

Female parity, male aggression, and the Challenge Hypothesis in wild chimpanzees

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Received: 22 March 2012 / Accepted: 24 September 2012
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Abstract The Challenge Hypothesis proposes that testosterone mediates aggression during periods of heightened conflict between males, especially episodes that have important fitness consequences. Considerable evidence from seasonally breeding species provides support for this hypothesis, but few data exist in animals that mate year-round. We tested predictions generated by the Challenge Hypothesis in chimpanzees, a non-seasonally breeding primate, through a study of individuals living in an exceptionally large community at Ngogo, Kibale National Park, Uganda. Results indicated that dominance rank had no influence on testosterone levels. Instead of rank influencing testosterone production, additional analyses revealed an important role for reproductive competition. Male chimpanzees displayed more aggression when they were in the same party as parous estrous females than when reproductively active females were unavailable. Male chimpanzees competed more intensely for mating opportunities with parous females than with nulliparas, and as a consequence, males displayed more aggression around the former than the latter. When males accompanied parous estrous females, their urinary testosterone concentrations were significantly higher than baseline concentrations. In contrast, urinary testosterone concentrations did not exceed baseline when males associated with nulliparous estrous females. These differences in testosterone levels could not be attributed to mating per se because males copulated

equally often with parous and nulliparous females. Furthermore, variation in testosterone concentrations were not due to males gathering together in large parties, as their levels in these situations did not exceed baseline. Taken together, these findings, derived from a relatively large sample of males and estrous females, replicate those from a prior study and furnish additional support for the Challenge Hypothesis. Our results suggest that the Challenge Hypothesis is likely to be broadly applicable to chimpanzees and increase our understanding of the physiological costs to males who compete for estrous females.

Keywords Chimpanzee · Testosterone · Aggression · Challenge Hypothesis

Introduction

Aggression has been a traditional focus of ethological study (Lorenz 1966), with considerable research devoted to explain its causation, development, function, and evolution (Archer 1988; Nelson 2005). Studies regarding the hormonal correlates of aggression have featured prominently in discussions of the causal mechanisms underlying the aggressive behavior of vertebrates. While testosterone plays a primary role in reproduction, it has long been known to mediate aggression, as revealed by early studies that showed castration limited the manifestation of aggression in males of several species, including humans (Baum 2002; Soma 2006). Continued research, however, has complicated this picture. While differences between the sexes and between adult and juvenile males furnish additional support for a putative relationship between testosterone and aggression (Nelson 2011), in studies where only intact adult males are considered, a connection

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between testosterone and aggression does not always exist (Hirschenhauser and Oliveira 2006).

The Challenge Hypothesis helps resolve these conflicting findings by proposing that testosterone is associated with aggression only in specific social and reproductive contexts (Wingfield et al. 1990). Because chronically elevated testosterone levels can have deleterious effects on health, its production is limited to aggression that has particularly important fitness consequences. Aggression associated with competition for mates is a prime example, especially in many seasonally breeding primate species. For example, breeding season testosterone levels of male rhesus monkeys (*Macaca mulatta*) vary positively as a function of how frequently they display aggressive behavior (Higley et al. 1996). These same rhesus males exhibit relatively high levels of aggression while competing for estrous females and display correspondingly high levels of testosterone compared with male muriquis (*Brachyteles hypoxanthus*) who compete less intensely for mating opportunities (Strier et al. 1999). Additional observations reveal that males in several species of strepsirrhines and haplorrhines predictably increase their production of testosterone during the mating season and female conception cycles (strepsirrhines: Cavigelli and Pereira 2000; Fichtel et al. 2007; Gould and Ziegler 2007; Ostner et al. 2008; haplorrhines: Lynch et al. 2002; Bales et al. 2006; Girard-Buttoz et al. 2009; Ostner et al. 2011).

The Challenge Hypothesis was originally developed to explain the relationship between male aggression and testosterone secretion in seasonally breeding birds, which display pronounced temporal variation in their aggressive behavior (Wingfield et al. 1987). More evidence is needed to assess its applicability in non-seasonally breeding taxa that lack large seasonal changes in behavior (Archer 2006; Beehner et al. 2009). In this regard, chimpanzees represent a relevant species to test the Challenge Hypothesis as they mate throughout the year and are non-seasonal breeders. Despite the absence of breeding seasons, however, they still provide an opportunity to compare male testosterone concentrations in situations involving intense male reproductive aggression and other contexts. This opportunity exists because male competition varies over time and as a function of the individual identity of females (Muller and Wrangham 2004; Muller et al. 2006). Individual females mate during discrete estrous periods where they develop sexual swellings that last about 12–13 days (Furuichi and Hashimoto 2002). Moreover, females give birth only once every 5–6 years (Goodall 1986; Boesch and Boesch-Achermann 2000; Nishida et al. 2003; Sugiyama 2004), leading to an operational sex ratio that is skewed heavily toward males (Emlen and Oring 1977). As a consequence, males compete vigorously to obtain mating and reproductive opportunities with estrous females who are available only

rarely (Boesch et al. 2006; Inoue et al. 2008; Wroblewski et al. 2009; Newton-Fisher et al. 2010). Aggression associated with reproductive competition can be extremely intense, especially for older, parous females, who have already reproduced successfully (Muller et al. 2006). Males compete less intensely for young nulliparous females, who represent less attractive mating partners (*ibid.*).

