ORIGINAL PAPER

Reproductive competition and fecal testosterone in wild male giant pandas (*Ailuropoda melanoleuca*)

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Abstract The giant panda (*Ailuropoda melanoleuca*) is notoriously difficult to study in the wild, but its interesting reproductive ecology makes the effort worthwhile. Perhaps more than most species, the panda is energy-limited, which alters the cost/benefit analysis of its reproductive ecology. Using global positioning system/very high frequency radio-collars to locate mating aggregations, we used behavioral observations and fecal testosterone assays to gain insight into male panda reproductive effort and strategies, and test theories relating to reproductive competition. Male pandas initially competed fiercely for access to females that were about to be fertile, but once male competitive status was determined, aggression rates declined. Contact aggression was only observed during the first 2 days of mating aggregations; thereafter, it was replaced with noncontact aggression and avoidance. Agonistic interactions were highly asymmetrical, with contest losers (subordinates) showing less aggression and more avoidance than contest winners (dominants), both before and after contest outcome was established. The competitively superior male displayed mate-guarding tactics and secured all observed copulations. Contrary to theoretical predictions, testosterone levels did not predict aggression levels or contest winners and also were not affected by winning or losing a contest. Body size appeared to be the primary determinant of contest outcome. We discuss our findings in light of theoretical predictions, such as those arising from the "challenge hypothesis," in the context of the giant panda's foraging and nutritional ecology.

Keywords Giant panda · Reproductive behavior · Fecal testosterone · Aggression · Competition · Challenge hypothesis

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Introduction

Reproductive competition among males is a prominent feature in polygynous mating systems, where the distribution of potential mates on the landscape governs male reproductive strategies (Emlen and Oring 1977; Clutton-Brock 1989). Wide dispersion of females, as in solitary species, makes male monopolization of multiple females difficult, resulting in less male reproductive skew than in social species. In solitary species, infrequent interaction between males may preclude the establishment of enduring male social status (dominance hierarchies). In these species, male reproductive strategies

often include a combination of scramble competition to locate fertile females, and competitive interactions to secure access to females (Sandell 1989; Schwagmeyer 1995; Fisher and Lara 1999). These search and compete strategies can entail considerable energetic costs and risk of injury, so males must balance the costs of energy expenditures and risk of injury with the benefits of paternity.

The role of testosterone in supporting reproduction, and particularly reproductive competition, is well established (Wingfield et al. 1990; Archer 1988). In addition to supporting courtship and copulation with females, testosterone affords competitive advantages in male–male competition. The "challenge hypothesis" provides a theoretical framework predicting the temporal patterning of testosterone across various ecological and life-history contexts (Wingfield et al. 1990). This hypothesis posits that, in seasonally breeding species, male testosterone should increase to levels intermediate above baseline at the onset of the breeding season, but that maximum testosterone levels are only realized when males are challenged by competitors. Elevated testosterone supports aggressive behavior, but comes with an energetic cost and other negative consequences for fitness (Marler and Moore 1988; Wingfield et al. 1990; Briffa and Sneddon 2007). According to theory, testosterone is elevated to provide a competitive advantage in male–male competition and, as such, testosterone levels are predicted to correlate with aggressive levels and winning contests (Wingfield et al. 1990; Ros et al. 2002; Muller and Wrangham 2004; Hirschenhauser and Oliveira 2006; Mills et al. 2007). Males with higher testosterone should then be better competitors and secure more copulations, but pay other fitness costs associated with elevated testosterone, such as energy costs, suppressed immunocompetence, and reduced paternal care. Testosterone and competitive status should be correlated primarily during periods of social instability, such as territory establishment or reproductive competition (Wingfield et al. 1990; Beehner et al. 2006). Testosterone levels may also be affected by the outcome of competitive contests, which can affect behavior and competitive ability in subsequent contests (review in Rutte et al. 2006). In the "winner effect," contestants experience increased assessment of their own competitive ability and modify their subsequent behavior in a manner consistent with greater confidence in winning the next contest. In many cases, this behavioral change appears to be supported by changes in testosterone levels, with winners showing increased testosterone and losers showing decreased testosterone. One interpretation of these findings is that increased expectations for winning competitive encounters merits greater investment of energy resources devoted to competition, mediated by testosterone.

