



Preference and Seasonality of Allogrooming Posture and Body Site of Wild White-Headed Black Langurs (*Trachypithecus leucocephalus*) in Guangxi, China: Functional Implications

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ABSTRACT

Allogrooming behavior is ubiquitous among nonhuman primates and considered to be highly preferences regarding to allogrooming postures and body sites. In order to investigate the allogrooming preference and seasonality of the white-headed black langurs (*Trachypithecus leucocephalus*), we studied the allogrooming posture and body site of the animal via focal animal sampling and continuous recording in the Chongzuo White-Headed Langur National Nature Reserve from February 2016 to January 2017. Results showed that totally proportions of non-eye contact and eye contact allogrooming postures of the animals accounted for 47.86% and 52.14%, respectively. The most frequently used allogrooming posture in the dry season was sprawl (32.73%), and that of in the rainy season was sit side (33.56%). There were significant differences among allogrooming postures throughout the year ($p < 0.001$). Proportion of allogrooming in inaccessible area in the dry season was higher than in the rainy season. The grooming preference index was greater than 0 in the dry season and less than 0 in the rainy season. The proportion of difficult to reach area was opposite and there was a significant difference between dry season and rainy season ($p = 0.04$), and both grooming preference index was greater than 0. The grooming preference index of easy to reach area was less than 0 in dry season and rainy season. Animals were selective in allogrooming sites, the anogenital area had the largest grooming preference index in both dry and rainy seasons. The allogrooming of white-headed black langur appeared to be consistent with the social function hypothesis. In addition, allogrooming was in line with the hygiene function hypothesis during dry season, but not in rainy season. The reason may be associated with variation of food supply between the two seasons. It is necessary to further study before generalizing the function of allogrooming of the langur.

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Key words

Allogrooming, Seasonal variations, Grooming postures, Grooming areas, *Trachypithecus leucocephalus*

INTRODUCTION

Grooming behavior is ubiquitous among primates, accounting for 2% ~ 10% of the daily time budget (Tahir *et al.*, 2017; Maurice *et al.*, 2019). Grooming can be divided into autogrooming and allogrooming

(Hutchins and Barash, 1976; Pérez and Baró, 1999). The former is an individual combing their own hair, sometimes picking up small particles from the hair and putting them in their mouths to chew or use their mouths to bite. The latter, as a supplement to autogrooming, is to comb the hair between individuals, and occasionally exposed skin to pick up small particles into the mouth to chew or bite directly with the mouth, thus it has a more complex social function (Vea *et al.*, 1999). Most studies of grooming in primates (Wolovich *et al.*, 2017; Jiang *et al.*, 2019), and in several other mammals, such as bats (Carter and Leffer, 2015), deer (Heine *et al.*, 2017), horses (Shimada and Suzuki, 2020), mice (Lawande *et al.*, 2020), coati (Hirsch *et al.*, 2012), cows (de Freslon *et al.*, 2020) show that grooming serves important social functions. Two popular ecological hypotheses, the hygienic function hypothesis

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and the social function hypothesis, can better explain the important biological significance of primate grooming behavior.

The hygienic function hypothesis holds that social primates groom each other to remove salt and parasites from the body surface to control disease (Barton, 1985; Borries, 1992; Grueter *et al.*, 2013). Therefore, regarding grooming body sites, allogrooming mostly occurs in areas of the body that are inaccessible to groomee and are susceptible to parasite infection (Borries *et al.*, 1994; Grueter *et al.*, 2013). For example, the allogrooming behavior of Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) focuses on parts of the body that are difficult to reach by groomees (Zhang *et al.*, 2014). This is also true in wild black capuchin monkeys (*Sapajus nigritus*), which preferentially groom inaccessible body sites (e.g., back and head) (Pfoh *et al.*, 2021). Also, the anogenital area and the corpus callosum are more frequently groomed among individuals of the narrow-nosed monkeys (Pérez and Baró, 1999; Li *et al.*, 2002; Allanic *et al.*, 2021) and François' langur (*Trachypithecus francoisi*) (Zhou *et al.*, 2006), which possibly since the area is difficult to autogrooming, and also contains some information about reproductive status and social status (Moser *et al.*, 1991). Wild Bonobos (*Pan paniscus*) mothers groom more frequently to their pups body site that are less accessible to themselves to prevent ectoparasite-related diseases (Allanic *et al.*, 2020). In addition, the number of grooming partners in vervet monkeys (*Chlorocebus aethiops*) is associated with hookworm infection, and the number of grooming partners in vervet monkeys vary significantly by hookworm infection and sex (Wren *et al.*, 2016).

