



Relaxed selection for tick-defense grooming in Père David's deer?



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ABSTRACT

Relaxed selection following extirpation of predators or as a consequence of captive breeding may result in the loss of adaptive antipredator behavior. We propose that it is equally possible for relaxed selection to result in the loss or reduced effectiveness of parasite-defense behaviors such as grooming. In both cases, the reintroduction of captive-bred animals into a predator- or parasite-rich area could have disastrous consequences for the survival of the population. Tick infestation is a powerful force for the evolution of adaptive tick-defense grooming behavior in the wild. The regulation of anti-tick grooming has been well-studied in ungulates and consists of endogenous and exogenous mechanisms, corresponding to the programmed and stimulus-driven grooming models. These models predict that (1) smaller animals will groom more frequently than larger ones, (2) breeding males will groom less than females, (3) animals will groom more during the high-tick season, and (4) animals will groom more in tick-rich vs. tick-sparse habitat. We studied the grooming behavior of Père David's deer (*Elaphurus davidianus*), which has experienced genetic bottlenecks and possible relaxed selection as a result of hundreds of years in captivity and was recently reintroduced into the wild in China. Our results indicate that most but not all patterns of adaptive anti-tick grooming behavior have been retained, indicating that some aspects of parasite defense have not undergone relaxed selection even after many years of captivity. Nonetheless, we propose that plans to reintroduce captive-bred animals should take into consideration the historical tick exposure and present grooming behavior of the species in selecting suitable habitat for reintroduction.

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1. Introduction

Relaxed selection occurs when populations experience the weakening or removal of a selection factor important for the maintenance of a trait. Current investigation asks whether adaptive behavior is lost or reduced under relaxed selection, and how fast this can happen (Lahti et al., 2009). An important applied aspect of relaxed selection involves small populations of endangered species that are bred in captivity for later reintroduction into the wild. If relaxed selection in captivity results in the loss or reduction of traits important for survival, individuals that are later released back into the wild may suffer high mortality and the reintroduction exercise may fail (Lahti et al., 2009). Recent studies have focused on the potential loss of antipredator defenses when predators are extirpated from the wild, or absent in a captive breeding situation. McPhee (2004) found that the more generations of Old-field mice (*Peromyscus polionotus*) were kept in captivity, the less

likely they were to seek refuge from a predator as do their wild-caught counterparts, indicating that reintroduced mice would experience increased mortality and reduced survivorship.

Whether a species quickly loses antipredator behavior following relaxed selection depends on the degree to which the behavior is experience dependent (i.e., learned or innate) and the cost of performing the behavior (Blumstein, 2002). In some cases, antipredator behavior may persist despite long absence of a predator because other predators remain, the trait retains a different function, or the trait is more 'hard-wired' (Blumstein, 2002; Lahti et al., 2009). However, when behavior is dependent upon learning, antipredator defenses may be lost in a single generation of isolation from predators. In the absence of predators, prey species may experience loss or weakening of visual, acoustical, or chemical recognition of predators (Lahti et al., 2009). For example, moose (*Alces alces*) living where predators have been extirpated are less vigilant and do not respond to cues from scavengers to avoid predators (Berger, 1999; Berger et al., 2001). Tamar wallabies (*Macropus eugenii*) isolated from predators on islands for up to 1000 years no longer respond to the sounds or smells of predators (Blumstein et al., 2000; Blumstein and Daniel, 2005), while black-

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tailed deer (*Odocoileus hemionus*) respond to current predators but not to those now extinct (Stankowich and Coss, 2007).

Ticks are the most important ectoparasites of wild animals, and the cost of these ectoparasites for host animals has been well documented (Sutherst et al., 1983; Norval et al., 1988; Kaiser et al., 1991; Allan, 2001). Just as conservation managers must understand the mechanisms underlying antipredator behavior before releasing predator-naïve animals into predator-rich areas (Blumstein, 2002), so managers must understand parasite-defense mechanisms before releasing tick-naïve animals into tick-rich areas. Will the released animals retain the adaptive tick-defense grooming behavior of their ancestors, or will this behavior be lost and/or take time to be re-learned? Oldfield mice kept in captivity for up to 35 generations not only lost antipredator behavior, but they also performed reduced grooming with increasing generations in their tick-free captive setting (McPhee, 2004). Bighorn sheep (*Ovis canadensis mexicana*) of the Chihuahuah Desert of New Mexico have been isolated from ticks for thousands of years and as a result groom at an extremely low rate in their tick-free environment, even lower than size-matched ungulates in tick-free zoological parks (Mooring et al., 2006a).

