

## Evaluation of Fecal Testosterone, Rank and Copulatory Behavior in Wild Male *Macaca thibetana* at Huangshan, China

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**Abstract.-** Although male testosterone levels are thought to be linked with the mating system, ecological and social factors complicate the big picture of the function of testosterone in wild nonhuman primates. We examined testosterone correlates with rank and copulatory behavior in male Tibetan macaques (*Macaca thibetana*) at Mt. Huangshan, China. A total of 426 fecal samples and approximately 453h of behavioral data were collected on five adult males from October 2005 to September 2006. Copulatory behaviors were significantly different between high and low ranking males in the mating season, but not during the non-mating season. No significant correlation between testosterone levels and a male's David's score indicated that testosterone was independent of social rank. Also, copulatory behaviors were positively correlated with fecal testosterone levels in both seasons, regardless of social rank. The present study represents a preliminary assessment of the relationship between testosterone level, rank, and copulatory behavior in a wild environment. The results enrich the picture of testosterone, rank, and copulatory relationships in non-human primates, and may also provide insight into what mechanisms link physiological factors to male reproductive success in natural circumstances.

**Key words:** *Macaca thibetana*; fecal testosterone, copulatory behavior, social rank, non human primates.

### INTRODUCTION

Testosterone, a steroid hormone important to male reproductive physiology, can facilitate male reproductive success by modifying behaviors like competition and sexual motivation in mammals (Muehlenbein and Bribiescas, 2010). The paucity of reproductive physiology in wild subjects (*i.e.*, non-human primates, NHPs) has been due in part by obtaining effective samples for measurement from feral populations. As Barrett and his colleagues stated "... ethical issues arise whenever invasive techniques such as restraint, anesthesia, and venipuncture are applied, particularly in studies of non-human primates..." (P29) (Barrett *et al.*, 2002b). Recently, non-invasive methods of assessing hormone concentration via fecal or urinary samples

has been well developed and successfully applied in NHPs (Barrett *et al.*, 2002b; Czoty *et al.*, 2009; Beehner and Whitten, 2004; Bergman *et al.*, 2005; Beehner *et al.*, 2009; Sobolewski *et al.*, 2012; Sobolewski *et al.*, 2013).

The importance of testosterone in regulating some aspects of male reproductive physiology has been widely documented in several primate taxa, for example, rhesus macaques (*Macaca mulatta*) (Wallen, 2001; Jahan *et al.*, 2011), chimpanzees (*Pan troglodytes*) (Klinkova *et al.*, 2004; Muller and Wrangham, 2004; Sobolewski *et al.*, 2013), howler monkeys (*Alouatta palliata*) (Critobal-Azkarate *et al.*, 2006), long-tailed macaques (*Macaca fascicularis*) (Czoty *et al.*, 2009), and Japanese macaques (*Macaca fuscata*) (Barrett *et al.*, 2002a). Previous studies have demonstrated that testosterone is a good candidate for a physiological factor that may link male dominance rank to potential reproductive success in NHPs (reviewed in Prall and Muehlenbein, 2014). However, most studies have

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focused on the relationships between testosterone and social rank, or rank-related behaviors (*i.e.*, aggression) (Critobal-Azkarate *et al.*, 2006; Lynch *et al.*, 2002; Muroyama *et al.*, 2007; Sobolewski *et al.*, 2013; Teichroeb and Sicotte, 2008), attempted to verify “the challenge hypothesis” proposed by Wingfield *et al.* (1990). The challenge hypothesis proposes that, as competition for mates intensifies, testosterone levels reach a physiological maximum to promote responses to social ranks and aggressive challenges (Wingfield *et al.*, 1990).

