Tick-defense grooming patterns of two sympatric Tibetan ungulates

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Abstract
Grooming is an important behavioral defense against tick infestation for ungulates. The ‘programmed grooming model’ explains the endogenous regulation of tick-defense grooming and predicts different inter- and intra-specific grooming patterns owing to the body size principle. Here, we studied the summer grooming behaviors of two sympatric Tibetan ungulates, to explore whether or not body size principle works inter- and intra-specifically. The ungulates were sexually and body-size dimorphic Tibetan antelopes (Pantholops hodgsonii) with body weight of c. 33 kg and sexually dimorphic but body-size monomorphic Tibetan gazelle (Procapra picticaudata) with body weight of c. 14 kg. Group size was also included in our negative binominal regression model to determine whether or not the ungulates groomed more or less with the increasing group size. Results showed that large Tibetan antelopes groomed much less than small Tibetan gazelles inter-specifically. Intra-specifically, sex-age significantly affects the grooming rate of Tibetan antelopes. The largest adult males groomed the least, whereas the smallest fawns groomed the most. However, this sex-age effect is not found in Tibetan gazelle: males and females groomed similarly. These findings indicate that body size principle is fully supported inter- and intra-specifically. Positive group size effect on grooming is observed in Tibetan gazelle, suggesting that released vigilance time from group size effect is probably transferred to grooming. From a conservation point of view, we suggest further studies on the testosterone effect on grooming patterns during the rut as well as on tick biology on the plateau.

Introduction
Ticks are the most important ectoparasites of large mammals, and the cost of tick infestation for host animals is well documented (Sutherst et al., 1983; Norval et al., 1988; Kaiser, Sutherst & Bourne, 1991; Allan, 2001). Grooming is the first line of behavioral defense against tick infestation for mammals, and animals with poor grooming are vulnerable to excessive tick infestation (Koch, 1981, 1988; Mooring, McKenzie & Hart, 1996; Mooring & Samuel, 1999).

Two grooming models explain the endogenous and exogenous regulation of tick-defense grooming. The ‘programmed grooming model’ postulates that a central control mechanism periodically evokes a bout of grooming to remove ticks before they attach and suck blood (Hart et al., 1992; Mooring, 1995), whereas in the ‘stimulus-driven grooming model’, bouts of grooming are responses to peripheral stimulation from tick bites (Riek, 1962; Willadsen, 1980; Wikel, 1984). Although ample evidence attests to central control of grooming (Roth & Rosenblatt, 1967; Nelson et al., 1975; Colbern & Gispen, 1988; Fentress, 1988; Spruijt, Vanhooff & Gispen, 1992), the two models are likely to operate concurrently as a complementary system (Mooring, Blumstein & Stoner, 2004; Li, Beauchamp & Mooring, 2014).

An important prediction of the programmed grooming model is the ‘body-size principle’, which is based on the recognition that smaller host animals incur higher costs for a given density of ticks compared with larger ones (Hart et al., 1992). Small animals have a greater surface area-to–mass ratio but relatively smaller volume, so they support more ticks per unit blood resource compared with larger-bodied animals. Thus, 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. The body size prediction can be applied inter-specifically among species of different body sizes or intra-specifically among conspecifics of different age–sex classes. Furthermore, we can predict that sexual difference would be small if males and females are of a similar body size or monomorphic. Both the inter- and
intra-specific models of the body size principle have been supported by comparative studies of ungulates in tick-free zoological parks where differential tick challenge was controlled (Mooring et al., 2000, 2002, 2004).

Five endemic ungulates live in the ‘roof of the world’, Qinghai–Tibet plateau (Schaller, 1998), including Tibetan antelope (Pantholops hodgsonii), Tibetan gazelle (Procapra picticaudata), Przewalski’s gazelle (Procapra przewalskii), Tibetan wild yak (Bos mutus) and Kiang (Eguas kiang). Here, we study two sympatric ungulates, Tibetan antelope and Tibetan gazelle, in Celincuo National Nature Reserve, Tibet. Ticks are common on the plateau, and at least 41 tick species have been reported in Qinghai and Tibet (Yang et al., 2008; Zhang et al., 2009). All these ungulates face threats from not only natural predators and human activities but also from ticks. The goal of this study is to investigate whether two species of Tibetan ungulates groom in a manner which reflects the body size principle. We predict the following:

(1) Interspecifically, grooming rate will be negatively associated with body size, and Tibetan gazelle grooms more frequently than Tibetan antelope.