In some cases tick-defense grooming may not evolve fast enough to avoid mortality and morbidity from ticks, just like in North American moose, which are susceptible to tick-induced alopecia, loss of blood, and even death from infestation by as many as 100,000 ticks per animal (Mooring and Samuel, 1998a). The vulnerability of moose to ticks has been attributed to their poor grooming behavior, only responding to adult ticks biting them but less sensitive to larvae and nymphs crawling on them, which contrasts with the highly efficient grooming of sympatric white-tailed deer and mule deer (*Odocoileus virginianus*, *O. hemionus*). The difference may be due to *odocoiline* deer being under natural selection from the winter tick (*Dermacentor albipictus*) for ~3.5 million years, compared to perhaps 10,000 years since moose migrated from Asia and encountered the winter tick (Mooring and Samuel, 1998a). Moose harbor up to 25 times greater tick burdens than deer, and suffer mortality from other parasites to which they have been recently exposed and that deer are well adapted to (meningeal worm *Parelaphostrongylus tenuis*; liver fluke *Fascioloides magna*). It appears that this species has still not evolved the grooming behavior it needs (Mooring and Samuel, 1998a, 1999). This begs the question as to whether a small population that has gone through a genetic bottleneck and centuries in captivity may have lost the essential tick-defense grooming behavior needed to survive and thrive when introduced into a tick-rich area.

1.1. Père David's deer

Père David's deer (*Elaphurus davidianus*) is an endangered species that was extirpated in the wild and has survived in captivity for perhaps the last 800 years. Descended from a population kept in the Nanyuang Royal Hunting Garden since as early as 1205, the world population descends from 18 individuals that survived in Woburn Abbey, UK during the early 20th century (Zeng et al., 2007, 2013), resulting in significant inbreeding and genetic bottlenecks. Thirty-nine individuals were reintroduced to Dafeng, China from 7 British zoos in 1986, and now the population has increased to about 2000 in 2013, making it the world's largest population. We presume that ancestral Père David's deer practiced the same adaptive tick-defense grooming seen in other species of closely related *Cervus* deer, but it is unclear what the level of tick exposure was during their long period of enforced captivity. Although both Woburn Abbey and Beijing Milu Park (present day site of the Nanyuang Royal Hunting Garden) were not tick-free, they may have had low densities of ticks (Ding, 2004). Furthermore, because the *Hoxb8* gene is critical to the expression of grooming behavior

(Greer and Capecchi, 2002), the low genetic diversity resulting from bottlenecks and founder effects (Zeng et al., 2007, 2013) raises the possibility that tick-defense grooming patterns have been lost or compromised regardless of relaxed selection. The death of two male Père David's deer from heavy tick infestation in 1995 and 2004 suggests that this might be the case (Shen et al., 1996, 2007).

1.2. Adaptive grooming behavior

Grooming is the first line of defense against tick infestation for wild mammals, and animals with restricted or poorly developed grooming behavior are vulnerable to excessive tick infestation (Koch, 1981, 1988; Mooring et al., 1996a; Mooring and Samuel, 1999). Two grooming models have been proposed to explain the endogenous and exogenous regulation of tick-defense grooming. 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, 1995). There is ample evidence for central control of grooming (Roth and Rosenblatt, 1967; Nelson et al., 1975; Colbern and Gispén, 1988; Fentress, 1988; Spruijt et al., 1992). 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 two models are not mutually exclusive, and indeed are likely to operate concurrently as a complementary system (Mooring et al., 2004).

