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Interactions in male baboons: the importance of both males' testosterone

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Abstract To date, research on testosterone and behavior has focused on individuals, even when studying social behaviors that necessarily involve multiple participants. Here, we explore male responses to other males of different dominance ranks and testosterone levels in a population of wild baboons. In chacma baboons (*Papio hamadryas ursinus*) of the Okavango Delta, a male's testosterone is related to his rank trajectory and, therefore, the threat he poses to other males. To examine the effects of testosterone and rank on male–male interactions, we used playback experiments to measure how a target male responded to the simulated approach of another male, scoring responses by whether or not the subject moved away from the speaker in the first minute. High testosterone subjects did not move away from the speaker more often than low testosterone subjects, but high testosterone callers elicited a move more often than low testosterone callers. When the combined testosterone of the subject and caller was high, moves were most common. The rank relationship between subject and caller did not predict moves, but the effect of combined testosterone on moving was most pronounced in adjacently

ranked males. Adjacently ranked, high testosterone males are the most likely to be competing for each others' rank, and our experiments on these dyads elicited the most moves. Both behavioral and experimental observations indicate that testosterone may be more important than the rank relationship in predicting the outcome of male–male interactions. Furthermore, combined information on the testosterone of both males was the best predictor of results, highlighting the utility of dyadic analyses when relating testosterone to behavior.

Keywords Testosterone · Playback experiments · Aggression · Baboon · Male–male interactions · Dyadic interactions

Introduction

Testosterone is the primary androgen in vertebrates: it is essential for the development of male secondary sexual traits (Wickings and Dixson 1992; Woodley 1994), is associated with muscle mass (Bhasin et al. 1996), is involved in male sexual physiology, behavior, and motivation (Terasawa and Fernandez 2001), and is often associated with aggression and social status (Bouissou 1983; Monaghan and Glickman 1993). The challenge hypothesis, initially proposed for birds, attempts to explain temporal variation in testosterone levels by positing that testosterone is correlated with aggression associated with mate acquisition (Wingfield et al. 1990). Recent work has shown that the challenge hypothesis also applies to some primate species. For example, in chimpanzees, testosterone is more closely tied to mate guarding aggression than to mating per se (Muller and Wrangham 2004). Furthermore, among seasonally breeding males, seasonal increases in testosterone are more pronounced in species with high rates of male–male aggression during the mating season (Brockman et al. 1998; Strier et al. 1999; Cavigelli and Pereira 2000; Barrett et al. 2002; Ostner et al. 2002).

Behavioral studies across many vertebrate taxa have indicated that the relationship between testosterone and

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behavior is complex, and that no simple cause–effect relationship exists. Experimental and observational studies have demonstrated that external stimuli can effect a change in male testosterone levels (Rose et al. 1971; Bernstein et al. 1979; Wingfield 1988; Mazur et al. 1992; Mazur and Booth 1998), and in many cases, rapid testosterone elevations were observed in direct response to simulated challenges from a conspecific male (Greenberg and Crews 1990; Buck and Barnes 2003; Remage-Healey and Bass 2005). Conversely, experimental evidence has shown that artificial elevations in testosterone (using implants) can produce heightened behavioral responses to simulated territorial intrusions or aggressive stimuli (Albert et al. 1986; Moore 1988; Hunt et al. 1997; Van Duyse et al. 2002).

Despite the numerous studies that examine testosterone in relation to aggressive behavior, very little work has considered the interactive effect of both participants' testosterone profiles in a social interaction. Such dyadic analyses may be particularly relevant in primates that live in large groups with highly differentiated social interactions. In such cases, a male's behavior may be influenced not only by his own testosterone titers but also the testosterone of the individual with whom he is interacting.

Dominance relationships are salient features of many primate societies that influence how two individuals interact. In baboons (*Papio hamadryas* spp.), as in many primate species, male dominance hierarchies mediate access to fertile females (Packer 1979; Bulger 1993). Because high-ranking males monopolize mating and baboons mate throughout the year, competition for social status serves as a proxy for competition for mates creating the potential for complex relationships among behavior, dominance rank, and testosterone. Rank-based mating skew might explain why, among male baboons, high-ranking males have higher testosterone than low-ranking males during periods of instability when ranks are actively contested (Sapolsky 1991, 1993). Furthermore, studies across several different primate taxa have shown that testosterone is related to aggression only during periods of instability (Bernstein et al. 1979; Steklis et al. 1985; Nieuwenhuijsen et al. 1987; Cavigelli and Pereira 2000).

Dominance rank and instability may be especially important in chacma baboons (*P. hamadryas ursinus*) living in the Okavango Delta of Botswana where rank-based mating skew is particularly strong (Bulger 1993). These baboons live in large social groups that usually include between 3 and 15 adult males. Upon reaching maturity, natal males typically rise in rank above older adult males, occasionally achieving alpha status. Although most natal males disperse at some point during this period (at 8–10 years of age), some males remain in their natal groups for their entire lives (Hamilton and Bulger 1990; Kitchen et al. 2003). More commonly, however, an immigrant male holds the alpha position, rapidly rising to achieve the alpha rank shortly after his arrival in the group (Hamilton and Bulger 1990).