The endangered giant panda presents an opportunity to study male reproductive ecology in a solitary, seasonally breeding mammal. As an energy-limited species specializing on nutrient-poor bamboo (Schaller et al. 1985), male panda behavior and hormone patterns should strike a balance between investing in mating competition and the need to minimize the risk of injury and the energetic costs of competition (Nie et al. 2011). Despite several observations of giant pandas mating in the wild (Schaller et al. 1985; Pan et al. 2001; Yong et al. 2004), the mating strategy remains elusive. It is well known that females experience a single estrus during each spring and males show seasonal rut-like behavioral and physiological changes in captivity and in the wild (Kleiman 1983; Kersey et al. 2010; Nie et al. 2011). Schaller et al. (1985) and Pan et al. (2001) reported that males occupy large home ranges that overlap several female and male home ranges, and that males engage in significant competition for females before and during each female's brief 3-day fertile period. Despite the clear role for intrasexual male competition, it is possible that female pandas exercise indirect choice (sensu Wiley and Poston 1996), where female behavior sets the stage for male–male competition. Female pandas advertise reproductive condition through scent beginning up to several weeks before the fertile period (Swaisgood et al. 2000, 2002, 2004) and vocal signals (Charlton et al. 2010), which may recruit males and incite competition, ensuring that the female mates with the best available male (Cox and Le Boeuf 1977).

We used behavioral observations and noninvasive endocrine assays to examine some unknown aspects of male panda reproductive strategies. We made the following predictions for the present study. (1) Large males will have a competitive advantage over small males, winning more contests and securing more mating opportunities with females (McElligott et al. 2001; Kovach and Powell 2003; Costello 2009) and may have more energetic resources to support higher testosterone levels (Poole 1989). (2) Male aggression levels will predict contest outcome. (3) Males with higher testosterone will display more aggression (Mehlman et al. 1997; Wingfield et al. 1999; Hirschenhauser and Oliveira 2006). (4) Male testosterone levels should predict contest outcome, with high-testosterone males winning contests, and/or testosterone levels should increase in winning males and decrease in losing males (e.g., Oyegbile and Marler 2005; Rutte et al. 2006). Because giant pandas do not appear to form enduring dominance relationships, we assumed that males competing for a fertile female would conform to the predictions supposing social instability.

Materials and methods

Study site and animals

This study was conducted in Foping Nature Reserve, in the Qinling Mountains of Shaanxi, China from 2007 to 2010. The study site was in the core area of the reserve; preliminary

analysis of molecular census data using fecal DNA indicates a total 13 to 15 individuals residing in the area, with no significant sex bias (Wei, unpublished data). As the most dense population of this endangered species (State Forestry Administration, China, 2006), we speculate that the population density in Foping better reflects historical levels. Therefore, the reproductive and competitive behavior and ecology studied here will more likely reflect the norm during the panda's evolutionary history, making it an ideal location to test evolutionarily derived hypotheses.

Four adult pandas, two of each sex, were located opportunistically based on tracks and spoor, and fitted with global positioning system (GPS)/very high frequency radiocollars (GPS4400M, GPS7000M, Lotek Wireless, Ontario, Canada) with approval from the State Forestry Administration in China. Radiocollars made it possible to locate mating sites and conduct behavioral observations and fecal sampling during the mating season. Capture and anesthesia for pandas was conducted by a qualified veterinarian and field biologists during the winter months between November and March. This avoids impacting pandas during the breeding and birth seasons and ensures that pandas are anesthetized during cool weather, avoiding the risk of heat stress during anesthesia. To attach GPS satellite transmitters, we tracked and darted pandas using a compressed-air gun delivering approximately 5 mg ketamine/kg body weight. While under anesthesia, routine dental examinations and morphometrics were taken; this takes approximately 5 min. The location where the panda was darted was selected with care to avoid any risky terrain such as steep mountainsides. Once anesthetized, the panda was moved to a suitable position to avoid the flow of saliva backward into its trachea. Heart rate was monitored via femoral pulse and respiration was monitored visually and by placing the hand near the nose to feel the breath. Collars were adjusted to fit correctly, allowing some space between the collar and the neck. Use of expandable break-away collars ensured that pandas lost to the study remained unharmed. The panda was observed and tracked as it was coming out of anesthesia for a period of 2–3 h until it was moving and behaving normally.

We observed a total of six individual males (two collared, four un-collared) competing across three mating sites with one estrous female per site; other mating sites were located but were not included in the analysis because only a portion of the mating and competitive behavior was observed.

Behavioral observations and collection of fecal samples

Giant pandas are a challenging species to observe, and behavioral data collection on wild pandas remains rare. Researchers can spend months in habitat occupied by pandas without seeing them. Much of a panda's life is spent deep in the bamboo thickets where observations are nearly impossible. The mating season is the exception, when pandas emerge from the bamboo, spend more time in open areas along ridges, and engage in frequent and loud vocalization. Behavioral data was collected from visual observation of males during mating aggregations at the three mating sites during the March–May mating season (for details, see Nie et al. 2011).

