

## Do social rank and food supplementation affect grooming behavior? A test in Père David's deer

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**Abstract:** Previous studies have shown that several factors including sex, age, tick density, and season affect oral grooming behavior of reintroduced Père David's deer in Dafeng Nature Reserve, China, and reintroduction of wild animals should consider if they retain regular grooming patterns thus to control ectoparasites especially the ticks. In this study we further examined two more factors, social rank and food availability, which might influence grooming behavior of Père David's deer. Harem masters, who usually have a higher level of testosterone, are predicted to groom less than bachelors during the rutting season. However, we did not find any differences in rate of grooming behavior between the harem master and bachelors probably due to the late rutting season when the testosterone levels have fallen off. Food supplementation might affect grooming behavior since released foraging pressure would provide much more time for other behaviors such as grooming. However, we did not find an effect of food availability, probably due to an increase of vigilance instead of grooming in the pavilion population. Our results suggest that more explorations are needed such as changing observation time and excluding the interference of visitors.

**Key words:** Dafeng; Food availability; Programmed grooming; Social rank; Ticks

## 社会等级和食物供给会影响搔痒行为？麋鹿中的验证

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**摘要:** 先前的研究表明, 性别、年龄、蜱虫密度、季节等因素会影响大丰自然保护区中重引入麋鹿的口部搔痒行为, 因此野生动物重引入需要考虑该种群是否仍保留搔痒行为以便有效抵御皮外寄生虫尤其是蜱虫的侵扰。在本次研究中, 我们进一步探讨了两个可能会影响麋鹿搔痒行为的因素: 社会等级和食物供给。在发情季节, 麋鹿群主通常具有较高水平的睾酮水平, 进而抑制搔痒行为, 所以我们预测群主的搔痒频率应该会低于单身汉。但是群主和单身汉的搔痒频率并没有差别, 这可能是因为观察期处于发情末期, 睾酮水平已经下降。食物供给也可能影响搔痒行为, 因为其可以减轻采食压力, 从而释放了其他行为如搔痒的时间。然而我们也没有发现食物供给的影响, 这可能是因为围栏小种群的游客较多, 使得警戒行为占据了采食压力释放后的行为时间。我们建议未来改变行为观察时间, 并排除游客干扰, 从而进一步探讨这两个因素对麋鹿搔痒行为的影响。

**关键词:** 大丰; 食物供给; 程序性搔痒; 社会等级; 蜱虫

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### 1 Introduction

Ticks are the most important group of ectoparasites of wild mammals (Allan, 2001). Tick infestations may cause many diseases. Père David's deer in the Dafeng Milu Nature Reserve are suffering from infestations of ticks. Grooming plays an important role in

health, reproduction and social life in mammals (Sachs, 1988; Mc Lean and Speakman, 1997; Carter and Wilkinson, 2015). Grooming behavior is commonly observed among rodents, felids, ruminants and primates, and it is highly effective in removing fitness compromising ectoparasites (Hart *et al.*, 1992). Grooming is the first line of defense against tick infestation

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for wild mammals (Koch, 1981; Hart *et al.*, 1992; Hart, 1994; Mooring *et al.*, 1996a).

Two grooming models have been proposed to explain the endogenous and exogenous regulation of tick-defense grooming (Hart *et al.*, 1992; Mooring and Hart, 1995b). The “stimulus driven grooming model” states that grooming rate is regulated as a response to peripheral stimulation from tick bites triggered by the release of histamine from dermal mast cells at the site of the bite (Riek, 1962; Willadsen, 1980; Wikel, 1984). The “programmed grooming model” postulates a type of central control that periodically evokes grooming behavior so as to remove ticks before they attach and blood-feed (Hart *et al.*, 1992; Mooring and Hart, 1995b). The programmed grooming model has been proved in some wild ungulates including Père David’s deer (Hart *et al.*, 1992; Mooring and Hart, 1995b; Mooring *et al.*, 1996a, 1996b; Mooring *et al.*, 2002; Mooring *et al.*, 2006a, 2006b; Li *et al.*, 2014).

From a programmed grooming model vigilance principle is predicted. The vigilance principle states that in sexually dimorphic species, males will groom less than female in the same area during breeding season to maintain high levels of vigilance for rival males or estrous females (Hart *et al.*, 1992). Physiologically, testosterone was proved to depress grooming behavior in an experiment which showed that oral self-grooming in long-term castrated male goats was significantly down-regulated to one-third of the baseline grooming rate by testosterone supplementation (Kakuma *et al.*, 2003).