Another hypothesis of grooming is the social function hypothesis. It argues that grooming is crucial for promoting social cohesion (Kanngiesser *et al.*, 2011). Since grooming can reduce the tension and thus maintain the intimacy, the social hierarchy relationship between individuals, as well as the stability of the community (Terry, 1970; Dunbar, 1991). In white crowned mangabeys (*Cercocebus torquatus lunulatus*), low-rank females groom to reduce the frequency of attack (Vea *et al.*, 1999). Similarly, Japanese macaque (*Macaca fuscata*) females tend to invest in grooming of high-rank females (Kurihara, 2016). Female mandrills (*Mandrillus sphinx*) will give priority to grooming individuals of high status in order to gain advantage in social competition (Schino and Lasio, 2018). In wild gelada (*Theropithecus gelada*) females, infant handling affects grooming exchanges to strengthen society (Caselli *et al.*, 2021). Primate grooming is often thought of as a kind of currency that can be exchanged for other services or goods in biological market theory, such as alliance support (Borgeaud and Bshary, 2015), infant care (Jiang *et al.*, 2019; Pereira *et al.*, 2019), tolerance

for food sources (Wubs *et al.*, 2018), positive food sharing (Wolovich *et al.*, 2017), or mating opportunities (Rathinakumar *et al.*, 2017).

In gregarious primates, grooming behavior varies with ages (Tombak *et al.*, 2019), genders (Lhota *et al.*, 2019), dominance hierarchy (Wu *et al.*, 2018), kinship (Wu *et al.*, 2018; Allanic *et al.*, 2020), seasonal food availability (Jasso del Toro *et al.*, 2020), grooming postures (Zhao *et al.*, 2019) and other factors. Grooming of different body sites can mean different costs and benefits (Schino and De Angelis, 2020). Many primates have seasonality in grooming time. For instance, the grooming time of captive hamadryas baboons (*Papio hamadryas*) is reduced in the cold winter (Chen, 2016). Similarly, grooming time in Sichuan snub-nosed golden monkey (*Rhinopithecus roxellana*) in autumn and winter is shorter because it had less food to eat and was reduced to more indigestible bark, which led to longer rest periods and shorter grooming periods (Li, 2004).

The white-headed black langur (*Trachypithecus leucocephalus*) is a rare and endangered species endemic to the limestone forests in southwest Guangxi, China (Huang *et al.*, 2008). Lime forests are dominated by evident seasonal variation of food supply and rainfall; therefore, activity of limestone-associated langurs vary accordingly (Zhang *et al.*, 2020). To date, increasingly studies, including diet (Li *et al.*, 2016; Lu *et al.*, 2021), habitat use (Liu *et al.*, 2022), ranging behavior (Huang *et al.*, 2017), activity pattern and time budget (Zhang *et al.*, 2020) of white-headed black langurs have been conducted. However, limited information concerning allogrooming seasonality and preference of the free-ranging group is available. The white-headed black langur had a distinct social structure and obvious mutual grooming behavior, which was conducive to our observation. Thus, we conducted field observations on the allogrooming postures and body sites to explore the following questions: (1) What are the allogrooming characteristics of the white-headed black langur? (2) Are allogrooming preference and function of the white-headed black langurs vary with seasonality?

MATERIALS AND METHODS

Study site

The research site is located in the Jiuchongshan in the Chongzuo White-Headed Langur National Nature Reserve, Guangxi, China. The reserve is dominated by limestone landscapes, which can be divided into three parts from top to bottom: including hilltop, the cliff and the gentle slope (Li and Elizabeth Rogers, 2006). The elevation of the peak ranges 200~300 m (Tan, 2014). The climate belongs to

the north tropical humid monsoon climate, with an annual rainfall of about 1200 mm, which can be clearly divided into a rainy season (April to September) and a dry season (October to March of the following year) (Huang *et al.*, 2010). The average temperature is around 22.0 °C, and a humidity around of 78% (Tan, 2014).

Study subjects

The observation group G10 ranged several hills ca. 0.4 square kilometer. The group had 10 individuals at the beginning of the study. According to Huang (2002) criterion, the group included six adult females, one adult male, two sub-adult females, and one juvenile female. During the observation the adult male replacement happened twice, and hence group composition changed throughout the observation (Table I). In this area, photographers often observe and photo the langurs, and local farmers work on the flat land around the hills. The langurs are tolerant to observers within 50 m.