These findings complicated the picture of the variation of testosterone and the rank-testosterone relationships in social NHPs. Previous studies showed that testosterone co-varied with dominance status within species. Studies on chimpanzees at Kanyamwara, Kibale National Park in Uganda demonstrated that testosterone correlated positively with rank (Muehlenbein *et al.*, 2004; Muller and Wrangham, 2004), however, Sobolewski and coauthors found no such relationship at the same place (Sobolewski *et al.*, 2013). Some data revealed a negative relationship between testosterone and social rank. For example, some studies demonstrated a positive correlation between adult male testosterone and dominance rank in chimpanzees (*Pan troglodytes*) (Anestis, 2006; Muller and Wrangham, 2004), long-tailed macaques (*Macaca fascicularis*) (Czoty *et al.*, 2009), while other studies failed to indicate a relationship between the two variables in Japanese macaques (*Macaca fuscata*) (Barrett *et al.*, 2002a), bonobos (*Pan paniscus*) (Sannen *et al.*, 2004), colobus monkeys (*Colobus vellerosus*) (Teichroeb and Sicotte, 2008), and chimpanzees (*Pan troglodytes*) (Sobolewski *et al.*, 2013). Therefore, more case studies focused on NHPs can enhance our understanding about the intricate relationship between social rank and testosterone levels.

Previous studies indicated that the influence of ecological factors can complicate the relationship between testosterone and social rank. Field researchers tend to more or less discount the influence of environmental factors on the testosterone-copulation relationship. Few studies have directly measured the relationships between testosterone and copulation under wild circumstances (Muller and Wrangham, 2004, Wallen,

2001), and thus the mechanisms underlying these relationships are not well understood. One possible explanation for this is that copulatory behaviors are influenced by environmental and social factors (such as social rank). Bernstein *et al.* (1979) demonstrated that hormones are necessary but not sufficient predictors of copulatory behavior due to the complex nature of the social milieu inherent in NHP troops. Moreover, the understanding of how testosterone mediates reproductive behavior is complicated by findings that the two variables are not related in a number of primate taxa (*Macaca mulatta* in Wallen, 2001; *Macaca fuscata* in Barrett *et al.*, 2002a; *Pan troglodytes* in Klinkova *et al.*, 2004). One factor confounding interpretation of the association between testosterone and male copulatory behavior is potential inequity in social rank. Therefore, studying the interrelationship of testosterone, rank, and copulatory behavior in wild NHPs can provide insight into individual reproductive physiology in a wild milieu.

In the current study, we evaluated fecal testosterone, rank, and copulatory behaviors of wild Tibetan macaques (*Macaca thibetana*) at Huangshan, China. Tibetan macaques live in multi-male, multi-female social groups (mean group size =  $33.8 \pm 6.79$ ), plus offspring (Li, 1999). Male Tibetan macaques form linear dominance hierarchies, and exhibit a despotic dominance style (Berman *et al.*, 2004; Li, 1999). *M. thibetana* is described as a seasonal breeder with the mating season lasting from July to January (Li *et al.*, 2005; Xia *et al.*, 2010). Copulations in Tibetan macaques are reported to occur outside of the mating season, however these are nonreproductive, and principally involve high-ranking males (Li *et al.*, 2007). Males' testosterone concentrations, which reach their zenith in August and nadir approximately five months later, have been assessed and reported to be positively correlated with the environmental temperature for this population (Xia *et al.*, 2008).

The purpose of the present study was to examine if testosterone levels are associated with social rank and copulatory rates in a wild environment. To our knowledge, this is the first attempt to investigate these relationships in a group of Tibetan macaques. The following predictions were tested: (1) Testosterone levels in high-ranking

males would be higher than in low-ranking males both in the mating season and non-mating seasons, (2) Copulatory behaviors would be positively correlated with fecal testosterone levels in both the mating and non-mating season, independent of social rank.

## MATERIALS AND METHODS

All research protocols were approved by the Chinese Wildlife Management Authority. The study was observational in nature and did not involve any type of invasive experimentation on wild primates. Thus, no review from an institutional ethics committee in China was required. All of the work was carried out under the Wildlife Protection Law of the People's Republic of China. All research reported here comply with the regulatory requirements of the Huangshan Garden Forest Bureau, China, where the study took place.