The mating system of Père David’s deer is harem polygamy, in which a strong harem master dominates the harem group and monopolizes nearly all mating opportunities (Li *et al.*, 2001, 2004). Serum testosterone level in the harem master was higher than that in the bachelors (Li *et al.*, 2004). Testosterone is the mechanism behind sexually dimorphic grooming, with higher levels of testosterone resulting in a physiological suppression of programmed grooming (Hart, 1997; Mooring *et al.*, 1998; Kakuma *et al.*, 2003). The time budget of grooming behavior of the harem master should be reoriented toward other behaviors like vigilance against other males.

Foraging pressure or food availability might also affect the grooming budgets of ungulates (Mooring and

Hart, 1995a). For captive animals, foods are usually fully provided, that would leave enough time for other behaviors, such as vigilance and grooming behavior. Previous studies indicated that the time available for grooming was limited by increased feeding time (Mooring and Hart, 1995a). In the pavilion zone of Dafeng, Père David’s deer were offered supplemental foods throughout the year, whereas in other zones, they were supplemented only from November to March. Thus, we wanted to explore if food supplementation would affect grooming behavior of the deer.

A recent study has shown that several factors including sex, age, tick density, season, affect oral grooming behavior of reintroduced Père David’s deer in Dafeng Nature Reserve, China (Li *et al.*, 2014), here we further explored the effect of social rank and food availability on grooming behavior of Père David’s deer. We predict that, (1) due to vigilance principle, harem masters would groom less than bachelors during the rutting season; (2) deer in the pavilion zones would groom more due to food supplemental.

## 2 Methods

### 2.1 Study site

The field study was conducted in the Dafeng Milu Nature Reserve (32°59′ - 33°3′N, 120°47′ - 120°53′E) in Jiangsu Province, China. The reserve is located on the Yellow Sea coast in eastern China and lies 2 - 4 m above sea level. Annual average temperature is 14.1°C, with an average temperature of 0.8°C in January and 27.0°C in July. Average precipitation is 1 068 mm with rain falling mostly between June and September. Dafeng Reserve consists of three core zones, two of which are enclosed by fences allowing Père David’s deer to range freely (Li *et al.*, 2014).

### 2.2 Study species

The mating system of Père David’s deer is harem polygamy, in which a strong harem master dominates the harem group and monopolizes nearly all mating opportunities (Li *et al.*, 2001, 2004). The rutting season starts in May and ends at July (Ding, 2004). We observed the deer in the first zone and the pavilion zone. The first zone is composed of grassland and woodland, whereas the pavilion zone is a separate area from the first zone which was opened to all visitors (Yu and He, 2011).

### 2.3 Tick census

Ticks are common in Dafeng Nature Reserve and the dominant tick species is *Haemaphysalis longicornis*. It is the main threat to Père David's deer. We measured tick density by flag sampling method. The sampling flag was made of a 100 cm × 100 cm piece of unbleached cotton muslin stapled to a wooden base. We sampled every week in summer and autumn. We randomly took 18 samples to cover these study areas each time. For each sampling, we dragged the flag for 10 m and then collected the ticks attached on the flag.

### 2.4 Behavioral sampling

This study was conducted in the first core zone and the pavilion zone of Dafeng Nature Reserve. In the first core zone, Père David's deer were semi-wild and offered supplemental food only from November to March. Permitted tourists are allowed to visit in cars with a regular route. In the pavilion zone Père David's deer were fenced and offered supplemental food all seasons, and they were open to all visitors. We used binoculars and telescope to observe Père David's deer. The distance between observer and deer was at least 150 m away from deer so as to reduce potential observer effects (Li *et al.*, 2007a, 2007b). The observation time was between 07:00 and 18:00. Before observation, activity types, zone (the first core zone or pavilion zone), sex and age, group size, social rank, composition, date and time were recorded. Focal individuals were randomly selected from groups or different parts of one same group to avoid pseudoreplication. Each focal individual was observed for 10 minutes. Three types of grooming behavior were recorded: oral grooming using tongue, tooth or lips to scrape through the fur and skin; scratching grooming using hind leg; antler grooming using antlers in male individuals. An uninterrupted sequence of grooming episodes was defined as grooming bout. We recorded grooming bouts during the observation session. Grooming bouts were used to evaluate grooming rate per hour.

We focused on adult male deer to evaluate social rank effects on observed grooming behavior in July. Deer were classified as either harem masters or bachelors. Harem masters can be distinguished by their appearance and behavior; the fur color around their neck is darker; they usually use mud and grass to decorate their antlers. Bachelors were randomly selected near the focal harem groups.

We observed the grooming behavior in the first core zone and pavilion zone to evaluate effects of food availability in summer (July 2014) and autumn (Late September to early October 2014). Deer were classified into 5 categories by size and general appearance (Li, 2013) as adult male, adult female, sub-adult male, sub-adult female and fawn.