One previous study has taken advantage of the temporal variation in mating behavior by individual females and differences in male mating preferences to test the Challenge Hypothesis in chimpanzees (Muller and Wrangham 2004). There it was found that male aggression and testosterone levels increased in the presence of parous estrous females. In contrast, aggression and testosterone were not elevated when males were around nulliparous estrous females and when estrous females were absent. Because mating frequencies were the same with both parous and nulliparous females, increases in male testosterone appeared to be related to aggression associated with mate acquisition, rather than the act of mating itself. Finally, a positive relationship between male dominance rank and testosterone concentrations existed, with high-ranking males possessing higher levels than lower ranking individuals during a period of rank stability. While these results support the Challenge Hypothesis, it is unclear whether they can be applied broadly to the behavior of chimpanzees because a relatively small number of females and males were observed. Because variation is a characteristic feature of chimpanzee behavior (Wrangham et al. 1994; Boesch et al. 2002), additional data from other chimpanzee communities, including larger samples of males and estrous females, are clearly needed to evaluate the generality of these findings.

One possibility not investigated by Muller and Wrangham (2004) in their previous study was that high male testosterone levels were a byproduct of large male group size. Large groups of males gather predictably around estrous females (Mitani et al. 2002), and in these, elevated rates of male aggression typically occur (Muller 2002). As a consequence, party size is a likely confound that requires examination and possible control. The presence of alpha males represents another potential confound that warrants investigation because these males are responsible for a disproportionate number of charging displays observed each day (Muller 2002). As a result, male testosterone concentrations might increase simply due to the heightened conflict that surrounds alpha males.

Chimpanzees at Ngogo, Kibale National Park, Uganda, live in an extremely large community containing over 150 individuals and many adult males and females. The unusually large size of this community creates ample opportunities to observe male–male aggression and matings between males and females that differ in their

reproductive states and parity. In this study we extend the findings of previous work by examining whether associations exist between male aggression and testosterone during interactions with females who differ in parity. Our observations of male chimpanzees, as they compete for matings with multiple parous estrous females over several cycles, provide empirically sufficient samples to test the Challenge Hypothesis in this non-seasonally breeding primate. Specifically, we test the prediction that the presence of parous estrous females leads to heightened rates of male–male aggression, which elevates male chimpanzee testosterone levels above their baseline concentrations. In contrast, we do not expect a similar rise in urinary testosterone levels in males when they associate with nulliparous females. Although males mate nulliparas, these females do not generate high levels of male competition.

Methods

Study site and subjects

We observed chimpanzees at Ngogo, Kibale National Park, Uganda. The 30 km² study area lies at an altitude of about 1,400 m above sea level and consists primarily of mature, evergreen tropical forest (Struhsaker 1997). The Ngogo chimpanzees have been under observation since 1995 (Mitani 2009). As a result, they are well habituated to human presence and can be followed easily and observed closely. The Ngogo chimpanzee community is the largest described in the wild thus far and consisted of approximately 150 individuals at the time of study, including 27 adult males and 19 cycling females. The latter comprised eight parous females and 11 nulliparas.

Behavioral observations

MES conducted behavioral observations over 14 months during three field seasons, May–July 2006, May–November 2007, and February–May 2008. She observed 27 adult males for 1,378 h, recording their identities and noting whether they followed estrous females. For purposes of the following analyses, we defined large groups of males to form on days that the party included more than half of all of the adult males in the community, i.e. ≥ 14 males. Although chimpanzees live in fission–fusion communities, whose members split apart and come together throughout the day, at Ngogo, large parties of males are fairly cohesive as they predictably form during periods of high food availability and whenever several females come into estrus simultaneously (Mitani et al. 2002).

Male aggression was scored by recording charging displays directed at specific individuals. These displays occur

frequently and are particularly conspicuous and easy to observe and record. As a result, they were recorded ad libitum. In prior research, displays have been used to assay male aggression as they correlate positively with male dominance rank, with high-ranking males engaging in this behavior often (Muller 2002). During charges, males become piloerect, shake and break trees and foliage, pull branches in their wake, and run quickly in a straight line through the forest on the ground. Charges typically elicit screams from recipients at whom charges are directed and nearby bystanders; they occasionally result in displaying individuals physically attacking others. We calculated rates of aggression for each male by counting the number of times individuals charged relative to the number of hours they were observed.