Because dominance relationships among males are influenced primarily by age, size, and fighting ability

(Hamilton and Bulger 1990; Bulger 1993; Kitchen et al. 2003), changes in the male hierarchy are common, occurring on average 1.5 times per month in a group of 10–15 males, with a change in the alpha position on average every 6–7 months (Cheney et al. 2004).

The frequent changes in rank suggest that instability may be the norm rather than the exception (Bergman et al. *in press*). In a recent study of these same males, male testosterone measures were correlated with rank and even more strongly correlated with *changes* in rank (Beehner et al. 2005). Males ascending the dominance hierarchy had the highest testosterone concentrations, and male testosterone predicted future dominance rank and mating activity for up to 11 and 7 months, respectively (Beehner et al. 2005). These results indicate that a male's current testosterone is a fairly accurate predictor of his rank trajectory and, consequently, the threat (or lack thereof) that he poses to other males. If males are sensitive to this threat, they may behave differently towards a male with high testosterone than towards a male with low testosterone.

To test this hypothesis, we designed a playback experiment to simulate the approach of a specific male. We were interested in how baseline testosterone concentrations predicted subject responses to brief simulated encounters. Our experiments were modifications of previous studies in birds pairing testosterone analyses (and, in some cases, the manipulation of testosterone) with simulated approaches by intruding males. Some of these studies found that responses were positively correlated with subjects' testosterone measures (Herting and Belthoff 1997; Van Duyse et al. 2002), whereas others found no association (Meddle et al. 2002; Moore et al. 2004; Foerster and Kempnaers 2005). We are unaware of any experiments that have attempted to simulate the approach of a familiar rival, thereby allowing responses to be analyzed in relation to the testosterone profiles of both the subject and the simulated intruder. This is the approach we use here.

In our experiments, subjects were played the grunts of a particular male (the “caller”) to simulate the caller's approach. We chose male grunts because they are non-threatening and typically used in friendly encounters with females (Palombit et al. 1999). Thus, the subject was made aware of the proximity of a particular male in a non-threatening situation. We measured whether or not the grunts of particular callers caused a subject to move away from the area. In chacma baboons, interactions among males are frequently aggressive, and males often appear to avoid confrontations with rivals by leaving the area when they are approached. By controlling for visual cues and the recent history of interaction between males, the experiments allowed us to focus on how the naturally occurring testosterone levels of both the caller and the subject affected the responses to our simulated approaches.

In addition to testosterone profiles, two other factors may affect a subject's decision to move away from the playback stimulus. First, we know that adjacently ranked males have more aggressive interactions than more disparately ranked males (Kitchen et al. 2003); therefore, the relative rank relationship between the subject and the

caller may be important. We considered that four different rank relationships might be important in determining the outcome of male–male interactions: mainly (1) whether the subject ranked above or below the caller and (2) whether the subject was adjacent in rank or more disparate in rank to the caller. Second, males that have a history of aggressive interactions may avoid each other. Therefore, we also considered that the rate of aggression between a pair of males would predict each male's response to simulated approaches from the other male.

As a background for our interpretation of the playback experiment results, we examined the behavioral interactions between the 42 pairs of males that were used in experiments. Because adjacently ranked males were more likely to be aggressive (Kitchen et al. 2003), we expected that adjacently ranked males would approach each other at low rates and interact aggressively at high rates. We also examined both individual and combined testosterone measures in relation to aggression rates. We predicted that higher testosterone measure for both males would be more likely to result in aggression in pairs of males.

We made several predictions for the experimental responses. (1) Based on previous observations by Kitchen et al. (2003), subjects would be more likely to move away from the speaker when they heard grunts of higher-ranking males than when they heard grunts of lower-ranking males, and subjects would be more likely to move away from the speaker when they heard grunts of adjacently ranked males than when they heard grunts of disparately ranked males. (2) Because high testosterone males are more likely to be rising in rank, all males would move away from high testosterone males. Thus, the caller's testosterone would be a significant predictor of responses. (3) The testosterone of both males involved in the experiment would be the best predictor of responses. A high testosterone male is most likely to be competing with another high testosterone male for high rank; therefore, moves will be most common when a high testosterone subject hears the grunts of a high testosterone caller.