Two predictions emerge from the programmed grooming model. The body-size principle is based on the recognition that smaller animals, with a greater surface area-to-mass ratio, incur higher costs for a given density of tick infestation relative to larger ones (Hart et al., 1992). Assuming an equal rate of infestation, small-bodied animals should groom at a higher rate and consequently maintain a lower density of ticks compared with larger animals. Intraspecifically, subadults have been observed to groom more than adults (Mooring and Hart, 1997a, 1997b; Mooring and Samuel, 1998c, 1998b; Mooring et al., 2002, 2006a; Hart and Pryor, 2004) and harbor fewer ticks as a result (Gallivan et al., 1995). The vigilance principle predicts that males of polygynous species will groom less than females in the same herd during the breeding season (sexually dimorphic grooming) in order to maintain high levels of vigilance for rival males or estrous females (Hart et al., 1992). Testosterone is the mechanism behind sexually dimorphic grooming, with higher levels of testosterone resulting in a physiological suppression of programmed grooming (Mooring et al., 1998, 2004, 2006a; Kakuma et al., 2003). Sexually dimorphic grooming as a consequence of the vigilance principle is independent and additive to grooming rate differences among sex-age classes due to the body size principle (Mooring et al., 2004). A corollary of the vigilance principle is that such males should carry more ectoparasites. Sexually dimorphic grooming has been observed in a wide range of ungulates (Hart et al., 1992; Mooring and Hart, 1995; Mooring et al., 1996b, 1998, 2002, 2006a, 2006b; Mooring and Samuel, 1998b), and breeding male ungulates carried many more ticks than females (Main et al., 1981; Drew and Samuel, 1985).

Two predictions also emerge from the stimulus-driven grooming model. The tick challenge principle predicts that grooming rate will track the intensity of tick exposure (Mooring, 1995). Because tick challenge may vary dramatically over time and space and grooming behavior has costs, animals will adjust their grooming rate on a seasonal or geographical basis. This prediction has been supported for African and North American ungulates (Mooring, 1995; Mooring and Hart, 1997b; Mooring and Samuel, 1998c). Related to the tick challenge principle, the habitat principle

predicts that because habitats with a greater density of ticks expose hosts to a higher risk of infestation, animals that inhabit such areas will groom more frequently than those utilizing habitats of lower tick density. A broad generalization is that closed habitats, such as woodland and forest, have a greater abundance of ticks than open ones, such as grassland or savannah (Barnard, 1986; Garris et al., 1990; Carroll and Schmidtman, 1996).

The goal of this study is to investigate whether effective tick-defense grooming patterns have been retained in Père David's deer subject to potential relaxed selection and loss of genetic diversity over hundreds of years of captivity. We observed the grooming behavior of two populations of deer, one introduced to the Dafeng Milu Nature Reserve, where tick challenge varies by season and habitat, and the other housed at the tick-free San Diego Zoo Safari Park. We predicted that if relaxed selection has resulted in the loss of effective anti-tick grooming behavior, tick-naïve deer will not groom according to the patterns observed in other ungulates in accordance with the predictions of the programmed and stimulus-driven grooming models. The reduced responsiveness of moose grooming to tick infestation mentioned above (Mooring and Samuel, 1998a) suggests that relaxed selection may result in a quantitative reduction in grooming rather than its elimination. If so, we might expect that tick-naïve Père David's deer will respond to increased tick challenge with a slightly increased grooming rate, but perhaps not of the magnitude required to avoid heavy tick infestations.

If Père David's deer still retain adaptive tick-defense grooming, we predicted that their grooming would reflect the principles outlined above. Specifically:

- (1) Grooming rate will be negatively associated with body size, with fawns and subadults grooming more frequently than larger adults (intraspecific body size prediction).
- (2) Grooming rate will be higher for adult females compared with breeding adult males (vigilance prediction).
- (3) Grooming rate will be elevated for all deer during the high-tick spring/summer seasons compared with the low-tick fall/winter seasons (tick challenge prediction).
- (4) Grooming rates will be elevated for all deer in higher tick habitat (the first zone) compared with lower tick habitat (habitat prediction).

2. Methods

2.1. Field study site

The field study was conducted in the Dafeng Milu Nature Reserve (32°59'–33°03'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, 27.0 °C in July and 217 frost-free days. Average precipitation is 1068 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.

2.2. Study species

Père David's deer have pronounced sexual dimorphism: adult males are about 35% heavier than adult females (Ding, 2004). The mating system is harem polygamous, in which a strong harem holder dominates the harem group and monopolizes nearly all mating opportunities (Li et al., 2001, 2004). The rutting season extends from May to July, and the calving season is typically from March to May. In the area, ticks become active in the spring and reach a peak in summer, becoming inactive in autumn and winter

(Wang et al., 1997; Ding, 2004). The study population occupies two separate zones with different habitat types and tick densities. The first zone is composed of grassland and woodland, whereas the second zone is completely grassland (Yu and He, 2011).