Daily tracking of collared males allowed us to discover mating aggregations on the first day of their formation and follow them through their conclusion. We were able to locate one to two mating aggregations each year using this method. Mating aggregations can also be detected by the noisy vocalizations associated with mating and male–male competition. One or more of our collared males was present at each mating aggregation that we detected, suggesting that we missed few such mating aggregations in the vicinity of our study area each year. Un-collared male pandas were identified by physical characteristics such as pelage and facial markings.

We used all-occurrence sampling (Altmann 1974) to record selected behaviors. Observations commenced as soon as males aggregated with a female were located and continued continuously until nightfall approached. Written field records were verified and behaviors scored using video recording from two Sony video cameras capturing the behavior simultaneously from different vantage points. Male–male interactions were divided into three categories: initiated noncontact aggression, initiated contact aggression, and avoidance. Noncontact aggression included the vocalizations huff, snort, chomp, bark, growl, roar (sensu Kleiman and Peters 1990), and aggressive proximity-seeking behaviors: chase (rapid pursuit of a retreating individual) and charge/lunge (rapid approach of another individual). Escalated contact aggression included bite (grasping opponent with the teeth) and swat/grab (vigorous contact with opponent with paw/claws or holding onto opponent with paws). Avoidance behaviors included retreat (rapid movement away from opponent in response to aggressive act) and submit (panda crouches down and remains quiet but does not flee in response to aggressive act of another panda).

Individual daily behavior rates were calculated by dividing the number of agonistic acts committed by each male by total observation hours per day. The average rate of avoidance was calculated by dividing the number of avoidance acts by the rates of aggression received from other males. Contest outcome was determined once contact aggression ceased and one male was given clear priority of access to the female; invariably, the competitively superior male (dominant) remained close to the female, while the other males (subordinates) remained at a distance and avoided the dominant male. Here, we use the term "dominant" and "subordinate" as convenient labels to describe winners and losers of contest, but make no inference regarding the establishment of dominance social

relationships that endure beyond the period of the few days surrounding an individual mating event. Data on aggression and avoidance were categorized as before/after contest outcome was determined. In the first day of the mating event, prior to determination of competitive status, we estimated relative body size among competing males visually, providing an evaluation of body size unbiased by knowledge of contest outcome. Observers had significant prior experience with giant pandas and so were capable of distinguishing variation in body size in the field.

Fresh feces (0–3 h old) that could reliably be assigned to an identifiable individual from our observations were collected opportunistically while conducting behavioral observations. In nearly all cases, we were able to collect two to three samples for each male for each observation day; on two occasions, we collected only a single sample for a male in a day. Fresh fecal samples were stored on ice in plastic bags in the field, and frozen at -20°C within 4 h until laboratory analysis.

Hormone analysis

For details of hormone analysis, see Nie et al. (2011). We used a technique modified after those described by Wasser et al. (1994) and Li et al. (2001) to extract fecal testosterone from wet fecal samples (Barrett et al. 2002; Lynch et al. 2002; Cristobal-Azkarate et al. 2006).

The concentration of testosterone metabolites that cross-react with the antibody (testosterone immuno-reactivities) was determined using a commercially available iodinated radioimmunoassay kit designed for serum or plasma testosterone analysis (Equate Testosterone RIA kit, Puerweiye Biological Technology, Beijing, China); the testosterone antiserum is known to cross-react less than 0.01% with dihydrotestosterone, androstanedione, estradiol, progesterone. The lowest detectable dose of testosterone metabolites (sensitivity) of the assay was 0.02 ng /ml. There was no detectable cross-reactivity to corticoids.

For the assay, each sample contained 50 μl of unknown samples matched with 50 μl aliquots of testosterone standards. We then added 100 μl aliquots of antiserum to the standards and samples. For the steroid assay, we added a 100 μl I^{125}-testosterone solution and incubated the samples for 1 h at 37°C, before adding 500 μl charcoal dextran and spinning them at 3500 g for 15 min at 25°C; we then used an automatic radio-immunoassay r-counter to count the radioactivity of the supernatant fraction for 2 min. We estimated the loss of testosterone during the extraction procedure by the addition of ^{125}I-testosterone prior to extraction and measurement of radioactivity in the appropriate methanol fraction after separation and achieved an average recovery rate of testosterone of 87%. The intra- and inter-assay coefficients of variation for androgen were 4.9% ($N=10$) and 11.4% ($N=10$), respectively.