Table I. Group composition of the white-headed black langur in this study.

Stage	Adult male	Adult female	Sub-adult male	Sub-adult female	Juvenile male	Juvenile female
1	1	6		2		1
2	1	6	2	2	3	1
3	1	6		1		1

Key: Stage 1: Initial study period. Stage 2: In March 2016, male replacement occurred, two sub-adult males and three juvenile males were added to the group. Stage 3: The second male replacement occurred in the observation group on November 21, 2016. After that the observation group consisted of one male and 8 females.

Behavioral observations

We used focal animal sampling and continuous recording to study the animals (Altmann, 1974). The sampling period was 15 min, we observed and recorded the duration of allogrooming behavior during the first 5 mins, followed by a 10 min interval, and then sampled again. An allogrooming start if the allogrooming time exceeded 30s, and end if it stopped for 30 s (Altmann, 1974).

Field observation was conducted from February 2016 to January 2017. The observation time was 59 days in total, ranging from four days in September 2016 to five days in the remaining months. The observation time in summer was 7:00-19:00, and 7:30-18:30 in other seasons. A monocular (Nikon, 20-40x, Japan) or a binocular (Zeiss, TERRA ED 10X42, German) were used to observe the animals from 50-200 m. Grooming sampling data record included time spent on body sites and grooming postures. The grooming postures consisted of six items, sitting in the same direction, sprawl, quadrupedal stand, back lie, sit face to face, and sit side, which were further assigned to two categories, with eye contact and without eye contact (Zhang *et al.*, 2014) (Table II).

In order to estimate the relative proportion of the body area of the langurs, following Ghiglieri (1984) and Boccia (1983), we divided the animal body site into 3 areas: Inaccessible area (IA), easy to reach area (ERA) and difficult to reach area (DRA) (Table III). Thus, we measured the body regions of a female white-headed black langur specimen in the Guangxi Normal University Biodiversity Herbarium and a male one in the white-headed black langur Exhibition Hall of the Chongzuo white-headed black langur Nature Reserve. The proportions of the relative body area were used to represent that of the animal in the field.

Table II. Definition of grooming posture of white-headed black langur.

Grooming posture	Definition
Eye contact	
Sit face to face	Groomer and groomee face each other abdomen
Back lie	The torso of the receiver is standing upright on a relatively horizontal support, and the body mass is mainly on the back
Sit side	Between the initiator and the receiver, the abdomen orientation of one individual is perpendicular to the abdomen orientation of the other individual, and the abdomen of one individual may be close to the body side of the other individual
Without eye contact	
Quadrupedal stand	The limbs of the receiver stand on a horizontal or sub-horizontal support; the elbow joints and knees are relatively extended, and the torso is close to level
Sitting in the same direction	The abdomen between groomer and groomee is facing the same, and the body mass is borne by the ischium and feet. The torso is vertical and may be curved
Sprawl	The torso of the groomee is standing upright on a relatively horizontal support, and the body mass is mainly in the abdomen

Table III. Body sites partition of white-headed black langur (from Ghiglieri, 1984).

Body sites	Body region
Easy to reach area (ERA)	
Hand	From wrist to fingertip, excluding wrist
Forearm	From wrist to elbow, excluding elbow
Tail tip	From the middle of the tail to the tip
Shank	From groin and buttocks to knees excluding knees
Thigh	From knee to foot stomp, including knee, excluding foot rash
Foot	From the foot to the toe, including the stomp
Inaccessible area (IA)	
Face	The front part of the head, including the eyes
Head	The part that covers the brain, including the ears and eyebrows
Neck	The annular part that connects the head to the torso
Upper back	The upper part of the back of the body
Lower back	The lower part of the back of the body
Difficult to reach area (DRA)	
Arm	From shoulders to elbows, including underarms
Abdomen	The frontal part of the body from the chest to the anogenital area
Chest	The front part of the thoracic cavity from the bottom of the neck to the ventral surface
Tail head	From the base of the tail to the middle of the tail
Flank	From the chest and abdomen to the side part of the back
Anogenital area	The part including the hip, umbilical body, sex skin and anal