(2) Intra-specifically, the smallest fawns of Tibetan antelope will groom the most, whereas the largest males will groom the least in Tibetan antelope.

(3) For sexual dimorphic Tibetan antelope, males will groom less frequently than females. For Tibetan gazelle in which both sexes have a similar body size, males will groom similarly as females.

We also predict that individuals in larger herds would groom more frequently than those in small groups which must perform higher levels of vigilance for predators (Lian et al., 2007; Li & Jiang, 2008; Li, Jiang & Beauchamp, 2009). If vigilance requirements restrict the time available to groom, the frequency of grooming should increase in large groups in which other individuals are also vigilant.

Materials and methods

Study site

This study was conducted in Celincuo National Nature Reserve, Shenzha County in Tibet, China. Shenzha County (30°02′39″–32°19′33″ N, 87°45′30″–89°47′49″ E) is in the central part of Tibet Autonomous Region. Elevations range from 4530 to 6448 m, with an average of more than 4700 m. Local climate is characterized by extreme cold and long winters, strong winds, and high levels of solar radiation. Mean annual temperature is 0.4°C. Annual precipitation is about 330 mm, and most rain falls between June and September. Alpine meadow is the main vegetation type, and no shrubs appear in this area.

Study species

The Tibetan gazelle resides in fragmented habitat patches on the Qinghai–Tibetan Plateau; however, the population is decreasing, and their range is shrinking (Schaller, 1998; Zhang and Jiang 2006). Although it is listed as Near Threatened in the IUCN Red list of Threatened Species, Tibetan gazelle is a Category II (Threatened in China) National Protected Wild Animal Species in China. Tibetan gazelle breeds from December to January and fawns from July to August (Li & Jiang, 2006; Li, Jiang & Beauchamp, 2010). Focal populations are estimated at a few hundred at Shenzha.

The Tibetan antelope is a flagship species to Qinghai–Tibet Plateau. The total population underwent a severe decline in the 1980s and early 1990s as a result of commercial poaching for the valuable underfur (Schaller, 1998). Rigorous protection has been enforced since then, and recent estimates were made at about 100 000–150 000 (Leslie & Schaller, 2008; Liu, 2009). Tibetan antelope has been classified as a Category I (Endangered in China) National Protected Wild Animal Species in China since 1989 and as Near Threatened by IUCN since 2016. Tibetan antelope breeds from December to January, and the fawning season is from June to July. The resident status of Tibetan antelope can be divided into two types, namely, migratory and resident. Our focal population in Shenzha is resident and does not migrate. The population size can reach 10 000.

The two species can be distinguished using morphological traits (Fig. 1). Tibetan antelopes are dimorphic and heavier than the Tibetan gazelles; adult male with an average body weight of 39 kg is larger than female with an average of 26 kg (Leslie & Schaller, 2008). Unlike Tibetan antelopes, Tibetan gazelles are monomorphic except that only the males bear horns (Schaller, 1998). Body weights range from 13 to 16 kg (Leslie, 2010). The horns of Tibetan antelope could reach 80 cm and are very strong, whereas the horns of Tibetan gazelle are short and tenuous (Fig. 2).

The most common mammalian predator in Shenzha is the wolf (Canis lupus), followed by the lynx (Felis lynx), and the brown bear (Ursus arctos), which are rarer. Tibetan fox (Vulpes ferrilata) are also common and may prey upon fawns of these ungulates. Large raptors including upland buzzard (Buteo hemilasius), cinereous vulture (Aegypius monachus), and lammergeier (Gypaetus barbatus) are common and are frequent scavengers of dead ungulates. Additionally, feral dogs might be an increasing threat to these rare ungulates.

Behavioral sampling

Daytime observations were carried out from sunrise to sunset between July and August 2016 and between June and July 2017 in Shenzha. We defined a group as a herd of gazelles or antelopes with no more than 50 m separating any two group members. Observations were carried out from the roadside using binoculars (8 × 42) or a telescope (20–60 × 63). We walked or drove regular routes to find targets for behavioral observations.