2.3. Tick census

Ticks are common in the nature reserve and the dominant tick species is *Haemaphysalis longicornis*, which is also the main threat for the deer. Tick densities were measured using the flag sampling method. The sampling flag was made of a 100 cm × 100 cm piece of unbleached cotton muslin stapled to a wooden base. For each sampling, we dragged the flag for 10 m and then collected the ticks attached on the flag. We randomly sampled about 15 flags to cover the study area each time and sampled two to three times in the first and second core zones in spring and summer. We did not measure tick densities in autumn and winter because previous studies indicated that ticks were inactive from late October till March (Wang et al., 1997; Ding, 2004).

2.4. Behavioral sampling at Dafeng Reserve

The study at Dafeng Reserve was conducted in the first and second fenced core zones during four consecutive seasons (Summer: July 2012; Fall: late October to November 2012; Winter: January 2013; Spring: April 2013). We walked daily along trails looking for deer groups. From one to a dozen individuals were selected from each group depending of group size (ranging from 1 to 340). Focal individuals were randomly selected from different groups or different parts of the same group to avoiding re-sampling the same individuals (pseudoreplication), and each individual was observed for 10–15 min, during which the focal subject was in full view, using binoculars (8 × 56) or a telescope (20–60 × 63). At the beginning of each focal observation, zone (first, second), sex and age (fawn, subadult female, subadult male, adult female, adult male), as well as group size and composition (male group, female group, and mixed-sex group), date, and time (morning, afternoon), were recorded. Observations were usually made from a shelter or 150 meters away from the focal individuals so as to reduce potential observer effects (Li et al., 2007). Although the animals were unmarked, given the large population size it was unlikely that the same individuals were sampled more than once on a given day.

Using size and general appearance, deer were classified into one of five age-sex categories: fawns less than 1 year (12.6 ± 1.4 kg), subadult females between 1 and 3 years (100.4 ± 5.7 kg), subadult males between 1 and 4 years (130.1 ± 2.8 kg), adult females older than 3 years (139.0 ± 7.6 kg), adult males older than 4 years (184.2 ± 17.2 kg) (Li, 2013). For males, we distinguished ages through antler structures: subadult males are one main beam with 0–2 branches while adult males are one main beam with 3 or more branches. Two types of grooming behavior can be distinguished: oral grooming using the lower incisors to scrape through the pelage and scratching with the antler or hoof of the hind leg, but we mainly focused on oral grooming because there is evidence that scratching is largely a temporary response to peripheral stimulation from ticks or biting flies (Hart et al., 1992; Mooring, 1995; Mooring et al., 1996b; Mooring and Hart, 1997b). We did not find allogrooming behaviors in this deer. The number of grooming bouts, defined as an uninterrupted sequence of grooming episodes, was recorded during the observation session. Grooming bouts were then extrapolated to grooming rate per hour.

2.5. Behavioral sampling at the San Diego Zoo Safari Park

We conducted observations on Père David's deer at the San Diego Zoo Safari Park (formerly the San Diego Wild Animal Park)

in June 2001. Animals at the Safari Park were free of ticks and other ectoparasites, which have not been detected during drag censuses or examination of animals routinely immobilized by veterinary staff. Although biting insects may cause irritation, insect-repelling movements (e.g. tail switching, head shaking, et al.) are readily distinguished from tick-removal oral or scratch grooming (to be conservative, single-episode oral grooming bouts were discarded in case they were directed at insects). We conducted observations on 22 marked individuals (10 adult females, 5 adult males, and 7 subadults).

We used spotting scopes and binoculars to conduct focal animal observations of 10 min duration in which grooming bouts were continuously recorded and 1-min instantaneous scans recorded activity budgets. We conducted three observations per individual, each observed on a different day when they were standing and active. We then calculated mean grooming rate for each animal and took the grand mean to compute the mean grooming rate for females, males, and subadults (there were no fawns).

2.6. Statistical analysis

For the tick data at Dafeng Reserve, we performed 140 flagging censuses during the study period. Because of the relatively high prevalence of zero counts, we used the Mann–Whitney test to examine the effects of season and zone on tick density.