Statistical analysis

Our data come from six different males that were observed at three different mating sites with a different estrous female per site. When the same male was observed at additional mating sites ($N=2$ radiocollared males), we included this in the analysis as a separate event. For analysis, we obtained average values for each male at each site before and after contest outcome was determined. Although these variables are not likely to be statistically independent among males competing for the same female at the same mating site, our small sample size and research question requiring that we focus on individual males does not allow us to address this lack of independence adequately in the analysis. In fact, our hypotheses suggest that male behavior and testosterone should be influenced by other males. After ensuring that the distributional assumptions of normality and homogeneity of variance were met, we used linear mixed models (LMMs) to explore the differences between dominant and subordinate males before and after contest outcome was established. We fitted models with aggression, avoidance, and testosterone levels as separate dependent variables, dominance status, time period (before/after dominance established), and dominance × time period interaction as fixed factors, and subject, mating sites, and subject × dominance status interaction as random effects factors. A Wilcoxon paired test was used to analyze the paired data of each male before and after contest outcome was established. We used LMM to evaluate the effects of average daily fecal testosterone concentrations on rates of aggression by fitting the model with aggression as the dependent variable, dominance status and testosterone levels as fixed factors, and subject as the random variable. In all cases, we included the full model with all possible interactions included. Because fecal steroid hormone concentrations reflect serum concentrations 12 h earlier (Kersey 2009) and fecal samples were collected during various times of the day, we correlated same-day fecal testosterone levels with aggression levels to better address the ability of testosterone to predict behavior. Data are presented as mean±standard error of the mean, with the statistical significance level set at $P<0.05$. Analyses were done with SPSS 16.0.

Results

We recorded 558 agonistic acts between males in three mating sites during a total 16 days of observation (5–6 days at each mating site). Each male was observed for 20.5 to 30.8 h at each mating site for a total of 235.6 subject observation hours, and we collected 9–15 fecal samples per male, per mating site, for a total of 93 fecal samples. At these three mating sites, all the females showed similar

behavioral patterns and spent the majority of the time sequestered in the branches of a tree above the males before the day of mating. In each case, two to three adult males competed for reproductive access to the female, and there was always a clear victor by the end of day 2 of the competition. For the two males fitted with GPS satellite collars, the mating sites were within the boundaries of their home ranges.

The daily means for behaviors and testosterone are provided for descriptive purposes to illustrate the temporal dynamics of male competition in Fig. 1. The clear majority of the aggressive acts were noncontact aggression such as roar and chase (97.7%). However, fighting was vigorous initially and all males received injuries during the contest. Observed injuries included bloodied faces and open wounds on the legs and back, some of them several centimeters in length. Contact aggression was only observed during the first 1–2 days of the contest period and was notably absent thereafter, suggesting avoidance of escalated aggression once competitive status was determined (Fig. 1a). Initiation of contact aggression appears equally likely for dominants and subordinates in day 1 of competition, but only dominants initiated contact aggression in day 2, again suggesting rapid establishment of sustained dominance. Noncontact aggression decreased gradually across the mating event,

but appeared to increase again on the day of mating, suggesting greater aggressiveness on the part of the dominant or perhaps more approaches by subordinates (although clearly subordinates did not initiate more aggression on the day of mating, as evident in Fig. 1b.) In all three cases, the eventual winner showed higher rates of aggression across the entire mating period and was the largest male competitor present (Table 1), suggesting body size is a determining factor in male panda contests. Prior knowledge about most contestants was not known, but there was 100% agreement among all experienced observers (a minimum of three present) regarding relative body size. The individual that eventually attained dominance was larger and appeared to be in better body condition. In striking contrast to behavior, testosterone levels appear little affected by competitive status or temporal dynamics of the contest (Fig.1d).