Methods

Study site and subjects

Research was conducted in the Moremi Game Reserve in the Okavango Delta of Botswana. Baboon density in the area is one of the highest reported for this species (Cheney 1987; Hamilton and Bulger 1992). The study group, C, has been observed since 1978 (e.g., Bulger and Hamilton 1987). Since mid-1992, the group has been under almost daily observation (Cheney et al. 2004). The ages and matrilineal relationships of all natal individuals are known, as are the origins and destinations of many immigrant males. The group is fully habituated to humans on foot. During the course of this study, the group contained 82–91 individuals, including 9–10 adult males (>8.5 years of age), 29–31 adult females (>6 years of age), and their immature

offspring. A total of 11 adult males were resident in the group during the time when experiments were conducted.

Behavior

Rank relationships were scored daily from approach–retreat interactions (supplants) recorded during ad libitum and focal animal sampling. From these interactions, we were able to (manually) construct a linear hierarchy that we used to determine the rank relationship (e.g., adjacent or disparate) between males at the time of a playback. Approach–retreat interactions were extremely consistent with the current hierarchy, ranks were extremely linear, and changes were abrupt and salient. When male ranks changed, we waited at least 1 month before using males affected by the changes in playback experiments. Behavioral data were collected using 10-min focal animal samples (Altmann 1974). The data reported here were gathered during two periods (January 2–March 23, 2002 and September 14, 2002–February 1, 2003), separated by a period of extreme instability in the male dominance hierarchy that precluded experiments.

During focal observations, all approach interactions involving the subject were recorded. An approach occurred whenever a male moved to within 2 m of another male. We also scored all incidents of aggression involving the focal subject. In addition to overt fighting, aggressive interactions were scored whenever a male supplanted, threatened, chased, or exhibited submissive behavior toward another male. Threats included quick head movements, eyelid flashes, lunges, and threat–grunt vocalizations. Submissive behavior included crouching, fear grimacing, and retreating. If several behaviors were given in sequence, each behavior was recorded separately.

In each dyad, interactions during a given experimental period were summed from both males' focal observations, and rates of behavior were calculated using the combined observation time for both individuals. Behavioral rates were calculated separately for each period. We collected an average of 7.8 and 14.8 h of focal animal sampling per dyad in playback periods 1 and 2, respectively. To characterize relations within a dyad, we focused on two primary behaviors: approaches and aggression.

Playback experiments

Playback experiments have been used to study many aspects of chacma baboon behavior (e.g., Cheney et al. 1996; Palombit et al. 1997; Rendall et al. 2000; Fischer et al. 2001; Bergman et al. 2003; Kitchen et al. 2003). In all cases, responses to playbacks are similar to responses to naturally occurring behavioral interactions, and results indicate that baboons individually identify vocalizations (e.g., Cheney and Seyfarth 1997). To simulate the approach of a particular male, we used recordings of grunts given by that individual to females and their infants during

the past 3 months. Grunts given in this context are harmonically rich, individually distinctive calls that appear to function to signal benign intent (Cheney et al. 1995; Owren et al. 1997; Palombit et al. 1999; Rendall et al. 1999). In our experiments, grunt playbacks served simply to indicate the proximity of a particular male, allowing us to measure subjects' responses to the apparent approach of different individuals.

Each playback stimulus consisted of a natural sequence of five grunts in which the timing and intergrunt interval were preserved from the original recording. Grunts were recorded using a Sennheiser ME-66 directional microphone and a Sony WM-D6 Professional Walkman recorder. After recording, vocalizations were digitized to WAV files using CoolEdit software (Syntrillium, Phoenix, AZ). Playback stimuli were matched for duration and amplitude and calibrated to the amplitude of naturally occurring grunts. No male heard the same stimulus twice.

In each trial, we waited until the subject was resting away from other males (>20 m), and the male caller was out of the subject's sight and in a known location. One observer played the stimulus grunts from a loudspeaker (Bose Roommate II) using a Nomad Jukebox III. The speaker was placed between 5 and 7 m away from the subject in the direction that the caller had last been seen. The speaker was hidden from the subject by trees, bushes, or other vegetation. A second observer video-recorded the subject's responses for 1 min. Following each playback, the subject was observed for 15 min.

Because previous observations on this group suggest that males often respond to the approach of another male by leaving the area, we used this as our experimental response. We scored trials categorically based on whether or not the subject moved more than 5 m away from the speaker within the first minute following the playback of all five grunts. We reasoned that moving away from the speaker was more likely to be in response to the playback stimulus when it immediately followed the playback. Analysis of the distribution of moves over 15 min (Fig. 1) indicates that moves were common in the first minute but then dropped considerably to a low and fairly constant level, suggesting a natural cutoff point for our classification.

We conducted 16 trials between January and March 2002 and 26 trials between September 2002 and February 2003. Nine males served as subjects in the first period (including one male that disappeared halfway through the period); eight males served as subjects in the second period. Between the periods, one male disappeared, one new male entered the group, and several males, including the alpha male, changed rank. The 42 trials involved 39 unique subject/caller combinations. The three repeated subject/caller pairs were separated by at least 2 months and a change in the male's rank relationship. Trials involving the same subject were always separated by at least 3 days.