For the grooming data at Dafeng Reserve, we collected a total of 1247 behavioral samples, and the total observation time was 242 h (14496 min) with an average (\pm SD) observation length of 11.6 ± 0.1 min. We used a generalized linear model with negative binomial distribution and log link using the logarithm of focal observation length as offset and the group id as a random factor. The independent fixed factors considered were: zone (first vs. second core zone), sex and age composition (adult and subadult males, adult and subadult females, and fawns), activity type (bedding vs. standing), season, group size and time of day (am vs. pm). The final model was attained by sequentially removing non-significant factors. A priori contrasts were made to determine differences among levels of each independent variable.

For the zoo data, we analyzed the scan sampling data using a logistic regression model with age class as the independent variable (adult vs. subadult). Data from adult males and females were pooled together given the rare occurrence of grooming.

3. Results

3.1. Dafeng Reserve

3.1.1. Tick density

Tick density varied between zones (Mann–Whitney test, $Z = -7.437$, $p < 0.001$) and seasons (Mann–Whitney test, $Z = -2.829$, $p = 0.005$), being much higher in the first zone (43.67 ± 7.90 ticks per flag) than in the second zone (1.94 ± 0.53 ticks per flag), and much higher in summer (31.94 ± 6.12 ticks per flag) than in spring (4.14 ± 1.51 ticks per flag).

3.1.2. Grooming

We collected a total of 1247 grooming samples. Of them, 374, 204, 272, 192 and 205 were respectively observed for adult male, subadult male, adult female, subadult female and fawn; 294, 398, 316 and 239 were respectively observed in spring, summer, autumn and winter; 765 and 482 were observed in the first zone and second zone.

The expected frequency of oral grooming varied significantly with age–sex combination ($\lambda = 19.0$, $p = 0.0008$; Fig. 1), season ($\lambda = 22.0$, $p < 0.0001$; Fig. 2), zone ($\lambda = 8.0$, $p = 0.005$), activity type

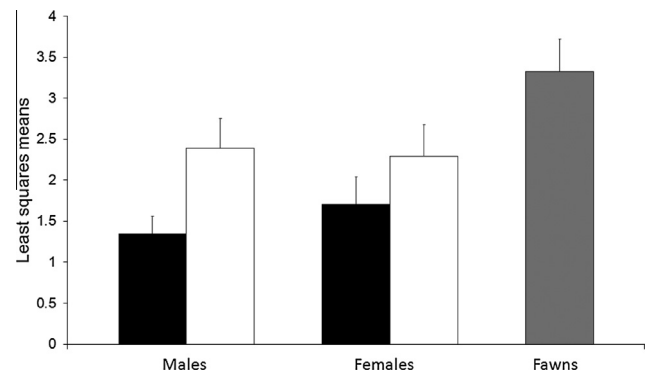


Fig. 1. Least-squares means of the expected number of oral grooming bouts per hour as a function of age and sex (black bars: adults; white bars: subadults). Bars show one standard error.

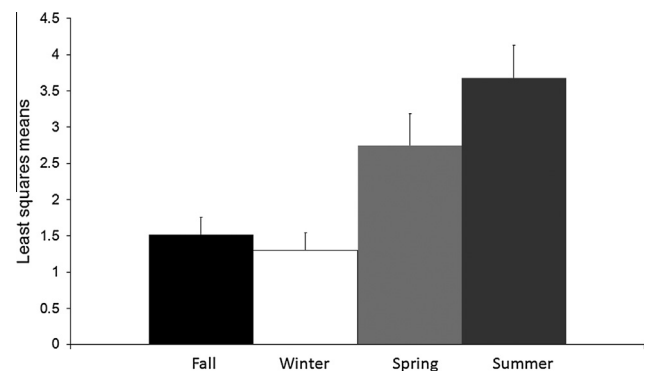


Fig. 2. Least-squares means of the expected number of oral grooming bouts per hour as a function of season. Bars show one standard error.

($\lambda = 6.9$, $p = 0.009$) and time of day ($\lambda = 4.3$, $p = 0.04$) but not with group size (when added to the above model: $\lambda = 0.27$, $p = 0.60$).