### 2.5 Statistical analysis

All statistical analyses were performed using Rv3.2.2. We used packages: car, lme4 and Remdr for data analyses. For tick density data we performed 216 flags during the study period. Because of the relatively high prevalence of zero counts, we used the Mann-Whitney-Wilcoxon Test to examine the effects of season and zone on tick density.

For the effects of social rank, we used a generalized linear mixed model with negative binomial distribution and the group ID as a random factor. The independent fixed factors considered were: weather (rainy and rainless), zone (first core zone and pavilion zone), social rank (harem master and bachelors), activity type (bedding *vs.* standing) and group size.

For the effects of food availability, we used a generalized linear mixed model with negative binomial distribution and the group ID as a random factor. The independent fixed factors considered were: weather (rainy and rainless), zone (first zone and pavilion zone), sex and age levels (adult and sub-adult males, adult and sub-adult females, and fawns), activity type (bedding *vs.* standing), season, food supplement (offer or not) and group size.

## 3 Results

### 3.1 Tick density

We did not find any significant difference of tick density between the first zone ( $89.5 \pm 17.1$  ticks per flag) and the pavilion zone ( $77.9 \pm 16.7$  ticks per flag,  $W = 876$ ,  $P = 0.84$ ) in summer. We did not find ticks in autumn.

### 3.2 Grooming behavior

We collected a total of 487 grooming samples: 196, 58, 73, 121 and 39 were observed for adult males, sub-adult males, adult females, sub-adult females and fawns, respectively; 371 and 116 were observed in summer and autumn, respectively; 312 and 175 were observed in the first zone and the pavilion zone, respectively.

With the mixed linear model of social rank, we found activity type significantly antler grooming ( $\beta = -1.48 \pm 0.50, P = 0.003$ , Table 1), overall grooming behavior ( $\beta = -1.08 \pm 0.46, P = 0.018$ , Table 4) and nearly significantly affected oral grooming ( $\beta = -1.01 \pm 0.8, P = 0.083$ , Table 2). Social rank only affected scratching grooming-masters scratched more than bachelors ( $\beta = 1.22 \pm 0.61, P = 0.046$ , Table 3).

With the mixed linear model of food availability, we did not find any significant effects of weather, group size and food availability, except sex-age influenced oral ( $\beta_{\text{MALE}} = -1.42 \pm 0.39, P < 0.001, \beta_{\text{FAWN}} = 1.00 \pm 0.48, P = 0.036$ , Table 5), and overall grooming ( $\beta_{\text{MALE}} = -0.90 \pm 0.35, P = 0.011, \beta_{\text{FAWN}} = 1.44 \pm 0.51, P = 0.005$ , Table 6), and activity type influenced oral grooming ( $\beta = -0.88 \pm 0.37, P = 0.016$ , Table 5).

**Table 1** Effect of social rank on antler grooming of Père David's deer in Dafeng Nature Reserve. Parameter estimates were made at 0 when rainless day, standing, first zone, and bachelor. Significant levels were set at  $P = 0.05$

	Estimate	S. E.	Z-value	P
(Intercept)	-0.79	0.94	-0.842	0.400
Rainy	0.09	0.54	0.163	0.871
Bedding	-1.48	0.50	-2.937	0.003 *
Pavilion zone	0.59	0.77	0.767	0.443
Master	0.12	0.63	0.187	0.852
Group size	0.001	0	0.274	0.784

**Table 2** Social rank effect on oral grooming of Père David's deer in Dafeng Nature Reserve. Parameter estimates were made at 0 when rainless day, standing, first zone, and bachelor. Significant levels were set at  $P = 0.05$

	Estimate	S. E.	Z-value	P
(Intercept)	-0.46	0.92	-0.505	0.614
Rainy	0.33	0.58	0.568	0.570
Bedding	-1.01	0.58	-1.734	0.083
Pavilion zone	-0.82	0.78	-1.054	0.292
Master	-1.13	1.06	-1.064	0.287
Group size	-0.002	0	-0.476	0.634

**Table 3** Social rank effect on scratching grooming of Père David's deer in Dafeng Nature Reserve. Parameter estimates were made at 0 when rainless day, standing, first zone, and bachelor. Significant levels were set at  $P = 0.05$

	Estimate	S. E.	Z-value	P
(Intercept)	-1.23	1.00	-1.225	0.221
Rainy	0.43	0.61	0.715	0.474
Bedding	0.077	0.73	0.107	0.915
Pavilion zone	-1.34	0.82	-1.634	0.102
Master	1.22	0.61	1.994	0.046 *
Group size	-0.004	0.004	-1.105	0.269