Marking or recognizing individuals through particular features was not feasible. Therefore, upon encountering a group, we randomly selected one or two focal individuals per group. Owing to the random selection process, focal individuals could occur anywhere in the group in terms of spatial location. The same individuals were likely monitored more than once during the study period because they were unmarked, and the population size of the Tibetan gazelle was small. By focusing on one or two
individuals per group, we reduced the chances of sampling the same individuals twice on the same day. Sometimes we added a few samples when collecting data from extreme large groups (more than 100 individuals), like in Tibetan antelope, but we tried to sample them from different parts of the same group to avoid resampling. The pseudo-replication for Tibetan antelope is rather small owing to the extremely large population.

At the beginning of each focal observation, we recorded the date, time of day, location, group type, group size, and the number of fawns present. Only three stable group types (single–male groups which were made up by only males, single–female groups which were made up by only females, and mother–fawn groups which were made up by females and fawns) could be found during summer (Li & Jiang, 2006). Focal individuals were classified into four categories: adult male, sub-adult male, adult female and fawns. Distinguishing sub-adult females from adult females was difficult, so they were combined as adult females.

Behavioral events were videotaped or dictated to an MP3 recorder. Observations lasted for 30 min unless we lost sight of the focal individual. Actual observation duration was 5–30 min. Grooming behavior was defined as oral grooming using the lower incisors to scrape through the pelage or scratching with the horns or the hoof of the hind leg. The number of grooming bouts, defined as an uninterrupted sequence of grooming episodes, was recorded during the observation session.

### Statistical analysis

Grooms could be considered as count data, and they were negative binomial distributed with a test of fitdistplus package (Delignette-Muller & Dutang, 2015). The average of number of grooms was 4.3 per observation, much smaller than the variance, 38.4; and a negative binomial regression model was then used to evaluate the possible effects of sex–age and group size. Observation length and observation time (Morning, 10:00–13:00; Noon, 13:00–16:00; and Afternoon, 16:00–19:00; all in Beijing Time, local time was 2 h later than Beijing Time) were also included in the model. The independent fixed factors were: species (Tibetan gazelle, Tibetan antelope), sex and age composition (adult male, adult female, sub-adult male and fawn), daytime (morning, noon and afternoon), group size (continuous variable) and observation length (continuous variable). Sex–age, daytime and group size were nested in each species in the model. Non-parametric Spearman correlation between group size and groom rate was also used to evaluate if these ungulates groom more with increasing group size. All these analyses were done with MASS package (Venables &
Ripley, 2002) using R Core Team (2013), data were shown with Mean ± se, and significant levels were set at 0.05.

**Results**

We collected a total of 416 behavioral samples, and the total observation time was about 100 h (5982 min) with an average (± SE) observation length of 14.4 ± 0.2 min. Among them, 218 and 198 were observed for Tibetan gazelle and Tibetan antelope, respectively. Group sizes ranged from 1 to 15 for both Tibetan gazelle and 1–400 for Tibetan antelope.

Based on the final negative binominal regression model, we found a significant effect of body size on grooming rates, both inter- and intra-specifically (Table 1). For the inter-specific body size prediction, Tibetan antelope, about twice the weight of Tibetan gazelle, groomed at a much lower frequency by a factor of 2.112 ± 0.338 than Tibetan gazelle ($Z = -6.252, P < 0.001$). For the intra-specific body size prediction, we did not find any effect of sex-age on grooming rate in Tibetan gazelle, but we found a significant effect on that in Tibetan antelope. Adult females ($Z = 2.957, P = 0.003$) and fawns ($Z = 5.181, P < 0.001$) groomed much more than adult males.