### *Study site and subjects*

This study was conducted over a one-year period from October 2005 to September 2006 at Mt. Huangshan in Anhui Province, China. The sampling area is a well-known research site for the study of Tibetan macaques (*Macaca thibetana*). Details on this study site can be found in Xia *et al.* (2010).

At the study site, there currently are two distinct social groups of macaques, identified as Yulinkeng 1 (YA1) and Yulinkeng 2 (YA2). These two groups are located in an area within the reserve known as the "Valley of the Wild Monkeys" (N30° 04' 25.1" / E118° 08' 59.3"). These monkeys have been studied since 1987. Group YA2, which fissioned from YA1 in 1996 (Li *et al.*, 1996), was the focus of the present study. A detailed account of the groups' history and demography can be found in Li (1999).

During the study, group size increased from 37 to 42 animals and age-sex classes included 7 adult males, 7 adult females, 22 subadults/juveniles, and 2-7 infants (5 were born during the study period). The five subjects (GBZ, CT, YX, JT, and BL) were selected from among 7 adult males of the troop, all of which were thoroughly habituated to observers and readily distinguishable by physical features (*i.e.*, scars, hair color patterns, or

facial/body contours) without need to disturb or capture them. The two adult males not included in this study were one non-habituated male and one male that had suffered physical trauma to one testicle in a fight several years earlier. It was thought that this loss might impact his testosterone levels and the exhibition of normal sexual behavior in unknown ways, and therefore his data were not included.

### *Behavioral data collection*

Behavioral data and fecal samples were collected concurrently from the five adult males in the YA2 group throughout the one-year study period. All behavioral data were collected by a single observer (Xia), during an intensive study period totaling 303 days from October 2005 to September 2006 (mean=25 days/month, range=15~31). The group was followed from dawn to dusk, with behavioral observations beginning at approximately 0700 - 0800 and ending at 1700 -1800 each day (depending on the time of year). Data were collected by the observer who maintained an observation distance of 5-10 m from the subject monkeys. Focal animal sampling and continuous recording (using a digital voice recorder, model News my RV50) were used to score copulatory behavior of the males (Altmann, 1974). The order of observation for focal animals was determined daily via random selection. Focal sample duration was set at 20 minutes (Li *et al.*, 2007). If the focal male could not be followed or was lost from view during the sampling interval, then another male was randomly selected (Li *et al.*, 2007), and an effort was made to locate and record the behavior of the lost adult male during the next 20 minute sampling period (Li *et al.*, 2007; Xia *et al.*, 2010).

Focal sampling yielded a total of 452 h of data, evenly distributed between the breeding season (226 h, monthly mean $\pm$ SD = 45.2 $\pm$ 2.4h, n=5) and non-breeding seasons (226 h, monthly mean $\pm$ SD = 45.2 $\pm$ 3.7 h, n=5). Data collected were approximately equally distributed among the five males (range: 80-94.3 total h per male).

As described by Li (1999) and Li *et al.* (2007), copulatory behavior in this study was defined as an instance in which a male mounted a female with intromission and thrusting, but did not

necessarily ejaculate.

#### *Dominance hierarchy*

We calculated a David's Score (DS) for each male dyad based on the directionality of agonistic (aggressive/submissive) interactions that occurred over the study period (Gammell *et al.*, 2003; Li, 1999) (Table I). The greater DS, the higher an individual's social rank. The individual with the highest DS was defined as the alpha or dominant male. Aggressive and submissive behaviors were scored *ad libitum*. Aggressive interactions were defined as an individual's threatening, chasing, slapping, grabbing, or biting another individual (Berman *et al.*, 2007). Submissive behaviors were scored when an individual showed fearful interactions, such as fear grin, cower, mock leave, avoid, flee, or scream (Berman *et al.*, 2004).

#### *Fecal sample collection and hormone analysis*

A total of 426 fecal samples (of known identity) were collected non-invasively from the five subjects (mean = 86/individual, range = 74-111/individual). One to two samples per week were collected from each male using a targeted collection schedule. For most weeks, we obtained a fecal sample from each male before any male was sampled twice. The total number of samples from each male varied (GBZ = 81; CT = 74; YX = 74; JT = 86; BL = 111), however, fecal samples were evenly distributed across males for any given period of time.