For statistical analysis, we divided data into periods before and after competitive superiority was established, so that we could test whether behavior and testosterone predicted or was influenced by contest outcome. Contact and noncontact aggression was pooled for analysis. Total aggression levels were higher for dominant males than subordinate males during the periods both before (LMM: $F_{1.6}=27.44$, $P=0.002$) and after ($F_{1.6}=41.40$, $P=0.001$; Fig. 2a) contest outcome was established. The analysis for
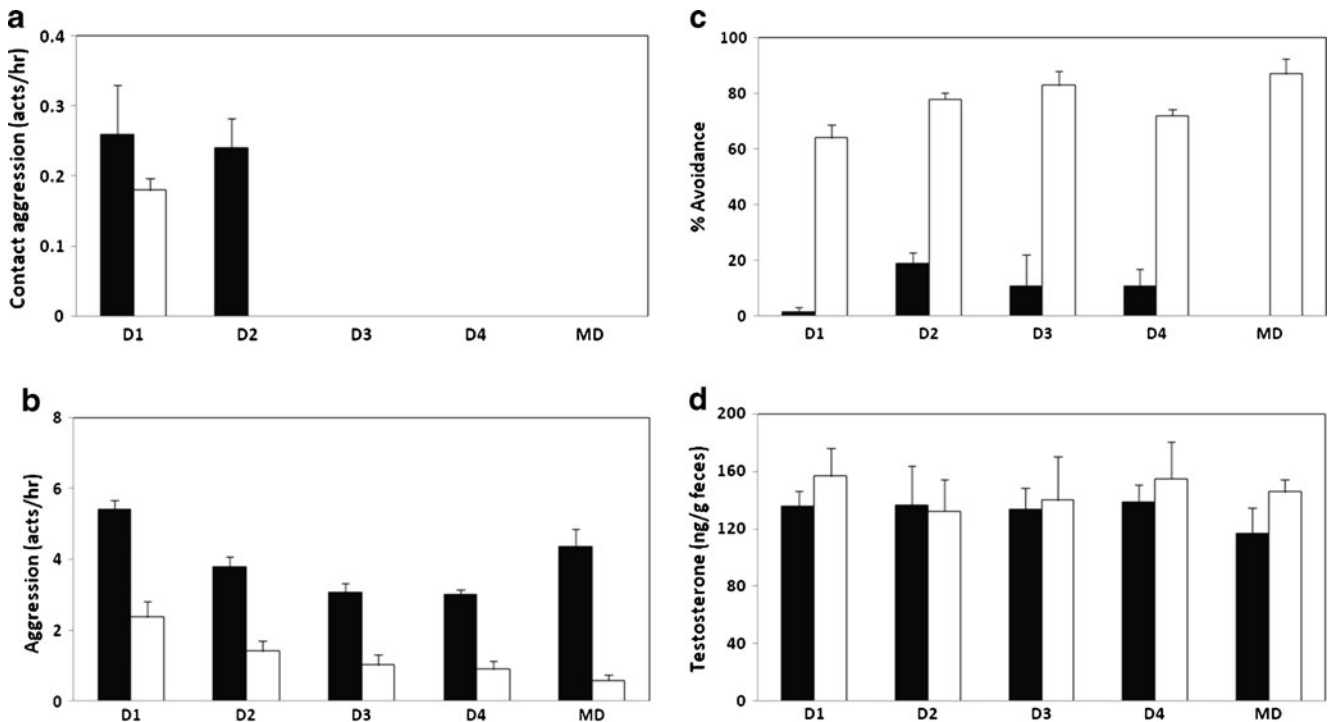

Fig. 1 Mean rates of **a** contact aggression, **b** noncontact aggression, **c** avoidance, and **d** testosterone in subordinate and dominant males across the 5 days of reproductive competition at three mating sites. [For the mating event that occurred over 6 days, we showed days 2–6 so that the last day in the figure always displays the day of a mating event, i.e., day of mating (MD)]. *Black bars* represent dominant individuals, while *white bars* represent subordinates

Table 1 Individual rank, body size ranking, rate of aggression and avoidance, and mean fecal testosterone concentration for each male

Mating site	Male	Rank	Body size ranking[a]	Initiated aggression (acts/h)	Avoidance (%)	Mean T (±SEM)[b]
MS1	UNM1	Dominant	1	3.58	6.06	121.49 (±8.75)
	CM1	Subordinate	2	0.88	74.51	194.39 (±11.00)
MS2	UNM2	Dominant	1	4.26	8.70	136.80 (±13.24)
	CM1	Subordinate	2	2.08	75.91	173.29 (±14.02)
	CM2	Subordinate	3	0.55	81.93	93.04 (±10.53)
MS3	UNM3	Dominant	1	3.96	6.25	133.18 (±9.75)
	CM1	Subordinate	2	1.22	69.14	150.75 (±10.05)
	UNM4	Subordinate	3	1.71	84.90	131.31 (±11.64)