**Table 1** Effects of sex-age, daytime and group size on grooming rates of sympatric Tibetan antelope and Tibetan gazelle in Tibet with a negative binominal regression model

<table>
<thead>
<tr>
<th>Model term</th>
<th>Estimate</th>
<th>se</th>
<th>Z value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.761</td>
<td>0.246</td>
<td>3.092</td>
<td>0.002</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibetan antelope</td>
<td>-2.112</td>
<td>0.338</td>
<td>-6.252</td>
<td>4.04E-10</td>
</tr>
<tr>
<td>Tibetan gazelle</td>
<td>0</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>0.052</td>
<td>0.01</td>
<td>5.055</td>
<td>4.30E-07</td>
</tr>
<tr>
<td>Tibetan gazelle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex-age$^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult female</td>
<td>-0.265</td>
<td>0.153</td>
<td>-1.736</td>
<td>0.083</td>
</tr>
<tr>
<td>Sub-adult male</td>
<td>0.281</td>
<td>0.35</td>
<td>0.803</td>
<td>0.422</td>
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<tr>
<td>Fawn</td>
<td>0.321</td>
<td>0.526</td>
<td>0.609</td>
<td>0.542</td>
</tr>
<tr>
<td>Adult male</td>
<td>0</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Daytime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noon</td>
<td>-0.356</td>
<td>0.181</td>
<td>-1.97</td>
<td>0.049</td>
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<td>Afternoon</td>
<td>-0.072</td>
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<td>0.67</td>
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<tr>
<td>Morning</td>
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<td></td>
<td>-</td>
<td>-</td>
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<tr>
<td>Group size</td>
<td>0.15</td>
<td>0.039</td>
<td>3.838</td>
<td>0.0001</td>
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<tr>
<td>Tibetan antelope</td>
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<td>Fawn</td>
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<td>Adult male</td>
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<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Daytime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noon</td>
<td>-0.408</td>
<td>0.322</td>
<td>-1.264</td>
<td>0.206</td>
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<tr>
<td>Afternoon</td>
<td>0.106</td>
<td>0.258</td>
<td>0.409</td>
<td>0.682</td>
</tr>
<tr>
<td>Morning</td>
<td>0</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group size</td>
<td>0.0002</td>
<td>0.001</td>
<td>0.203</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Bold indicates significant at $P < 0.05$. Dispersion parameter for Negative Binominal (1.1584) family was taken to be 1. Null deviance: 790.40 on 415 d.f.; Residual deviance: 447.92 on 401 d.f; AIC = 1854.9.

Group size effect on grooming rates was only found in Tibetan gazelle in which grooming rates increased by a factor of 0.15 ± 0.04 with increasing group size ($Z = 3.838, P < 0.001$). Spearman correlation also indicated a positive relationship between group size and grooming rate in Tibetan gazelle ($r = 0.237, N = 218, P < 0.001$). Group size had no effect on grooming rates of Tibetan antelope ($Z = 0.203, P = 0.839$; $r = 0.134, N = 198, P = 0.058$, Fig. 3). Daytime was included in the model, but its effect was significant only between noon and morning in Tibetan antelope ($Z = -1.97, P = 0.049$).

**Discussion**

We examined the predictions of the programmed grooming model and the group size effect in Tibetan gazelle and Tibetan antelope. We found that grooming rates followed the inter-specific body size prediction of the programmed grooming model, with grooming rate being negatively associated with species-typical body mass. The small Tibetan gazelle (13–16 kg) groomed much more than the large Tibetan antelope (26–39 kg). These results follow a large body of evidence supporting the inter-specific body size prediction. For example, Moor- ing et al. (2000) found a highly significant negative correlation between mass and mean grooming rate of 25 species of ungulates in a tick-free zoological park in which stimulus-driven grooming factors were ruled out. Species mass accounted for up to two-thirds of the variation in grooming rate across species (Mooring et al., 2000).

We also tested the intra-specific version of the body size principle and found a significant effect of sex-age on grooming rate in Tibetan antelope. As predicted, the grooming rate of different-sized conspecifics fell in the predicted direction, with fawns grooming the most and males grooming the least. The intra-specific body size prediction has been supported by an extensive survey of 53 species/subspecies of ungulates in tick-free zoological parks, with the grooming rate of juveniles declining as they grew larger (Mooring et al., 2002). Other studies have shown that sub-adults which groomed more than adults harbored fewer ticks as a result (Gallivan et al., 1995; Mooring & Hart, 1997a,b; Mooring & Samuel, 1998a,b; Scimonelli, Marucco & Celis, 1999; Mooring et al., 2006; Li et al., 2014), as predicted by programmed grooming (Hart et al., 1992).