We extracted testosterone from feces following the methods described by Barrett *et al.* (2002b) and Beehner and Whitten (2004). In cases where multiple samples were obtained from the same individual on the same day, only the first sample was analyzed. Fecal samples were placed in a methanol solution, homogenized, filtered, and loaded onto a reverse-phase C18 cartridge. Following solid-phase extraction, all samples were immediately frozen (-20°C) until transported to Anhui University for analysis. There, steroids were eluted from cartridges with methanol and subsequently frozen (-70°C) until the time of radioimmunoassay (RIA). Immediately prior to RIA, aliquots of samples were evaporated under nitrogen and reconstituted at a fivefold dilution in working

buffer.

#### *Radioimmunoassay*

All samples were assayed for testosterone using the testosterone RIA kit (Equate RIA <sup>125</sup>I Testosterone Kit, Beijing North Institute of Biological Technology, Beijing, China) previously validated for use in Tibetan macaques (Xia *et al.*, 2008). RIA was done at the Nuclear Medical laboratory of Anhui Medical University. We used the GC-911 radioimmunoassay gamma counter (Zhongjia Company of University of Science and Technology of China) and radioactivity counts were recorded for two minutes per sample. Inter-assay coefficients of variation were 9.13±0.89% (high control, N = 30) and 13.95±0.09% (low control, N = 30). The intra-assay coefficient of variation was 2.79±0.66% (fecal extract pool, N = 6). All samples were run in duplicate, and values are expressed as ng/g dry feces.

#### *Statistical analysis*

We report data as mean (± SE) rates of copulatory behavior (events/hour) and testosterone levels (ng/g). We used a one sample Kolmogorov-Smirnov test to examine whether the data conformed to a normal distribution ( $P > 0.05$ ). We square transformed all copulatory data to achieve normality before performing additional analysis.

We used a one-way ANOVA to check the differences of copulatory behavior or fecal testosterone levels among subject males (per male, n = 5), respectively. We used paired-samples T test to analyze the difference of testosterone levels and copulatory behavior between the mating and non-mating season (per male per season, n = 5), respectively. For testing the influence of social rank on copulatory behavior and fecal testosterone, we used the Pearson's correlation test to check the correlation of David's scores with copulations and testosterone levels (per male, n = 5), respectively.

We used general linear mixed-effects models (GLMM) in which testosterone levels were regressed against copulatory rates. Mixed models can also include multiple fixed effects, which allow for a number of potentially confounding variables to be controlled simultaneously. We included

copulatory rates and social rank of subject males may (DS values) as fixed effects. Each individual male

**Table I.- Copulatory behaviors (events/h, Mean±SE) and fecal testosterone levels (ng/g, Mean±SE) both in the mating season (MS) and non-mating season (NMS)**

Individuals	Ranks (DS values)	Age (yrs)	Copulatory behaviors		Testosterone levels	
			MS	NMS	MS	NMS
GBZ	1(10.00)	8	1.03 ± 0.08	0.15 ± 0.03	16.67 ± 0.63	11.45 ± 0.34
CT	2(4.45)	19	0.58 ± 0.06	0.07 ± 0.02	13.48 ± 0.69	10.50 ± 0.23
YX	3(-0.70)	28	0.30 ± 0.04	0.01 ± 0.01	4.75 ± 0.38	2.65 ± 0.18
JT	4(-3.75)	17	0.54 ± 0.05	0.10 ± 0.03	11.57 ± 0.88	11.02 ± 0.68
BL	5(-10.00)	9	0.27 ± 0.04	0.02 ± 0.02	13.65 ± 0.52	10.39 ± 0.63

**Table II.- Results of the linear mixed-effect models (GLMM) to test whether fecal testosterone levels is related to copulatory behavior and social ranks.**

Effects	Mating season				Non-mating season			
	Value	SE	<i>t</i> value	<i>P</i> value	Value	SE	<i>t</i> value	<i>P</i> value
Copulatory behavior	0.643	0.111	5.821	0.000	0.709	0.024	4.895	0.000
Social rank	-0.097	0.193	-0.672	0.904	-0.065	0.163	-0.579	0.896

not exactly contribute evenly to the database, and age may be a confounding factor for the correlation between testosterone and rank (Beehner *et al.*, 2009), thus age was included in the model as random factors. The model was fitted using maximum likelihood estimation. We also used Pearson's correlation test for analysis of the correlation between monthly (12 months) copulatory behavior and testosterone at individual levels (per male per month,  $n = 5$ ).