To examine the influence of the callers' testosterone on subjects' responses, our goal was to conduct experiments using high and low testosterone callers and high and low testosterone subjects. As hormonal data were not available to us at the time that the playbacks were conducted, we

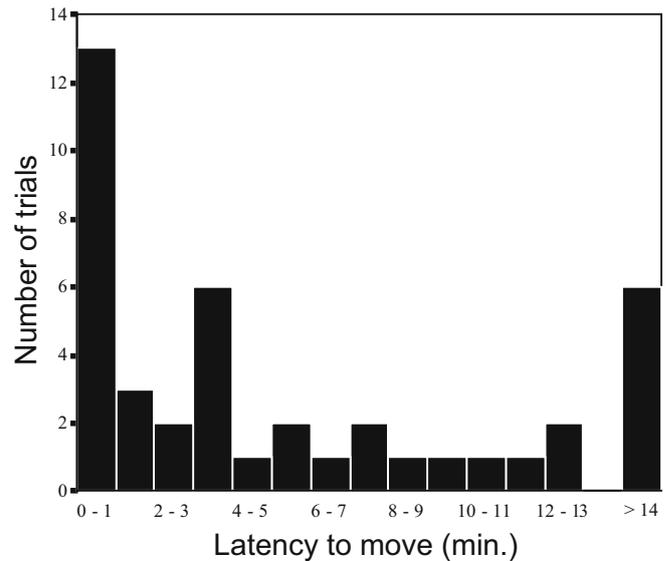


Fig. 1 The latency with which subjects moved away from the speaker in response to the simulated approach of another male

relied on rank information to approximate differences in testosterone levels. Because rank was positively correlated with testosterone concentrations, we ensured that each subject heard grunts from males of very different ranks in our effort to approximate high and low testosterone callers. In both periods, our goal was for each subject to hear grunts from up to four different individuals: the highest-ranking male, the lowest-ranking male, the male ranked immediately above him, and the male ranked immediately below him. The highest- and lowest-ranking males, by necessity, only heard two callers, and the next highest- and lowest-ranking males heard only three. When a trial involved a subject who was adjacent in rank to the highest- or lowest-ranked males, these trials were classified with the trials involving males ranked immediately above and below the subject, respectively. We completed 16 of 30 possible caller/subject combinations in period 1 and all 26 possible combinations in period 2 (Table 2). In period 1, each of nine subjects appeared in one to three trials, whereas in period 2, each of eight subjects appeared in two to four trials (Table 2).

Hormones

Fecal samples were collected in the morning from each male approximately once per week. We extracted hormones from feces in the field using a method described by Beehner and Whitten (2004), where samples are collected in a methanol/acetone solution and homogenized, filtered, and solid-phase-extracted in the field. Following shipment back to the USA, all samples were assayed for testosterone using a testosterone radioimmunoassay (RIA) kit (Equate RIA ^{125}I Testosterone Kit) previously validated for use in baboons (Beehner and Whitten 2004). All laboratory analysis was carried out at Emory University. Interassay coefficients of variation were $9.43 \pm 0.94\%$ (high control,

$N=36$) and $14.31\pm 0.11\%$ (low control, $N=36$), and the intra-assay coefficient of variation was $2.90\pm 0.78\%$ (fecal extract pool, $N=6$). All samples were run in duplicate.

In our analyses, we log-transformed testosterone values and then calculated a mean testosterone value for each male based on all samples from each period. We found considerable variation in mean testosterone (Table 2). For statistical analysis, we ranked the males according to their testosterone levels during that period (i.e., the highest testosterone male received a ranking of 1). We ranked male testosterone to control for the differences in mean testosterone between the periods and to enable data to be pooled across the two periods while simultaneously preserving information on the testosterone measure of each male relative to each other male at the time of the experiment. Furthermore, ranking testosterone values controls for the large variation in male testosterone within a period (particularly the high testosterone of male MG in period 1) and equalizes the contribution of each male to combined measures of testosterone used in dyadic analyses. Nevertheless, when we repeated the analyses using each male's mean testosterone for each period, the major results were the same.

To analyze dyads based on the testosterone levels of both the caller and the subject ("combined testosterone"), we summed the testosterone rankings for caller and subject. These testosterone rankings (individual or combined) were used in all statistical analyses. For descriptive purposes, individual testosterone rankings were divided at the median to categorize males as high or low testosterone (two males switched testosterone categories between the two periods). Similarly, we separated combined testosterone at the summed median to create high testosterone dyads (combined testosterone rank above the summed median, $N=15$) and low testosterone dyads (combined testosterone rank below the summed median, $N=27$).