Data analysis

Following Post (1981), in each month, we calculated the mean diurnal grooming time from a sampled individual, and then we averaged the values across all individuals sampled. The total grooming time was calculated by summing up the across individual mean of each month. In the same way, the diurnal proportion of time spent grooming body sites (hand, head and arm, etc.) and grooming postures were calculated from a sampled animal in each month, and a mean across all individuals was then calculated. The across means of time spent on body sites were further classified into the cross-individual means of rainy months and dry months were averaged to calculate the rainy season and dry season proportion of time spent grooming, respectively. Following Tweheyo *et al.* (2004), we assessed grooming preference using a grooming preference index (GPI) calculated by the following formula:

$$GPI = \log 10 \left[\frac{\% \text{ grooming time}}{\% \text{ body site}} \right]$$

Where % grooming time is the proportion of grooming time in one body site relative to total grooming time; and % body site is the proportion in size of one part to the total body site area. When the value of GPI was 0 there was no

preference of the body site; GPI > 0 indicated preference and GPI < 0 indicated avoidance the body site. The larger the GPI, the more preferred this part was.

We used the generalized linear mixed models (GLMM) to analyze the seasonal variation of grooming postures and body sites. The Kruskal-wallis test was used to analyze the annual variation of grooming postures. All data analysis was conducted in R 4.3.0 and SPSS 22.0. All tests were two-tailed, with significance levels of 0.05.

RESULTS

Grooming postures

The total grooming time in the dry season accounted for 56.88% ± 4.09% of the annual total grooming time. There were significant differences among monthly grooming postures throughout the year ($\chi^2 = 49.59$, $df = 5$, $p < 0.001$). With the exception of sit side, the grooming posture in dry season was lower than that in rainy season (Fig. 1).

The grooming posture with eye contact and without eye contact accounted for 52.14% and 47.86% of the total grooming time, respectively. The proportion of time without eye contact was 52.01% in dry season and 43.71%

in rainy season, and there was no significant seasonal variation throughout the year ($Z = -0.05$, $n = 18$, $p = 0.96$).

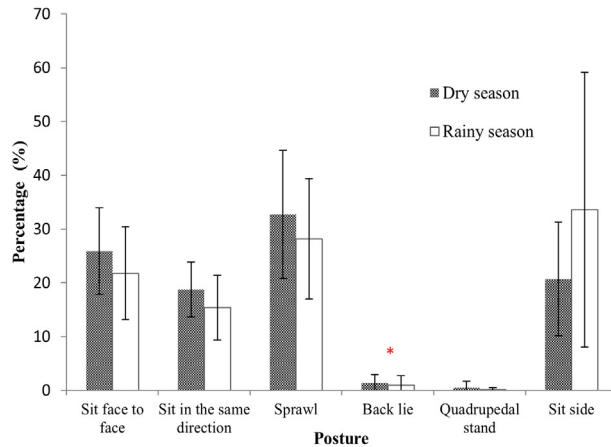


Fig. 1. Proportions of different postures of white-headed black langur in the dry and rainy season. Asterisks represent statistically significant differences between the dry and rainy season.

In the dry season, the dominant posture was sprawl ($32.73\% \pm 11.90\%$), while in the rainy season, the dominant posture was sit side ($33.56\% \pm 25.52\%$). Grooming postures did not significantly varied between the dry season and the rainy season (sit face to face: $\chi^2 = 3.43$, $df = 1$, $p = 0.06$, sitting in the same direction: $\chi^2 = 0.48$, $df = 1$, $p = 0.49$, sprawl: $\chi^2 = 0.80$, $df = 1$, $p = 0.37$, quadrupedal stand: $\chi^2 = 0.59$, $df = 1$, $p = 0.44$, sit side: $\chi^2 = 2.66$, $df = 1$, $p = 0.10$), but for the back lie posture ($\chi^2 = 4.18$, $df = 1$, $p = 0.04$).

Grooming body sites

The variation of grooming time between dry and rainy season was body sites-specific (Table IV). Among them, the proportion of grooming time in DRA in dry season ($39.83\% \pm 4.69\%$) was significantly lower than that in rainy season ($48.12\% \pm 5.31\%$) ($\chi^2 = 4.05$, $df = 1$, $p = 0.04$), but there was no seasonal significant difference between the other two body areas (ERA: $\chi^2 = 0.07$, $df = 1$, $p = 0.79$; IA: $\chi^2 = 0.98$, $df = 1$, $p = 0.32$).

Table IV. Time and body surface area ratio of different grooming areas of white-headed black langur. GPI is grooming preference index. The values are Mean $\pm$ SD.