Analyses were two-tailed and data analyses were carried out using the software SPSS 13.0 (SPSS Inc., Chicago, IL, USA) (Norusis, 2005), with the levels of significance set at 0.05.

## RESULTS

### Copulatory behaviors

A total of 449 copulatory events were recorded during the study period ( $89.8 \pm 27.8$  events/male, range = 40-194), including 404 copulations in the mating season and 45 in the non-mating season. Males differed significantly in the frequency of copulations across the year ( $F =$

$16.253$ ,  $df = 4$ ,  $P < 0.01$ ), and within the mating ( $F = 12.774$ ,  $df = 4$ ,  $P < 0.01$ ) versus the non-mating seasons ( $F = 10.451$ ,  $df = 4$ ,  $P < 0.05$ ) (Table I). Rates of copulatory behavior in the mating season ( $0.55 \pm 0.13$  events/hour) were significantly different from the non-mating season ( $0.07 \pm 0.02$  events/hour) (disparity =  $0.47 \pm 0.11$ ,  $t = 4.228$ ,  $df = 4$ ,  $P < 0.05$ ).

### Fecal testosterone

In Tibetan macaques, fecal testosterone levels were  $10.94 \pm 0.64$  ng/g for the entire year. Testosterone levels varied significantly among the subject males ( $F = 15.321$ ,  $df = 4$ ,  $P < 0.01$ ) (Table I), and were significantly higher during the mating season ( $12.13 \pm 0.92$  ng/g) than the non-mating season ( $9.27 \pm 0.74$  ng/g) (disparity =  $2.863 \pm 0.43$ ,  $t = 6.632$ ,  $df = 4$ ,  $P = 0.003$ ).

### Copulatory behavior and rank

Based on the copulation and DS score relationship, we found that more copulations were associated with higher ranking individuals ( $R = 0.917$ ,  $N = 5$ ,  $P = 0.029$ ). Further analysis showed

that there was a positive correlation between copulation and social rank in the mating season ( $R = 0.931$ ,  $N = 5$ ,  $P = 0.026$ ), but not in the

non-mating season ( $R = 0.694$ ,  $N = 5$ ,  $P = 0.194$ ) (Fig. 1).

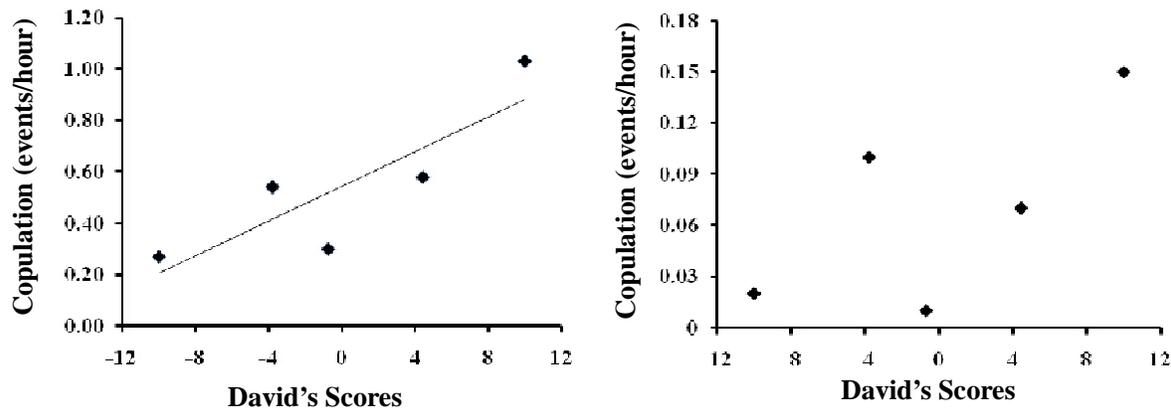


Fig. 1. Copulation and social rank in the mating (left) and non-mating season (right).