Data analysis

For most analyses, we used the experimental dyad ($N=42$) as the unit of analysis. We recognize that, because the same male appeared in multiple dyads, the dyads were not completely independent. However, our aim was not to analyze individual behaviors but rather interactions between pairs of males, and each dyad was represented by a

unique combination of male testosterone levels and rank relationships (the two independent variables of interest). To compare rates of behavior across rank relationships, we used ANOVA (all statistical procedures are from Sokal and Rohlf 1995). To analyze the relationship between male testosterone and behavior, we used Spearman's correlations. To assess the relationship between male testosterone and aggression after controlling for approach rate, we used partial correlation. We separated the contributions that different predictor variables made to aggression rates using stepwise linear regression. We conducted a forward conditional binary logistic regression with maximum likelihood estimators to determine whether the probability of a move was related to the predictor variables listed in Table 1. Finally, we used binary logistic regression analysis to further explore the variables that affected males' responses to the playback experiments.

Results

Male testosterone and rank changes

To confirm that male testosterone was, indeed, related to rank changes, we examined rank changes between the two periods for the seven males that were observed during both periods. By the start of the second period, the three high testosterone males in the first period had gained a total of six dominance rank positions (1, 2, and 3 positions), whereas the four low testosterone males had lost seven (0, -1, -2, and -4) positions, a significant difference (ANOVA: $F_{1,5}=8.08$, $p=0.04$). Furthermore, a male's testosterone rank in the first period was significantly correlated with his change in rank between periods ($r_s=-0.86$, $p=0.01$).

Behavior

Effects of rank

Approaches between males occurred at higher rates in adjacently ranked than in disparately ranked dyads (adjacently ranked dyads: $N=21$, disparately ranked dyads: $N=21$; $F_{1,40}=11.8$, $p=0.001$). Aggression was most common among adjacently ranked dyads ($F_{1,40}=5.7$, $p=0.022$).

Table 1 Name, type, and definition of variables included in the logistic regression analysis

Name	Type	Description
Dependent variable		
Move away from speaker	Categorical (yes/no)	Did subject move away from speaker in first minute?
Independent variables		
Subject's testosterone	Ordinal	Ranked T values of subjects (highest to lowest, 1–9)
Caller's testosterone	Ordinal	Ranked T values of callers (highest to lowest, 1–9)
Combined testosterone of subject and caller	Ordinal	Ranked T values of both caller and subject (highest to lowest, 1–17)
Caller's rank above subject's rank	Categorical (yes/no)	Was caller higher than subject in dominance rank?
Caller and subject adjacent in rank	Categorical (yes/no)	Were caller and subject adjacent in rank?
Dyadic aggression rate	Continuous	Aggression rate (incid/h) between caller and subject

After controlling for the rate of approaches, adjacent and nonadjacent dyads did not differ in rates of aggression ($F_{1,40}=0.45, p=0.51$).

Effects of testosterone

Combined testosterone was significantly correlated with the approach rate within dyads ($r_s=-0.63, p<0.001, N=42$); approaches were most common between the males of high testosterone dyads. It is not the case that high testosterone males were more aggressive, as we did not find a significant relationship between individual testosterone and aggression rates ($r_s=-0.40, p=0.11, N=17$, using data for the two periods separately). Rather, combined testosterone was significantly correlated with the aggression rate within dyads ($r_s=-0.70, p<0.001, N=42$), such that dyads with high testosterone had higher aggression rates. The significant relationship in the dyadic, as opposed to the individual, analysis did not result simply because of a larger sample size; a comparison limited to 17 dyads (chosen at random) was also significant ($r_s=-0.75, p=0.001$). Furthermore, the correlation between combined testosterone and dyadic aggression rate remained significant after controlling for approach rate ($r=-0.46, p=0.003$).

Combining rank and testosterone

In a stepwise linear regression with aggression rate as the dependent variable and combined testosterone and dominance rank adjacency as predictor variables, only combined testosterone was entered, resulting in a significant regression ($r^2=0.40, p<0.001$). Including rank adjacency in the equation did not increase the adjusted r^2 . Thus, the combined testosterone of the caller and subject explained a greater proportion of the variation in rate of aggressive interactions than did the rank relationship of the two males.

Experimental responses

Overall analysis

Overall, subjects moved away from the speaker within the first minute in 13 of 42 (31%) trials (Table 2). In a forward conditional logistic regression using the predictor variables in Table 1, a model composed of one variable, combined T , produced the best fit ($\chi^2=8.36, p=0.004$; $-2 \log \text{likelihood}=43.61$). Although this overall analysis showed that only combined testosterone was a significant predictor of responses [$\beta=-0.36, p=0.011, \exp(\beta)=0.70$], our small

Table 2 Summary of experimental responses based on whether the subject moved away from the speaker in the first minute (“Yes”) or not (“No”)