Sexual dimorphism is common in ungulates, where males are usually larger than females (Ruckstuhl, 2007). Therefore, as a prediction of body-size principle, larger males will groom less than females even in the non-breeding season, and males will groom at a similar rate as females if they are of a similar body size or monomorphic (Hart et al., 1992). As expected, we found a significant sexual effect on grooming rate in the sexual dimorphic Tibetan antelope and a similar grooming pattern between similar body-sized males and females in Tibetan gazelle. Sexual dimorphic grooming patterns have been found in many species, such as American bison (Bison bison) (Mooring et al., 2006) and Pere David’s deer (Elaphurus davidianus) (Li et al., 2014). Mooring et al. (2002) compared the oral grooming rate of 40 ungulate species with dimorphic body size (males larger than females) versus 11 monomorphic ungulate species and found a significant difference in their grooming.
behavior (Mooring et al., 2002). Of species with body size dimorphism, 91% exhibited grooming dimorphism (males groomed less than females) compared with 55% of monomorphic species, which reflects random variation. Whether a monomorphic species, such as Tibetan gazelle, can show a grooming dimorphism, is probably related to the testosterone levels. Owing to higher testosterone levels and the need to be vigilant for rival males and estrous females during breeding season, males are expected to groom less (Mooring et al., 2006). Although our current observations were made outside of the breeding season, more grooming data during the rut are needed to explore possible grooming dimorphism.

Group size effect on vigilance has been observed in Tibetan gazelle (Li & Jiang, 2008) and small groups (<30 individuals)

Figure 3 Group size effects on grooming patterns of sympatric Tibetan gazelle (a) and Tibetan antelope (b) in Selincuo National Nature Reserve, Tibet.
of Tibetan antelope (Lian et al., 2007). Living in large groups will allow animals more time for other behaviors, including feeding and grooming (Beauchamp, 2003, 2014). This group size effect on grooming occurred in the Tibetan gazelle. We did not find a significant effect of group size on grooming rate in Tibetan antelope, and it was probably because of the extremely large group size. The detection benefit owing to the group size effect was usually apparent when groups were small, and the detection effect would be minimized, and a small amount time can be released when the group size increased to a certain point (Beauchamp, 2003). The group size of Tibetan antelope in our observations ranged from 1 to 400, with an average of 55, which was much more than that of Tibetan gazelle, whose group size ranged from 1 to 9, with an average of 4. The released vigilance time owing to the group size effect was probably minimal in Tibetan antelope.

In conclusion, our study on the grooming pattern of two Tibetan ungulates strongly supports the body size principle. Inter-specifically, large Tibetan antelope groomed less than small Tibetan gazelle. Intra-specifically, adults groomed the least, and fawns groomed the most. Sexual difference in grooming patterns occurred in sexually dimorphic Tibetan antelope, whereas no difference was found between similar body-sized males and females of Tibetan gazelle. In the future, we expect to study the effects of hormones on grooming during the rut (Mooring, Gavazzi & Hart, 1998; Kakuma et al., 2003), especially in December and January, when the mate competition is intense, and the testosterone concentrations of the males are at their peak. The ‘testosterone effect’ (vigilance principle) could then be tested in these rare plateau ungulates.

From a conservation point of view, at least 41 species of ticks have been reported in Qinghai Province and Tibet (Yang et al., 2008; Zhang et al., 2009), of which at least five carry vector-borne diseases such as spotted fever (Han et al., 2018); thus, ticks are a threat to wildlife species. Although we did not measure the tick burden and cannot evaluate the parasite-defense efficiency of grooming, we showed that the grooming patterns in both rare species are consistent with what has been documented in many other species. Study on tick biology on the plateau should be carried on because our knowledge about the plateau ticks is very minimal. Tick species should be collected and identified, and the life cycle of these ticks should be studied. Research on the tick burden of these species should also be measured and monitored.

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Ethical approval
This is an observational study; all observations were made 150 m away from the focal animals to minimize observer effects. All the observational procedures in this study were approved by the Chinese Wildlife Management Authority.

Conflict of interest
The authors declare that they have no conflict of interest.

References