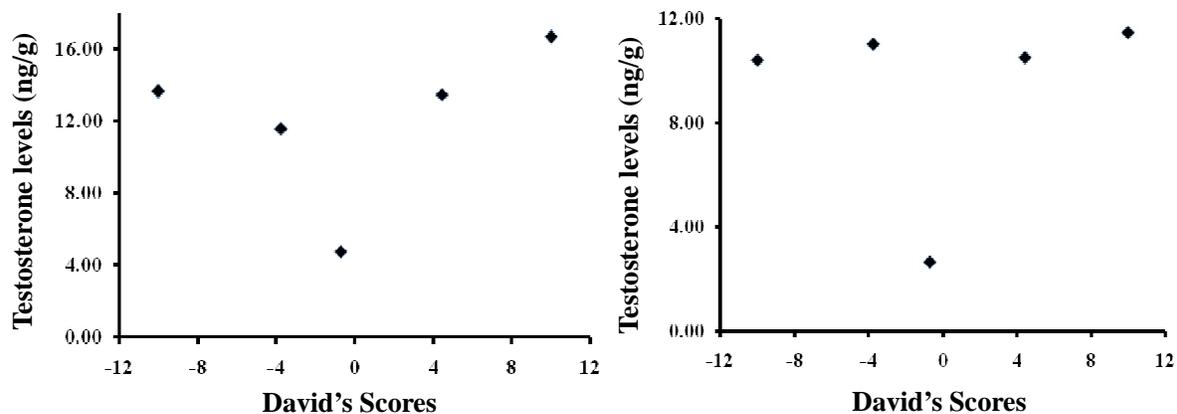


Fig. 2. Testosterone levels and social rank in the mating (left) and non-mating season (right).

#### Fecal testosterone and rank

The dominant male (GBZ) showed the highest testosterone levels in the mating ( $16.665 \pm 0.627$  ng/g,  $n=47$ ) and non-mating seasons ( $11.454 \pm 0.345$  ng/g,  $n=34$ ), respectively. At the individual level, there was no significantly positive correlation between testosterone and social rank ( $R = 0.300$ ,  $N = 5$ ,  $P = 0.624$ ), not only in the mating season ( $R = 0.319$ ,  $N = 5$ ,  $P = 0.601$ ), but also in the non-mating season ( $R = 0.512$ ,  $N = 5$ ,  $P = 0.381$ )

(Fig. 2).

#### Testosterone, copulatory behaviors and rank

The linear mixed-effect model showed a significant effect for fecal testosterone levels and copulatory behaviors in male Tibetan macaques (summarized in Table II). Season and social rank were not a significant predictor of the relationship between testosterone and copulation. Further analysis by month showed that, at the individual

level, both copulatory behavior and fecal testosterone levels went up or down in synchrony, and copulations were positively correlated with testosterone levels among subject males (GBZ:  $R=0.622$ ,  $N=12$ ,  $P<0.05$ ; CT:  $R=0.802$ ,  $N=12$ ,  $P<0.01$ ; YX:  $R=0.761$ ,  $N=12$ ,  $P<0.01$ ; JT:  $R=0.664$ ,  $N=12$ ,  $P<0.05$ ; BL:  $R=0.633$ ,  $N=12$ ,  $P<0.05$ ) (Fig. 3). The results showed that when an individual had a higher testosterone level, he mated more frequently. Conversely, lower testosterone level, less copulatory behavior. Or vice versa.

