potential behavioural difference of captive animals, the potential influencing factors such as season should be considered in addition to whole behavioural comparison, and the more detailed information may be extracted from the behaviour data.

For captive wildlife, there are many influencing factors, such as early rearing experience, hormonal pattern and intensity of abnormal behaviour, which could result in the occurrence of unsuccessful copulation (Mallapur et al., 2006; King and Mellen, 1994). Because of the limits of collected data and research planning, the present study just explored the potential behavioural difference between WC and SC
male musk deer, for which the undertaken mechanisms were not discussed; however, the reason for the behavioural differences between two classes should be related to the collective musk deer farming system, as Håkanssona et al. (2007) reported that breeding animals in captivity may lead to behavioural modifications, and modified behaviour could affect the individual’s reproduction. Therefore, to understand the undertaken mechanism for the behavioural differences between SC and WC males, a more detailed study should be conducted.

In conclusion, there were significant differences in tail-pasting behaviour between males with and without successful copulation during pre-rut and rut seasons. Therefore, the behaviour could be utilized as the behavioural criterion to predict the copulation success. In order to improve the reproduction rates and to economize the cost of male musk deer farming, the males with high tail-pasting frequency should be chosen to mate with females, and those with low tail-pasting frequency should be excluded and replaced.

Acknowledgements

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Różnice w zachowaniu kopulujących i niekopulujących samców piżmowca w warunkach niewoli

STRESZCZENIE

W okresie od czerwca 2004 do lutego 2005, stosując obserwację losowo wybranych osobników (metoda focal sampling) i konkretnych zachowań (metoda all occurrence) badano wzorce zachowań samców piżmowca (*Moschus sifanicus*), hodowanych w niewoli w Gospodarstwie Hodowli Piżmowców Xinglongshan w chińskiej prowincji Gansu. Celem badań było określenie różnic w zachowaniu kopulujących i niekopulujących samców dla znalezienia potencjalnych osobników do rozrodu. Wyniki wskazują, że w okresie przedgodowym (między sierpniem a październikiem), samce kopulujące częściej znakowały teren zapachem niż samce niekopulujące, natomiast w okresie godowym (między listopadem a listem następnego roku) samce kopulujące częściej znakowały teren zapachem, poruszały się, oddawały moczką i wykazywały agresję, natomiast samce niekopulujące rzadziej odpoczywały i obwąchwały teren. Częstość znakowania terenu zapachem wskazywała statystycznie istotne różnice pomiędzy samcami o różnej skuteczności kopulacji w okresie przedgodowym i godowym. Stwierdzono możliwość wykorzystania cech behawioralnych jako wskaźników użytkowości rozpłodowej u hodowanych w niewoli samców piżmowca. Uznano, że samce częściej znakujące teren zapachem będą potencjalnie skuteczniej kryć i powinny zostać rozплодnikami, natomiast samce rzadko znakujące teren zapachem należy wykluczyć z hodowli i zastąpić innymi osobnikami. Wyniki przeprowadzonych badań dostarczyły użytecznych informacji pozwalających na zwiększenie skuteczności krycia samiec piżmowca i zmniejszenie kosztów hodowli tych zwierząt.