Specifically, the frequency of grooming increased by a factor of 1.8 (95% confidence interval: 1.4–2.3) in fawns with respect to subadults and adults ($p < 0.0001$), by a factor of 1.3 (1.0–1.8) in subadult females with respect to adult females ($p = 0.04$) and by a factor of 1.8 (1.3–2.5) in subadult males with respect to adult males ($p = 0.001$). There was no significant difference in the frequency of grooming between females and males (1.1 (0.83–1.5), $p = 0.51$) or between subadult males and adult females (1.4 (0.95–2.1), $p = 0.09$). The frequency of grooming increased by a factor of 1.7 (1.2–2.5) when standing rather than bedding, by a factor of 1.7 (1.3–2.2) when in the first zone rather than in the second zone, and by a factor of 1.3 (1.02–1.70) in the morning rather than in afternoon. With respect to winter, the frequency of grooming increased by a factor of 2.1 (1.3–3.4) in the spring ($p = 0.003$), by a factor of 2.8 (1.9–4.3) in the summer ($p < 0.0001$), but only by a factor of 1.2 (0.72–1.9) in the fall, a non-significant increase ($p = 0.53$).

3.2. San Diego Zoo Safari Park

A bout of oral grooming occurred only once across all scan samples for adults. Oral grooming was recorded in four of the seven subadults between 1 and 3 times each. The odds of oral grooming decreased by a factor of 0.065 (nearly 15 times less) in adults relative to subadults (95% CI: 0.008–0.52; $p = 0.01$, Fig. 3).

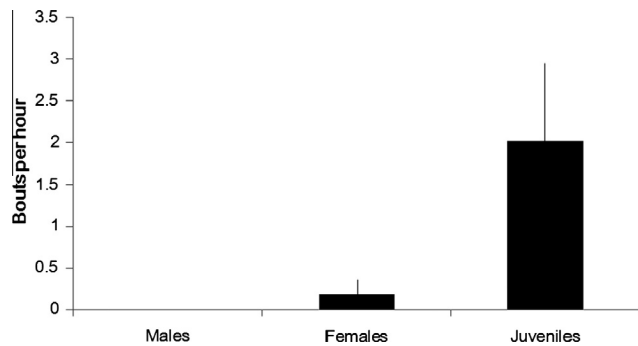


Fig. 3. Oral grooming bouts per hour at the San Diego Wild Animal Park for three different sex-age classes.

4. Discussion

We asked whether Père David's deer have retained adaptive tick-defense grooming patterns despite potential relaxed selection and loss of genetic diversity through inbreeding, bottlenecks, and founder effects during up to 800 years in captivity. This is an important question to ask whenever threatened species are captive-bred and later released into tick-infested habitat in the wild. Failure to respond to tick exposure with appropriate defensive behavior could potentially result in the failure of reintroduction efforts due to population decline and even mortality in extreme cases. Although conservation practitioners are beginning to consider the influence of relaxed selection on the antipredator behavior of reintroduced species, to our knowledge this is the first time that the influence of parasite-defense behavior has been examined in the context of in situ conservation. Our results suggest that Père David's deer have retained many (but perhaps not all) adaptive patterns of anti-tick grooming previously identified in other ungulates, including closely related *Cervus* species observed in the wild and captivity (Mooring and Samuel, 1998b; Mooring et al., 2000, 2002, 2004).

4.1. Tick-defense grooming patterns

We examined the predictions of the programmed and stimulus-driven grooming models. We found that oral grooming followed the body size prediction of the programmed grooming model for deer in both the Dafeng Reserve (tick-rich) and the Safari Park (tick-free). At Dafeng, fawns groomed more than subadults who groomed more than adults, while at the Safari Park, subadults groomed more than adults. Similar findings have been documented in other wild ungulate species, including closely-related elk (*Cervus elaphus*) (Mooring et al., 1998, 2006b; Mooring and Samuel, 1998b). These results provide strong support that the deer have retained tick-defense oral grooming regulated mainly by an intrinsic timing mechanism that operates in accordance with organismic factors associated with parasite vulnerability.