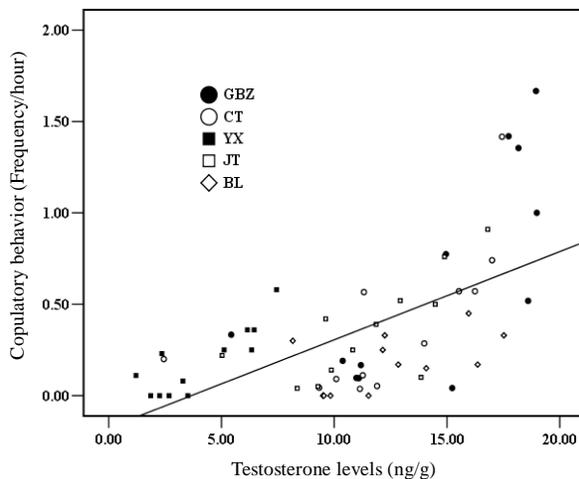


Fig.3. Testosterone levels and copulatory behavior in Tibetan macaques.

## DISCUSSION

To our knowledge, this is the first study to investigate the potential relationships between social rank, testosterone levels, and copulatory behavior in wild male Tibetan macaques. Overall, there were significant differences both in copulatory rates and fecal testosterone titers among the subjects. Testosterone levels did not correlate with social rank in either season. Therefore, prediction 1, stating that testosterone levels in high ranking male Tibetan macaques would be higher than in low ranking males both in the mating season and non-mating season was rejected. Examination of the data revealed that testosterone levels were significantly correlated with increased copulatory rates in both the mating and non-mating season, independent of

social rank. Therefore, prediction 2 was supported.

### *Testosterone and social rank*

Our finding in Tibetan macaques that testosterone did not correlate with social rank was opposite to wild *Cebus apella nigrinus* (Lynch *et al.*, 2002), wild *Eulemur fulvus rufus* (Ostner *et al.*, 2002), wild *Pan paniscus* (Sannen *et al.*, 2004), housed *Pan troglodytes* (Klinkova *et al.*, 2004), and wild *Colobus vellerosus* (Teichroeb and Sicotte, 2008), with no significantly different testosterone levels between higher- and lower-ranking males. However, our results were in line with Muller and Wrangham's (2004) findings in wild *Pan troglodytes* and Czoty *et al.* (2009) in housed *Macaca fascicularis*, which proposed that testosterone levels in higher-ranking males were higher than in lower-ranking individuals. Taken together, it is not difficult to find that testosterone and social rank relationships varied among species and environments. Our study provided a new piece of evidence for the variation of relationships between testosterone and social rank among species, and enriched the complex picture of these relationships in NHPs.

Testosterone can co-vary with dominance hierarchy, but not in all species under all conditions. For example, studies on chimpanzees at Kanyamwara, Kibale National Park in Uganda showed that testosterone correlated positively with rank (Muller and Wrangham, 2004; Muehlenbein *et al.*, 2004), however, Sobolewski *et al.* (2013) found no such relationship at the same place. Sapolsky (1993) has proposed that dominance rank may be a positive predictor of testosterone in some species only during periods of social "instability", for example competition by conspecific males for territory or access to the presence of receptive females (Sapolsky, 1993). During our study period, the dominance hierarchy was stable across the whole year. From this perspective, our results implied that dominance hierarchy might not be a predictor of testosterone in Tibetan macaques during a period where there is a stable social hierarchy. Future research should continuously observe subjects in order to clarify this point.

An alternative explanation is that maintaining high testosterone levels throughout the year is

potentially costly for males, and over the long term, high levels could be harmful (see Barrett *et al.*, 2002a). This similar perspective has been proposed by Beehner *et al.* (2006) "... sustaining high titers of testosterone for extended periods is costly..." (P469). Within the frameworks of Barrett *et al.* (2002a) and Beehner *et al.* (2006), we may find some supportive clues for our results. Aggression among male Tibetan macaques is more intense due to increased male-male competition for success to resources (*i.e.*, females) (Li, 1999). Higher testosterone is not independent of rank-related behaviors, such as aggression (Chen *et al.*, 2008). Either higher-ranking or lower-ranking males may maintain high or low testosterone levels by regulating testosterone secretion, and thus there may be no difference between higher-ranking and lower-ranking males.