Subject				Responses based on caller's dominance rank						Responses based on caller's testosterone (T)					
Name	Period	Dom. rank ^a	T mean \pm SE (ng/g) ^b	Highest		Lowest		Adjacent above		Adjacent below		High T		Low T	
				Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
MG	1	2	12.4 \pm 1.2	N/A ^c				0	1					0	1
FG	1	4	6.7 \pm 1.2			0	1	1	0			0	1	1	0
TH	1	5	6.2 \pm 1.2			0	1			1	0	1	1		
BJ	1	9	5.9 \pm 1.2	0	1	N/A				N/A				0	1
AU	1	8	5.6 \pm 1.2	0	1	N/A				0	1	0	1	0	1
RY	1	3	5.2 \pm 1.3	0	1			1	0			1	0	0	1
WA	1	7	4.4 \pm 1.2			0	1	0	1			0	1	0	1
AP	1	6	3.9 \pm 1.3	0	1	0	1			0	1	0	2	0	1
PO	1	1	3.6 \pm 1.2	N/A		1	0	N/A				1	0		
BJ	2	5	4.7 \pm 1.2	1	0	0	1	1	0	0	1	1	0	1	2
MG	2	2	4.6 \pm 1.2	N/A		0	1	0	1	0	1	0	1	0	2
AU	2	7	4.2 \pm 1.1	0	1	N/A		1	0	1	0	0	1	2	0
BG	2	1	3.8 \pm 1.1	N/A		0	1	N/A		1	0	1	0	0	1
PO	2	6	3.5 \pm 1.1	1	0	0	1	0	1	1	0	2	1	0	1
RY	2	4	3.4 \pm 1.1	1	0	0	1	0	1	1	0	2	0	0	2
TH	2	3	3.3 \pm 1.1	0	1	0	1	0	1	0	1	0	2	0	2
AP	2	8	2.6 \pm 1.2	0	1	N/A		0	1	N/A		0	2		
Total				3	7	1	10	4	7	5	5	9	13	4	16

^aDom. rank indicates the male's dominance rank at the beginning of the period (periods are described in the text)

^bTestosterone values were log-transformed before statistical analysis

^cN/A indicates trials that were not possible (e.g., the highest-ranking male could not be both subject and caller). Blanks indicate trials that were not completed

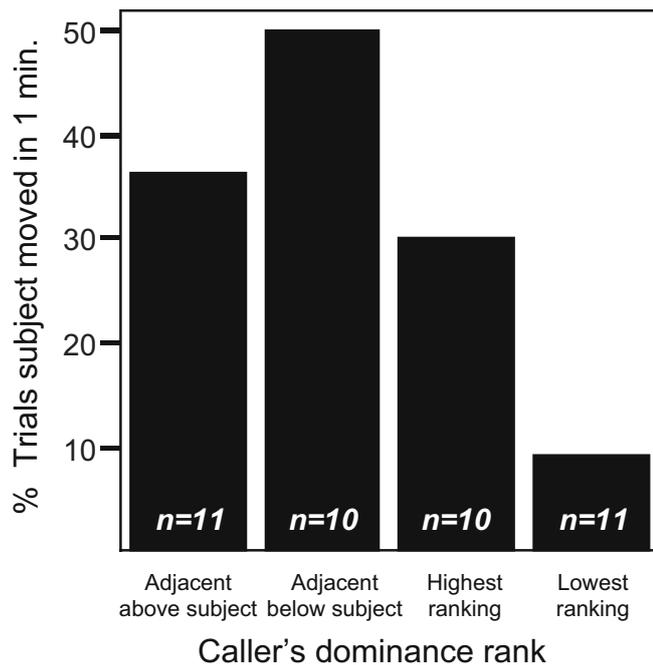


Fig. 2 Percentage of trials in which the subject moved away from the speaker in the first minute for the four subject/caller rank relationships

sample size (42 trials) may have precluded multiple variables from being simultaneously entered in the regression. Therefore, we explore other variables in further detail below.

Effects of rank

Subjects were most likely to move away from a caller that ranked immediately below them in the dominance hierarchy and least likely to move away from the lowest-ranking male (Fig. 2). Neither the rank relationship (above or below) between caller and subject (logistic regression, $\chi^2=0.11$, $p=0.738$, $-2 \log \text{likelihood}=51.86$) nor the rank adjacency ($\chi^2=2.84$, $p=0.092$, $-2 \log \text{likelihood}=49.13$) was a significant predictor of a male's response to playback stimuli. When the caller was higher-ranking than the subject, moves occurred in 7 of 21 trials (33.3%). Similarly, when the caller was lower-ranking than the subject, moves occurred in 6 of 21 trials (28.6%). Although not significant, subjects were more than twice as likely to move away from adjacently ranked callers (9 of 21 trials, 43%) than from disparately ranked callers (4 of 21 trials, 19%).