Body areas/ Body sites	Body surface area ratio (%)	Grooming time in		GPI in	
		Dry season (%)	Rainy season (%)	Dry season	Rainy season
Inaccessible area (IA)					
Face	0.49 ± 0.01	1.71 ± 1.54	0.12 ± 0.20	0.54	-0.61
Head	4.39 ± 0.06	2.53 ± 1.41	4.01 ± 4.98	-0.24	-0.04
Neck	1.93 ± 0.36	3.36 ± 1.16	2.01 ± 1.77	0.24	0.02
Upper back	7.34 ± 0.06	10.93 ± 5.94	6.45 ± 2.40	0.17	-0.06
Lower back	8.03 ± 0.29	9.45 ± 3.82	7.24 ± 2.02	0.07	-0.04
Total	22.17 ± 0.77	27.98 ± 10.02	19.82 ± 8.59	0.10	-0.05
Easy to reach area (ERA)					
Foot	4.51± 0.74	2.02 ± 2.03	2.13 ± 2.26	-0.35	-0.33
Shank	9.34 ± 0.13	7.34 ± 3.18	8.26 ± 2.20	-0.10	-0.05
Thigh	14.21 ± 0.54	8.15 ± 4.01	6.30 ± 2.55	-0.24	-0.35
Tail tip	5.71 ± 0.16	1.62 ± 1.38	5.14 ± 1.94	-0.55	-0.05
Forearm	7.90 ± 0.92	8.79 ± 3.57	9.42 ± 3.22	0.05	0.08
Hand	3.55 ± 1.41	2.14 ± 1.25	0.80 ± 0.31	-0.22	-0.65
Total	45.21 ± 0.72	32.18 ± 6.11	32.06 ± 5.47	-0.15	-0.15
Difficult to reach area (DRA)					
Arm	6.07 ± 1.05	10.50 ± 0.94	9.72 ± 3.32	0.24	0.20
Abdomen	4.53 ± 0.27	1.74 ± 1.26	0.77 ± 0.69	-0.42	-0.77
Chest	4.88 ± 0.27	2.20 ± 1.53	1.97 ± 1.33	-0.35	-0.39
Tail head	7.49 ± 0.81	6.30 ± 4.47	11.55 ± 3.57	-0.08	0.19
Flank	8.02 ± 0.22	6.79 ± 1.02	9.10 ± 1.72	-0.07	0.05
Anogenital area	1.63 ± 0.52	12.31 ± 3.00	15.00 ± 2.51	0.88	0.96
Total	32.62 ± 1.50	39.83 ± 4.69	48.12 ± 5.31	0.09	0.17

The anogenital area had the highest grooming time in both the dry season ($12.31\% \pm 3.00\%$) and rainy season ($15.00\% \pm 2.51\%$) (Table IV). Only 5 parts out of the 17 parts had significant difference between the rainy season and the dry season (tail head: $\chi^2 = 4.78$, $df = 1$, $p < 0.05$; tail tip: $\chi^2 = 9.41$, $df = 1$, $p < 0.01$; hands: $\chi^2 = 9.00$, $df = 1$, $p < 0.01$; thighs: $\chi^2 = 10.45$, $df = 1$, $p < 0.01$; flank: $\chi^2 = 6.180$, $df = 1$, $p < 0.05$).

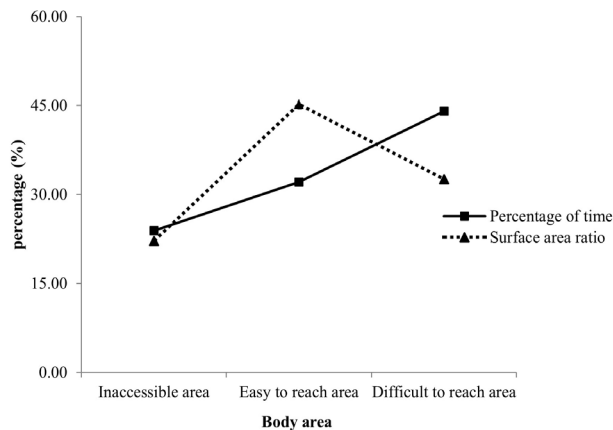


Fig. 2. Line chart of surface area and time percentage of different grooming areas of white-headed black langur. The dash line and solid line show the time spent was disproportion to body site area. Thus, difficult to reach area was preferred during grooming.