MS Mating site, *UNM* unknown male, *CM* collared male

[a] Body size ranking for males at each mating site are presented as 1>2>3

[b] Testosterone (T) values are expressed as ng/g feces (±SEM)

the period before contest outcome was established indicated that aggression levels predicted contest outcome, with dominant males displaying higher aggression than subordinates.
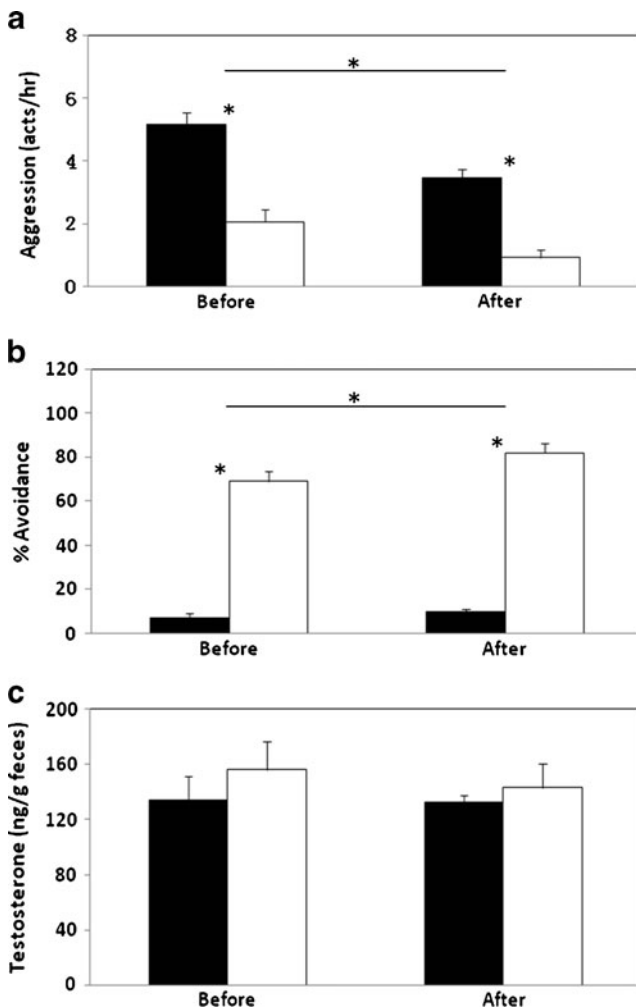

Fig. 2 Mean rates of **a** initiated aggression (contact and noncontact combined), **b** avoidance (proportion of aggressive acts evoking avoidance), and **c** fecal testosterone for dominant and subordinate males before and after competitive dominance was established. *Error bars* indicate standard error of the mean. *Black bars* represent dominant individuals, while *white bars* represent subordinates. *Asterisk* indicates $P<0.05$

Avoidance behavior also conformed to predictions: subordinate males displayed higher levels of avoidance than dominant males both before (LMM: $F_{1.6}=77.85$, $P<0.001$) and after ($F_{1.6}=136.23$, $P<0.001$; Fig. 2b) contest outcome was established. By contrast, testosterone levels were unaffected by dominance status before (LMM: $F_{1.6}=0.002$, $P=0.95$) and after ($F_{1.6}=0.001$, $P=0.97$; Fig. 2c) contest outcome.

After competitive status was determined, there was a marked difference in behavior. Subordinate males remained near the mating site, but stayed at a distance of 10–50 m from the dominant male. The dominant male engaged in behavior we label "mate guarding," remaining near the base of the tree containing the female and invariably deterring rival males, which occasionally approached the female or the dominant male. The dominant male also chased the female, back up the tree if she attempted to leave. The dominant male was the only one male observed copulating with females, and copulations only took place on the last day of the mating event. After copulation, all animals dispersed from the mating site. The rate of aggressive behavior declined once competitive status was determined (Wilcoxon paired test: $Z=2.52$, $N=8$, $P=0.01$; Fig. 2a). The proportion of aggressive acts evoking avoidance was affected by contest outcome, and the rate of avoidance increased after competitive dominance was established (Wilcoxon paired test: $Z=2.20$, $N=8$, $P=0.03$; Fig. 2b). Finally, we found no evidence for a "winner effect" in the temporal dynamics of testosterone: male testosterone levels did not change after winning or losing a contest (Wilcoxon paired test: $Z=0.98$, $N=8$, $P=0.33$; Fig. 2c). In no case did we find any significant interactions between competitive status and before/after contest outcome (aggression: $F_{1.6}=3.86$, $P=0.10$; avoidance: $F_{1.6}=2.96$, $P=0.42$; testosterone: $F_{1.6}=2.83$, $P=0.61$; Fig. 2a, b, c), suggesting that winning or losing a contest did not affect dominants and subordinates differently. For our behavioral measures, it would appear that contest outcome only served to reinforce behavioral patterns that were present at the outset of the competitive interactions.

Contrary to predictions, daily aggression levels were not affected by fecal testosterone, and they were not statistically correlated (LMM: $F_{1.39} = 1.51$, $P = 0.27$). Examination of Fig. 3 reveals a striking lack of relationship between these variables, suggesting that this result does not stem from low statistical power.