#### *Testosterone, rank and copulatory behavior*

We found supportive evidence for our previous finding that rates of copulatory behavior in the mating season significantly differ from the non-mating season in male Tibetan macaques (Xia *et al.*, 2010). We were surprised to find that high and low ranked males' copulatory behaviors were not significantly different in the non-mating season when males were categorized, although individual males did differ significantly in their copulatory rates. This finding is similar to the testosterone and rank relationship we found in the non-mating season. Taken together, the testosterone and copulatory behavior relationship may not be correlated. However, our study supported the notion of a strong link between these two variables. The prediction that testosterone levels would correlate with copulatory behavior in these monkeys was well supported by our data.

The linear mixed-effect models showed a positive correlation between testosterone levels and copulatory behavior, regardless of social rank. Analysis on individual level provided supportive evidence. For each monkey, the testosterone level was positively correlated with copulatory behavior. Perhaps due to constraints imposed by our research environment, we were unable to manipulate the subjects and testosterone. Therefore, we are unable to draw conclusions about the direction of a causal

relationship between fecal testosterone levels and copulatory behavior for male Tibetan macaques. Due to the small sample size ( $n = 5$ ) in the present study, we did not have enough data to determine if individual males had elevated fecal testosterone directly on the day after they copulated. As such, we could not determine whether there are instantaneous change in fecal testosterone due to copulatory behavior, or whether increased testosterone levels lead to increased copulations. Regardless, we provide a preliminary assessment on reproductive physiology for understanding male reproductive strategies and possible physiological correlates in Tibetan monkeys. The results implied that, in the wild environment, ecological and social factors might be an important indicator for testosterone or copulatory behavior, but might not for the relationship between testosterone and copulatory relationships.

Our results support a relationship between testosterone and copulatory behavior in some species (*e.g.*, Muller and Wrangham, 2004; Wallen, 2001), but differ from other findings in some respects. Barrett *et al.* (2002a) suggested that changes in testosterone promote aggression rather than facilitate sexual behavior in the wild Japanese macaques. There is, nevertheless, some evidence demonstrating that testosterone may also rise in response to real or perceived mating opportunities. In some primate species, several authors link elevations in testosterone to increased mating behavior but not to elevated male-male aggression (Lynch *et al.*, 2002 in *Cebus paella nigritus*). In Tibetan monkeys, copulatory behavior in the non-mating season is non-reproductive and rare (Li *et al.*, 2007). The cause of this pattern is that the number of receptive female in the non-mating season is much lower than in the mating season. Therefore, male Tibetan monkeys showed a lower level in both testosterone and copulatory behavior. This assumption was opposite to the study by Teichroeb and Sicotte (2008), which demonstrated that testosterone levels are negatively correlated with the number of receptive females in some groups of *Colobus vellerosus*.

Furthermore, the expression of male sexual behavior in most mammals depends on some threshold levels of testosterone (Barrett *et al.*, 2006),

and testosterone secretion is not only correlated with copulatory behavior, but also with many behavioral traits related to competition for copulations, such as reproductive motivation or male-male aggression (Teichroeb and Sicotte, 2008; Wallen, 2001). Additional research will be needed to examine more fully the potential relationship between testosterone and reproductive behavior as demonstrated by the Tibetan macaques at Mt. Huangshan.

With the restriction of the study condition in the natural environment, we could not do the experimental design to access the immediate response of copulatory behavior when testosterone changed, or vice versa. However, our study preliminarily assessed the correlation between copulatory behavior and testosterone levels in wild NHPs. Due to small sample size (n=5), generalizations regarding the exact nature of the relationship between testosterone levels and the temporal expression of copulatory behavior in *Macaca thibetana* should be limited to the current population. Nevertheless, we still found a relationship between mating frequency and T levels, although it has been difficult to control for time of day in regards to sample collection. Taken together, our study enhances the complex picture of the relationships between testosterone and social rank and may also provide insights into our knowledge of a physiological factor linked to male reproductive success.

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