We combined observations of charges, where the recipient was identified, with pant grunts to construct a dominance rank matrix. Pant grunts are vocal signals of submission given by low-ranking chimpanzees to high-ranking chimpanzees (Bygott 1979; Hayaki et al. 1989). We ordered individuals from top to bottom so as to minimize the number of reversals in the matrix. Only 12 reversals occurred in 872 interactions, producing a linearity index of 98.6 %.

Female chimpanzees display sexual swellings when they are in estrus. We considered only those females that exhibited full maximal swellings to be in estrus (Wallis 1992). While observing males during focal sampling sessions, MES recorded male copulations with females ad libitum. Copulations were recorded when males mounted estrous females followed by intromission and pelvic thrusting. We counted the number of times males mated females per day to determine male copulation rates.

Hormone analyses

Urine collection

Testosterone was assayed using urine collected non-invasively from male chimpanzee subjects. We analyzed samples from 26 of the 27 adult males (mean = 108 samples/male, SD = 42). Results of assays from one male (pi in Table 1) were inadvertently lost while these data were being transferred to the computer and were thus unavailable for subsequent analysis. Samples were collected from males while they were in the same party and associating with estrous females, including those who were parous (mean = 7.5 samples/male, SD = 3.5) and nulliparous (mean = 6.8 samples/male, SD = 4.3). As noted above, these samples were obtained only when females possessed full, maximal swellings. The eight parous females were observed during 12 cycles (mean = 1.5 cycles/female; SD = 0.8, range 1–3), while the 11

Table 1 Male dominance rank, age, aggression, and testosterone levels

Male	Rank	Age class	Aggression (charges/h)	Baseline testosterone (ng/mg Cr)
bt	1	Prime	1.04	69
ho	2	Prime	0.83	73
mi	3	Prime	0.97	113
bs	4	Prime	0.55	80
cr	5	Prime	0.58	71
mt	6	Prime	0.28	80
wb	7	Young	0.61	104
lo	8	Prime/old	0.36	99
br	9	Prime	0.38	85
mo	10	Prime/old	0.26	94
hr	11	Prime/old	0.11	88
mw	12	Old	0.13	89
bg	13	Prime	0.14	109
do	14	Old	0.05	77
mg	15	Prime/old	0.18	132
ga	16	Prime	0.57	123
pk	17	Old	0.11	95
or	18	Prime	0.24	119
ro	19	Young	0.29	107
pi	20	Old	0.20	No data
bf	21	Old	0.06	77
rh	22	Young	0.29	112
dx	23	Prime	0.04	82
ri	24	Young	0.10	112
ta	25	Young	0.23	111
gz	26	Young	0.12	62
di	27	Prime	0.13	112

nulliparas were observed during 20 cycles (mean = 1.8 cycles/female; SD = 0.8, range 1–3).

We collected 103 samples from 25 males on 25 days when more than half of all of the males were present in the daily party (mean = 4.13 samples/male, SD = 1.9). For these, samples were not collected on days males hunted, patrolled the boundary of their territory, and followed estrous females, as these behaviors are known or hypothesized to affect testosterone production (Sobolewski et al. 2012). Finally, the alpha male was not seen on 20 days that we observed parous females. We collected samples from males on these days (mean = 2.4 samples/male, SD = 1.1) to compare with others collected from them when both the alpha male and parous estrous females were present (mean = 6.0 samples/male, SD = 3.3). We also compared the former samples to their baseline levels.

Urine was collected from leaf litter on the ground with a pipette. Samples were occasionally caught in a plastic bag when males urinated overhead in trees. Samples cross-

contaminated with feces or blood were discarded. Those that were retained were placed in 5 ml tubes and labeled with the male's name, date, and time. Samples were frozen within 12 h after collection in the field and shipped to the United States on ice using certified transport equipment. All hormone analyses were conducted by MES working under the supervision of JLB at the Smithsonian Conservation Biology Institute, Front Royal, Virginia.