Although the programmed grooming model predicts that larger males will groom less than females in accordance with the body size and vigilance principles (Hart et al., 1992), we did not find any difference between males and females in general, and between subadult males and adults females which have a similar body mass. Previous studies on the reproductive and vigilance strategies of Père David's deer during the rut showed that male deer, especially the masters, compete intensely with rival challengers and need to be vigilant to hold their harems, detect females in estrous, and mate with them (Li et al., 2001, 2004; Jiang et al., 2004). In addition to this time constraint on grooming, testosterone levels in males are extremely high during the rut (Li et al., 2001, 2004),

which may have a suppressive effect on grooming (Mooring et al., 1998, 2004, 2006b; Kakuma et al., 2003). Nonetheless, adult females were more vigilant than expected as seen in a recent study (Zheng et al., 2013). Contrary to the vigilance prediction of the programmed grooming model, females usually spent as much time as males in vigilance when in mixed-sex and even more when in single-sex groups (Zheng et al., 2013). This is interesting when considering that the vigilance prediction has been supported for most other ungulates including closely-related *Cervus* species (Mooring et al., 2002). It is possible that the lack of evidence for the vigilance principle arises because our observations took place mostly at the end of the rut in July, when the adult males are not as aggressive and vigilant as in the rut peak in May and June.

As predicted by the stimulus-driven model, Dafeng deer groomed more frequently during seasons with higher tick densities. The main tick species *H. longicornis* at Dafeng, emerge from hibernation in March, nymphs peak in May, adult ticks peak in June and July, and larval ticks peak in August and September, and then the ticks become inactive and move underground for hibernation in late October (Wang et al., 1997; Ding, 2004). Our tick census also showed a significant difference between spring and summer. Deer thus suffer from heavier tick loads in summer, and therefore oral groom the most during this season, lower during spring, and lowest during non-tick autumn and winter.

Also in accordance with the predictions of stimulus-driven grooming, deer adopted higher grooming rates in areas with increased tick densities. Deer were observed in two fenced zones at Dafeng: the first zone is composed by grassland and woodland, while the second zone is completely grassland (Yu and He, 2011). Closed and complex habitats such as woodland are usually more suitable for tick survival than open habitat, such as grassland (Barnard, 1986; Garris et al., 1990; Carroll and Schmidtman, 1996), and this can explain why the first zone harbors more ticks. In addition, deer density in the first zone is nearly twice that of the second zone, and tick abundance is known to be closely related to host density because higher host density enables amplified transmission and reproduction of the tick population (Wilson et al., 1985, 1988; Stafford, 2007; Vor et al., 2010). As a result, deer in the first zone increased their grooming rate in response to the more frequent tick bites.

5. Conclusion

It is important to understand whether captive bred populations of threatened species have experienced a loss or attrition of adaptive tick-defense behavior before they are reintroduced into areas with significant tick challenge. Père David's deer are an ideal species with which to test this premise given their long period of captivity with unknown consequences for relaxed selection and documented loss of genetic diversity and potential loss of grooming behavior. This species was extirpated in the wild but now is being reintroduced into large fenced reserves where tick densities are high in certain habitats and seasons. Our observations in both tick-rich and tick-free environments support both the body size prediction of the programmed grooming model and the tick challenge and habitat predictions of the stimulus-driven model. These results suggest that Père David's deer have not lost their endogenous (centrally regulated) or exogenous (peripherally activated) tick-defense grooming behavior in the face of relaxed selection and loss of genetic diversity. It is likely that this species has retained effective grooming patterns because they were exposed to some level of tick challenge during much of their captivity. However, this may not be the case for all species. Furthermore, the failure to support the vigilance prediction and past mortality of tick-infested deer raises the possibility that some aspects of anti-tick

grooming may have been altered and perhaps compromised in this species. We therefore propose that for this and other small populations of endangered species, a consideration of the functionality of anti-tick behavior would be as relevant to in situ conservation efforts as the functionality of antipredator defense. Plans to reintroduce captive-bred animals should take into consideration the historical tick exposure and current grooming behavior of the species when selecting suitable habitat for reintroduction.

The following suggestions may be useful for tick burden management in this species at Dafeng. First, potential suitable areas should be selected to reduce both deer density and tick exposure, especially in the first core zone. Second, the reserve is now burning grass every spring to control tick population size, but the effect of burning needs to be explored in detail to determine the impact on tick population biology and vegetation structure. Finally, biological control of the tick population, with local bird species such as magpies (*Pica pica*) and cattle egrets (*Bubulcus ibis*), may be an effective means of controlling tick burden (Samish and Rehacek, 1999; Fernandez et al., 2014), but this approach needs to be investigated more specifically at Dafeng.

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