Effects of testosterone

The caller's testosterone was a significant predictor of a subject's response to playback stimuli [logistic regression, $\chi^2=6.57$, $p=0.01$, $-2 \log \text{likelihood}=45.40$; $\beta=-0.39$, $p=0.022$, $\exp(\beta)=0.68$], whereas the subject's testosterone was not ($\chi^2=1.15$, $p=0.28$, $-2 \log \text{likelihood}=50.82$). In the overall analysis, combined testosterone was the best predictor

variable, and this result is confirmed by the pattern of responses. High testosterone subjects were somewhat more likely to move (7 of 18 trials, 39%) than were low testosterone subjects (6 of 24 trials, 25%). High testosterone callers were more likely to elicit a move (9 of 22 trials, 41%) than were low testosterone callers (4 of 20 trials, 20%). These effects functioned additively, such that the highest rates of moving occurred in dyads involving both a high testosterone caller and a high testosterone subject (3 of 7 trials, 43%), and the lowest rates of moving occurred in dyads with a low testosterone caller and a low testosterone subject (0 of 9 trials, 0%).

Combining rank and testosterone

Combining rank and testosterone information shows that the majority of trials that resulted in a move involved adjacently ranked and high testosterone dyads (Fig. 3). Separating dyads by rank adjacency (into adjacently ranked and disparately ranked dyads) revealed that combined testosterone was a significant predictor of responses within adjacently ranked dyads [logistic regression, $\chi^2=4.54$, $p=0.03$, $-2 \log \text{likelihood}=24.15$; $\beta=-0.42$, $p=0.069$, $\exp(\beta)=0.66$] but not within disparately ranked dyads ($\chi^2=1.93$, $p=0.17$, $-2 \log \text{likelihood}=18.52$). Thus, the importance of male testosterone in predicting experimental responses was greatest for adjacently ranked dyads.

Combining aggression and testosterone

Dyadic aggression rate predicted experimental responses [logistic regression, $\chi^2=6.68$, $p=0.01$, $-2 \log \text{likelihood}=45.30$; $\beta=3.27$, $p=0.016$, $\exp(\beta)=26.40$]. However, a forward conditional logistic regression with combined

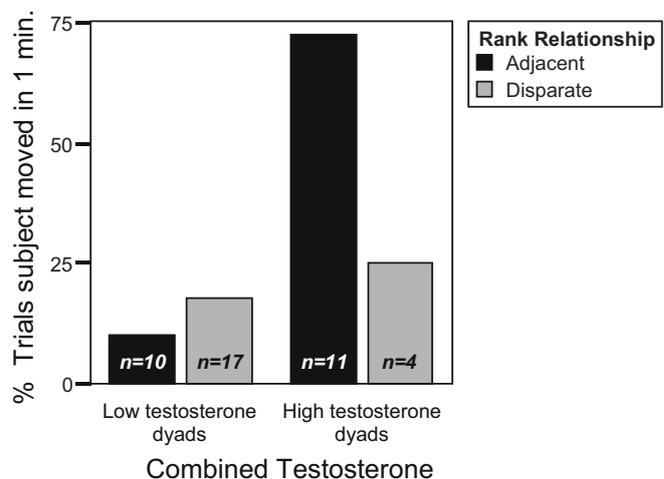


Fig. 3 Percentage of trials in which the subject moved away from the speaker in the first minute for high and low testosterone dyads, separated by caller/subject rank relationship (adjacent/nonadjacent)

testosterone and aggression rates as predictor variables entered only combined testosterone, resulting in the final significant model [$\chi^2=8.36$, $p=0.004$, $-2 \log \text{likelihood}=43.61$; $\beta=-0.36$, $p=0.011$, $\exp(\beta)=0.70$]. Combined testosterone explained more of the variation in moving than did rates of aggression.

Discussion

Both observational data and playback experiments indicated that naturally occurring variation in male testosterone predicted the outcome of male–male interactions better than dominance rank alone. Furthermore, the best predictor of both behavioral interactions and responses to playback experiments was the combined testosterone of the two interacting males. The testosterone of each male in the interaction exerted an additive effect on the behavioral outcome of dyadic encounters.

Similar to previous studies on this group (Kitchen et al. 2003), we found high rates of aggression among adjacently ranked males resulting from their high overall rate of interaction. However, the combined testosterone of both males in the dyad was a better predictor of aggression rates than the rank relationship of the two males. Dyads where both males had high testosterone exhibited higher rates of aggression than did other dyads, and this remained true after controlling for approach rates. Furthermore, the combined testosterone for a dyad was significantly related to the dyadic rate of aggression, even though individual testosterone did not predict individual rates of aggression. In a broader survey of testosterone variation in the same group, we found that male testosterone and aggression rates were only significantly correlated during periods of instability (Beehner et al. 2005). Because the experiments of the current study were mostly conducted during periods of stability, the positive relationship between testosterone and aggression found between dyads suggests that aggression occurs mainly between pairs of males that both have high testosterone, even when the hierarchy is stable.