**Table 4** Social rank effect on overall grooming (including oral, scratching and antler grooming) of Père David's deer in Dafeng Nature Reserve. Parameter estimates were made at 0 when rainless day, standing, first zone, and bachelor. Significant levels were set at  $P = 0.05$

	Estimate	S. E.	Z-value	P
(Intercept)	0.33	0.74	0.446	0.656
Rainy	0.12	0.44	0.269	0.788
Bedding	-1.08	0.46	-2.358	0.020 *
Pavilion zone	-0.31	0.60	-0.513	0.608
Master	0.61	0.50	1.231	0.218
Group size	-0.001	0	-0.243	0.808

**Table 5 Food availability effect on oral grooming of Père David's deer in Dafeng Nature Reserve. Parameter estimates were made at 0 when standing, rainless, first zone, and adult female. Significant levels were set at  $P = 0.05$**

	Estimate	S. E.	Z-value	P
(Intercept)	0.13	0.65	0.201	0.841
Bedding	-0.88	0.37	-2.398	0.016 *
Adult male	-1.42	0.39	-3.654	<0.001 *
Fawn	1.00	0.48	2.102	0.036 *
Subadult female	-0.17	0.44	-0.385	0.700
Subadult male	-0.25	0.38	-0.658	0.511
Rainy	0.11	0.29	0.372	0.710
Pavilion zone	-0.27	0.45	-0.583	0.560
Group size	0.001	0.002	0.565	0.572

**Table 6 Food availability effect on overall grooming (including oral and scratching grooming) of Père David's deer in Dafeng Nature Reserve. Parameter estimates were made at 0 when standing, rainless, first zone, and adult female. Significant levels were set at  $P = 0.05$**

	Estimate	S. E.	Z-value	P
(Intercept)	-0.13	0.61	-0.214	0.830
Bedding	-0.40	0.35	-1.166	0.244
Adult male	-0.90	0.35	-2.560	0.011 *
Fawn	1.44	0.51	2.840	0.005 *
Subadult female	0.29	0.43	0.676	0.499
Subadult male	0.18	0.36	0.498	0.619
Rainy	0.10	0.27	0.381	0.703
Pavilion zone	-0.37	0.42	-0.891	0.373
Group size	0.002	0.002	0.870	0.384

#### 4 Discussion

As a prediction of the vigilance principle, social rank should influence grooming behavior, and harem masters should groom less due to a physiological suppression by testosterone during the rutting season, assuming they are suffering a similar tick load (Hart *et al.*, 1992; Hart, 1997, Mooring *et al.*, 1998, 2006b; Kakuma *et al.*, 2003). Though we did not census the actual tick load of the deer, we found similar tick densities in both the first core zone and the pavilion zone. As grooming behavior is usually positively related to tick density (Li *et al.*, 2014), we can assume that all deer are facing similar tick loads. However, we did not find significant differences of grooming rate between harem masters and bachelors, and harem masters even scratched a little more than bachelors. A previous study indicated that the level of testosterone in Père David's deer reached a peak at the end of May and decreased toward the end of June (Li *et al.*, 2001). However, we collected our field samples at the end of the rutting season, when the adult males (and especially the harem masters) were not as aggressive or vigilant as in the

rutting peak in May and June (Ren *et al.*, 2011; Zheng *et al.*, 2013), and this might explain why they groomed similarly.

Food availability is predicted to affect grooming behaviors since released food pressure would leave much more time for other behaviors (Mooring and Hart, 1995a). However, our results showed little significant difference in grooming between the pavilion zone and the first zone. Tick densities were similar between the two zones, thus we can assume the main differences are food availability and human disturbance (ecotourism). It is possible that human disturbance serves a compound function in shaping grooming patterns of the deer. Deer behaved much more vigilantly in the pavilion zone which is open for all tourists and the distance between tourists and deer is less than 50 m, whereas in the first core zone, tourism is only allowed in a closed car with a regular route and human disturbance is low (Li *et al.*, 2007a). Then the released time budgets due to decreased feeding time in the pavilion zone were probably reoriented toward vigilance behavior instead of toward grooming.

We also found a similar grooming pattern between

sex-age levels, giving support to the programmed grooming model (Mooring, 1995). Activity type, meaning standing or bedding, affects grooming behavior in most cases; deer groom more when standing, which has been found in several other studies (Mooring, 1995; Mooring and Samuel, 1998; Mooring *et al.*, 2006a).

In conclusion, we did not find significant effects of social rank and food availability on grooming behavior of Père David's deer, probably due to mismatched sampling periods or a compound effect of human disturbance. We suggest more observation be conducted in the rutting peak during May and June; and controlling the interference of human disturbance to explore if food availability would affect grooming.

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