The GPI of IA was greater than 0 in dry season, but less than 0 in the rainy season; DRA was greater than 0 in both dry season and rainy season; and ERA was less than 0 in both dry season and rainy season (Table IV). Hence, according to the GPI, it could be seen that IA in dry season and DRA are more favored by monkeys, while ERA does not receive attention matching its area. In both dry and rainy seasons, the anogenital area has the greatest GPI of any part of the body, so monkeys preferentially groomed this area. The comparison chart of annual surface area and grooming time was shown in Figure 2.

DISCUSSION

The grooming posture adopted will affect the individual's eye contact during grooming (Zhang *et al.*, 2014). Some studies have pointed out that animals avoid eye contact when grooming each other to reduce tension and potential aggression (Boccia, 1983; 1989; Borries, 1992; Allanic *et al.*, 2020). In Sichuan snub-nosed monkeys, when high-ranking individuals stare or threaten low-ranking ones, the latter often lower its head or crouch to avoid eye contact (Ren *et al.*, 2000). However,

other studies suggest that eye contact between individuals makes it easier to identify each other, promotes emotional /attentional engagement, and facilitates body language communication (Zhang *et al.*, 2014; Zanolini *et al.*, 2021). It is likely that eye contact makes it easier to identify other individuals and facilitates body language communication though eye contact postures increase the chances of conflict (Zhang *et al.*, 2014). This slightly risky investment ensures that the group will reap the benefits of its social life (Boccia, 1989). In this study, the langurs used more without eye contact postures for grooming in the dry season, while in the rainy season they used more eye contact postures, but there was no significant difference. In general, there were more grooming posture with eye contact than without eye contact of the total grooming time. Thus, the grooming behavior of the langurs fit the social functional hypothesis.

On the basis of hygiene function hypothesis, the grooming time spent in different body sites are inconsistent, that is, the GPI of DRA and IA are greater than 0, while that of ERA is less than 0 (Pérez and Baró, 1999). In this research, GPI of DRA were greater than 0 but less than 0 of ERA in both dry and rainy seasons, suggesting that allogrooming of the animals is consistent with the hygiene function hypothesis, as is the case with François langur (Zhou *et al.*, 2006). Some studies suggest that the anogenital area (belonging to DRA) is highly selective during allogrooming, for example, François' langurs are more inclined to comb the anogenital area since this area is difficult to reach by groomee (Zhou *et al.*, 2006). Similarly, this has been seen in the narrow-nosed monkeys (Dunbar, 1991; Pérez and Baró, 1999; Li *et al.*, 2002). Social status and reproductive status can be checked through the anogenital area in primates, which may be the reason for the high selection of the anogenital area in addition to difficulty in autogrooming (Moser *et al.*, 1991). In our research, the white-headed black langur was also highly selective in the grooming sites in both dry and rainy season. This is consistent with other studies.

However, the GPIs of IA were greater than 0 in the dry season, and less than 0 in the rainy season (Table IV), suggesting that allogrooming function may vary with seasons. Firstly, rainfall has a significant impact on the behavioral and ecological characteristics of primates (Li *et al.*, 2018). The seasonal inconsistency of function of grooming in white-headed black langur may be related to this ecological factor. During the dry season, food availability decreases and animals spend less time searching for food to conserve energy and therefore have more time for grooming (Zhou *et al.*, 2012). On the contrary, during the rainy season, when food is abundant, animals spend more time searching for high quality food to maximize net energy income (Dunbar, 1992), thus

reducing grooming time.

Another factor influencing grooming is the number and gender of individuals that join in grooming, for example, the females form stable clusters with maternal kin-related female partners both during the mating and non-mating season, whilst, males were not included in the females' clusters during the mating season (Xia *et al.*, 2019). The third factors influencing preference of body site are age and kinship specific, e.g., mother groom more their offspring more to prevent disease linked to ectoparasites, and matures individuals spend more time grooming the inaccessible back than immature individuals (Allanic *et al.*, 2020). But to date, information concerning grooming variation between gender, among age groups and kinship are unavailable. Therefore, in the future, before we need more intensive study before generalizing the function of grooming of the langur.

CONCLUSION

In this study, we found that the animal was highly selective to the grooming site, preferring the difficult to reach area and the anogenital area. There was variation between the two seasons regarding the hygiene function hypothesis, but social function hypothesis has no different, which make it necessary to further study before generalizing the function of all grooming of the langur.

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Ethical statement

Data was collected in a noninvasive manner from free-ranging animals in the field. All research methods adhered to Chinese legal requirements, complied with protocols approved by the State Forestry Administration of China. Our field observations were approved by the state and local governments.

Statement of conflict of interest

The authors have declared no conflict of interest.

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