Playback experiments provided independent support for the testosterone profiles of both males in an interaction predicting the outcome of dyadic interactions. The combined testosterone of the caller and subject was the strongest predictor of responses to playback stimuli, and the males that were most likely to move away from a simulated approach were high testosterone subjects hearing the grunts of high testosterone callers. The subject's testosterone alone was not a significant predictor of responses; therefore, it was not simply the case that high testosterone subjects were just more responsive to playback stimuli. The interactive effect of both subject's and caller's testosterone profiles emphasizes the importance of dyadic analysis when studying hormone–behavior interactions.

Whereas the observational and experimental data largely supported each other, they also had some conflicting results. In particular, the observational data indicated that approaches were actually the most frequent between the males of adjacently ranked and high testosterone dyads,

and yet, these were the males most likely to move away from the simulated approaches. Several factors may account for this difference. In playback experiments, the “approaching” male always remained out of sight, and the absence of visual contact between caller and subject may be the critical factor. Males may use visual information to predict the behavioral intent of an approaching male and move away from the area if this information is lacking. In the vast majority of our observational approaches, approaching males were within eyesight of the subject. Alternatively, certain males may be found preferentially near desirable resources (such as food or females), which may increase their rate of approaches just by chance and override their preference to avoid each other. Additionally, some males may deliberately follow or “shadow” other males as a form of harassment. There is some evidence that adjacently ranked males interact at higher rates among red deer (Appleby 1983) and macaques (Matsumura and Okamoto 1997); however, no study has examined dyadic rates of interaction in combination with testosterone levels. Further study of proximity maintenance behavior among males is needed to resolve these issues.

With respect to dominance rank, subjects were most likely to move away from males that ranked immediately below them in the hierarchy. To our surprise, subjects were not more likely to move away from higher-ranking callers, unless they ranked immediately above them. These results suggest that males were less threatened by the prospect of having a high-ranking male supplant them than they were by the prospect of confronting an adjacently ranked (and therefore possible rival) male. Whereas combined testosterone of both males was the best predictor of experimental responses, this effect was limited to adjacently ranked males. As we show elsewhere, high testosterone males are generally ascending the dominance hierarchy (Beehner et al. 2005). As such, pairs of adjacently ranked and high testosterone males are most likely to be competing for each other's dominance ranks. As demonstrated here, these were also the males that were most likely to avoid each other following the playback stimuli.

One possible explanation for male responses might be the history of aggressive interactions between the caller and subject. If two males have a history of recent aggression, then they might seek to avoid one another in the near future. However, the testosterone of both males was a better predictor of responses than the aggression rates between them. Thus, male testosterone, and not male behavior, determined whether males viewed the presence of another male as a threat, suggesting that males may be able to monitor the testosterone of other males. In fact, we were able to demonstrate that it was mainly the caller's testosterone that predicted the elicited responses, with high testosterone callers eliciting the most moves by the subjects.

These results suggest that male baboons appear to be sensitive to the testosterone of other males. Although a mechanism for monitoring testosterone directly seems unlikely, it is possible that males are attentive to behavioral or morphological correlates of testosterone. One possibility is that males are able to monitor individual variation in the

loud “wahoo” vocalizations produced by other males during aggressive displays (Kitchen et al. 2003; Fischer et al. 2004). In many vertebrates, males produce loud calls that appear to function as displays of size, condition, or fighting ability (reviewed in Andersson 1994). For example, in red deer, males decide whether to escalate or retreat depending on the specific properties of an opponent’s calls (Clutton Brock and Albon 1979). Testosterone is thought to play a role in the roaring displays of red deer, and a similar relationship may exist in baboons. Loud calls, such as roaring or wahoos, are affected by the laryngeal muscles, which, like other secondary sexual characteristics, are controlled by testosterone (Lincoln 1971; Lincoln et al. 1972). In this population of chacma baboons, male dominance rank has been shown to correlate with both testosterone (Beehner et al. 2005) and some characteristics of wahoos (bout duration, “hoo” length, etc.; Kitchen et al. 2003). Therefore, it seems possible that these same acoustic properties of wahoos may also be mediated by testosterone.

We found that considering the testosterone of both males involved in both natural and simulated interactions provided the best predictive power of the outcome. A dyadic analysis of testosterone may have wide utility not just in species where male–male competition typically involves males that are familiar with each other (as in many primates), but also in numerous species where male–male competition is mediated by signals. The current dearth of dyadic hormonal analyses of male–male interactions in such systems is somewhat surprising. Numerous studies have evaluated the testosterone of the signaler, showing that male testosterone is correlated with the production of sexually selected signals (e.g., numerous studies that address the immunocompetence handicap hypothesis; Folstad and Karter 1992), including signals involved in male–male competition (Garamszegi et al. 2004). Additionally, other studies have examined the testosterone of the responder, demonstrating that male testosterone predicts responses to a challenge by another male (e.g., Van Duyse et al. 2002). As far as we know, no study has put these two pieces together to analyze responses to a signal in terms of both the responder’s and the signaler’s testosterone. Our results suggest that such a dyadic analysis can inform our knowledge of male rival interactions.

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