Discussion

This study is the first to provide quantitative behavioral and hormonal data bearing on intra-male competitive strategies in the giant panda in the wild. The panda's rarity, its secretive nature, and the dense habitat it occupies explain the absence of data on this subject. While our study provides novel and important insights, it is necessarily based on a limited number of subjects; thus, we extrapolate to the larger population with caution, noting, in particular, that these hormonal and behavioral patterns may differ for giant pandas living under different social and ecological circumstances. Our findings indicate that several male pandas locate a reproductive female and contest briefly, but vigorously, for priority of access for mating. All male pandas in this study sustained injuries during fighting, a finding consistent with other observations by our team and the frequent observation of escalated aggression by others (e.g., Schaller et al. 1985). Once competitive superiority is established, aggression, particularly injurious aggression, decreases between males, and the dominant male invests in mate guarding while the other males remain nearby but do not significantly contest the dominant male. Although aggression declines after contest outcome is decided, dominant males continue to maintain high levels of aggression toward rival males. This premating defense of estrous females probably increases reproductive success (Parker 1974; Yamamura 1987; Poole 1989).

The observed patterns of reproductive competition in male pandas suggests that while the rewards of reproductive competition merit risking injury to gain access to females,
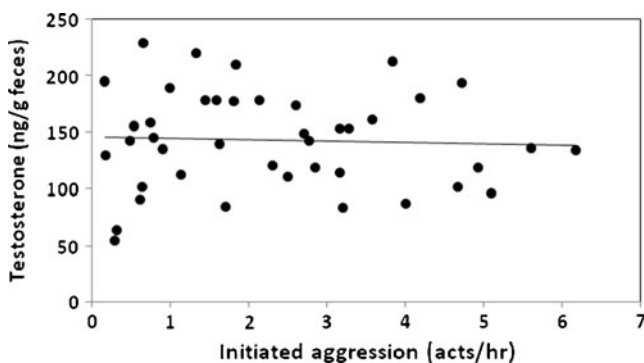
the costs of continuing escalated aggression outweigh this benefit once competitive status is determined. It would appear that pandas are capable of assessing competitive asymmetry early in the contest, as indicated by greater aggressiveness and lower avoidance by dominants *before* contest outcome is settled. Higher levels of aggression among dominant male pandas suggest that a chief benefit of heightened aggression is gaining access to mates through superior competitive ability. Body size was also related to competitive success, with larger males winning all contests in this study, consistent with results from bears (Kovach and Powell 2003; Costello 2009) and some other mammals (e.g., McElligott et al. 2001). If panda contest outcomes can be predicted by physical and behavioral differences between the opponents, pandas should also be able to make this determination and, according to theory, avoid escalated and injurious fighting (Huntingford and Turner 1987). That this is not the case indicates that the resource value (rare fertile females) is high enough to merit paying these costs to probe apparently superior opponents (cf. Clutton-Brock and Albon 1979; Parker and Rubenstein 1981). Alternatively, subordinate males may not be capable of assessing competitive asymmetry early in the contest and the sustained competition over the course of 1–2 days is required to determine competitive status. Either explanation, however, points to a willingness to risk injury to gain access to estrous females.

Although the numerical sex ratio in pandas shows no bias (Zhan et al. 2006), the operational sex ratio is highly skewed toward males because female pandas are only fertile for 1–3 days once every 2–3 years (Schaller et al. 1985; Wei and Hu 1994). The degree of female rarity is difficult to estimate. Our results suggest that a male may locate as few as 1–2 females each year in his home range (which expands during the mating season). Even though these mating aggregations are associated with loud vocalizations that we often detect independent of radiotracking collared males, we detected no aggregations that were not also attended by one or more of our collared males, suggesting that collared males may have located all estrous females in their home range. Thus, males may have access to fewer than three estrous females per year. The rarity of opportunities to fertilize females may explain the risks males appear to take even when probability of success is low.

It is both surprising and interesting that panda testosterone levels were not predictive of aggression levels or competitive ability. There was little variation in testosterone levels among males, and subordinates were as likely as dominants to sustain higher testosterone levels. The degree of variation in testosterone documented in our study may not represent the entire panda population. For example, males that do not participate in mating aggregations may have lower competitive ability and correspondingly lower testosterone levels. However, our own observations and

Fig. 3 Mean daily fecal testosterone levels relative to initiated aggression rates for each male in all the three mating sites

those of others (e.g., Schaller et al. 1985) indicate that even subadult males with clearly inferior competitive abilities often participate in mating aggregation and competition. There is much we do not know about panda mating strategies, but observations suggest that most males capable of locating a female participate in these competitive mating aggregations regardless of competitive ability.

As a seasonally breeding species with no paternal care, the challenge hypothesis predicts that pandas should maintain elevated testosterone throughout the mating season (Wingfield et al. 1990). However, in the only field study of panda testosterone, we found that wild male pandas reverted to baseline premating season levels of testosterone between mating opportunities (Nie et al. 2011). It seems plausible that, if pandas elevate testosterone levels during a few brief mating events each year at precisely the time it is most critical for reproductive competition, it may pay to always increase testosterone to the physiological maximum. Mating opportunities are so rare that the short-term energy costs associated with elevated testosterone may be outweighed by even a remote chance at reproduction. This explanation is also consistent with our failure to find a "winner effect" in pandas. In many other species, testosterone increases in contestants after winning a fight and may decrease in losers (Archer 1988; Oyegbile and Marler 2005, Rutte et al. 2006). That both winners and losers maintained consistently elevated testosterone before and after fighting suggests again that they may be at physiological maximum. Losers, who remain near the estrous female and are frequently chased away by the mate-guarding winning male, may maintain elevated testosterone in case circumstances change and they have the opportunity to contend for access to the female.

Together, our findings suggest interesting trade-offs between energetic constraints, risk aversion strategies, and vigorous reproductive competition supported by brief surges in testosterone levels, at least for the ecological conditions that prevailed during our research in the Foping Nature Reserve. In cases where our findings diverge from theoretical predictions and empirical results from other species, panda reproductive ecology may shed light on theory and expand our understanding of the ecological conditions in which theoretical predictions may or may not be met, leading to better theoretical models. If our explanations are correct, males of other species, (1) operating under severe energetic constraints during the mating season and (2) with infrequent opportunities for reproduction counter-selecting for energy mobilization for reproductive competition, may also confine their testosterone production to brief well-timed episodes to maximize reproductive success.

Of course, testosterone has biological effects that extend beyond effects on aggression and competitive strategies. Testosterone production is a required for spermatogenesis

and some of the observed patterns for wild pandas may be influenced by this essential function. Interestingly, previous studies on wild-living pandas (Nie et al. 2011) indicate that testosterone may be elevated in brief pulses when males participate in mating aggregations and fall to premating season baseline during intervals between these mating interactions. By contrast, captive male pandas have elevated testosterone for sustained periods for more than 5 months, arguably to support both continuous prodigious spermatogenesis seen in captive male pandas and fighting readiness (Kersey et al. 2010). How these wild pandas support spermatogenesis with apparently very low testosterone sustained levels is not known. Testosterone also does not act in isolation from the broader hormonal milieu during the mating season. Glucocorticoids are also elevated concurrently with testosterone during the mating season, and, in concert, testosterone and glucocorticoids increase both anabolic and catabolic pathways to sustain reproductive activity (Kersey et al. 2010). However, the few wild male pandas studied so far do not show evidence for a long period of testosterone-mediated anabolic investment in muscle buildup to prepare for competition (Nie et al. 2011). Since body size appeared to predict contest outcome, assimilation of muscle mass should be advantageous; we suggest that the nutritional constraints operating in the males included in our study precluded this strategy, perhaps to their disadvantage.

Our results also build on previous findings for this conservation-dependent species (reviews in Swaisgood et al. 2010, 2011). In small populations, understanding the mating system is of vital importance for conservation because of the negative impacts reproductive skew can have on effective population size and population viability (Anthony and Blumstein 2000). While evaluation of reproductive skew is best obtained from genetic studies, it is important to understand the underlying behavioral processes so that skew can be managed, when appropriate (Wedekind 2002; Fisher et al. 2003). Our findings contribute to this goal, demonstrating the significance of male reproductive competition in pandas and how such competition affects reproductive outcomes. These data are also making inroads into understanding how energetic constraints may affect testosterone production, with consequences for male reproductive strategies (see also Nie et al. 2011). Many aspects of panda life history appear to be constrained by the panda's energy-limited foraging strategy (Schaller et al. 1985), and it now appears plausible that male reproductive strategies could be negatively impacted by anthropogenic activities that reduce forage availability during the spring mating season. If more males enter the mating season in poor condition and are unable to sustain elevated testosterone, then there may be even fewer males competing for access to females, further increasing reproductive skew. Panda populations, which are highly fragmented and isolated,

may be vulnerable to even small changes that lead to further loss of genetic diversity.

Ethical standards The authors declare the experiments comply with the current laws of People's Republic of China and were approved by State Forestry Administration of People's Republic of China (2009-261).

Conflict of interest None

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