Testosterone analysis

We analyzed testosterone in unprocessed urine using a single antibody enzyme immunoassay (EIA) provided by Coralie Munro from the University of California, Davis (Kersey et al. 2010). Microtitre plates (96 well; Nunc-Immuno, Maxisorp) were coated with a polyclonal testosterone antiserum (R 156/157; 50 μ l per well; diluted 1:7,500 in coating buffer, 0.05 M NaHCO₃, pH 9.6) and allowed to set for 12–18 h at 4 °C. Unabsorbed antiserum was removed with wash solution (0.149 M NaCl, 0.5 % Tween 20). Testosterone standards (50 μ l, range 2.3–600 pg/well, diluted in assay buffer, 0.1 M NaPO₄, 0.149 M NaCl, 0.1 % bovine serum albumin, pH 7.0) in triplicate and samples (50 μ l) in duplicate were then added to the wells, followed immediately with testosterone-horseradish peroxidase (50 μ l, 1:80,000 dilution in assay buffer). Following incubation at room temperature for 2 h, plates were washed five times before 100 μ l substrate buffer (0.4 mM 2, 2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, 1.6 mM H₂O₂, 0.05 M citrate, pH 4.0) was added to each well. After incubation for 30–60 min, the absorbance was measured at 405 nm (540 reference filter) when the optical density in the total binding wells reached ~1.0. Intra-assay and inter-assay coefficients of variation (CV) for the internal controls (n = 124 assays) were below 10 and 15 %, with 9.34 % (mean binding, 23.6 %) and 11.89 % (mean binding, 69.5 %) for the high and low samples, respectively, while the CV for the 50 % binding point of the standard curve was 6.36 %. The assay was validated for chimpanzee urine by demonstrating that serial dilutions of pooled urine samples produced displacement curves parallel to those of the testosterone standard curve and that there was significant recovery (>90 %) of exogenous testosterone added to urine before analysis. We achieved high levels of recovery and accuracy of measurement and maintained strict controls for individual variation, sample quality, and assay variance.

Creatinine analysis

We indexed all urine samples for creatinine (Cr) to account for variations in water content (Taussky 1954). Creatinine is a by-product of muscle breakdown and under normal

conditions is excreted at a constant rate per individual. The creatinine concentrations in urine were determined using a Jaffe reaction. Samples with creatinine concentrations below 0.01 ng/ml were considered too dilute and excluded from analysis; this involved less than 5 % of all samples. Hormone concentrations were divided by creatinine concentrations and expressed as the concentration of testosterone (ng)/per mg Cr.

Statistical analyses

We determined baseline concentrations of testosterone for each male through an iterative process described by Moreira et al. (2001), with minor modifications. This technique has been used successfully in the past to identify baseline values from biologically relevant peaks (Moreira et al. 2001). We began by computing the mean value of all samples for each male. We then removed values that were above 2 standard deviations from the mean. We iterated this procedure until no values outside 2 standard deviations of the mean remained. We employed the resulting mean as the statistical baseline value for that male. We excluded urine samples collected before 0900 h from analyses to control for diurnal variation in testosterone production (Muller and Wrangham 2004).

Testosterone varies considerably among individual males (Kempnaers et al. 2008). Therefore, we used each male as his own control for statistical purposes. Specifically, we employed a non-parametric Wilcoxon matched-pairs signed-ranks test to determine if male testosterone concentrations assayed on days when estrous females were present differed from their baseline levels. We also used Wilcoxon tests to investigate whether male aggression and copulation rates varied as a function of female parity. For all of these tests, we separated estrous females into two categories, those who had given birth, i.e. parous females, and those who had not, i.e. nulliparous females. In two additional Wilcoxon tests we examined whether urinary testosterone levels were elevated on days males gathered in large parties compared to their baseline levels and whether male testosterone concentrations differed on days they followed parous estrous females in the presence and absence of the alpha male. Finally, we utilized the matched-pairs design to examine whether male testosterone levels were high on days they followed parous estrous females in the absence of the alpha male. We did so by comparing these to their baseline concentrations.

Results

Table 1 shows individual males, their dominance ranks, relative ages, aggression rates, and baseline testosterone

concentrations. In accord with previous studies (e.g. Muller 2002), high-ranking males at Ngogo displayed higher rates of aggression than did lower ranking males (Spearman $r = 0.70$, $p < 0.001$, $N = 27$). High-ranking males, however, did not possess high levels of testosterone. There was no relationship between male dominance rank and baseline testosterone levels (Spearman $r = 0.34$, $p > 0.05$, $N = 26$).

To test the Challenge Hypothesis, we examined the relationship between testosterone and aggression as males sought reproductive opportunities with females. Male chimpanzees compete for estrous females, as manifest by their aggressive behavior. Competition, however, varied as a function of female parity. Males displayed more aggression around parous estrous females than they did when no estrous females were present (Wilcoxon test: $Z = 4.55$, $p < 0.001$, $N = 27$ males; Fig. 1). In contrast, male aggression was not elevated in the presence of nulliparous estrous females compared to when estrous females were absent (Wilcoxon test: $Z = 1.33$, $p > 0.15$, $N = 27$ males; Fig. 1). As a consequence, male chimpanzees showed more aggression in the presence of parous estrous females than they did in the presence of nulliparous estrous females (Wilcoxon test: $Z = 4.13$, $p < 0.001$, $N = 27$ males; Fig. 1). Despite these heightened rates of aggression around parous females, male chimpanzees mated these females as frequently as they did nulliparous females (Wilcoxon test: $Z = 1.37$, $p > 0.10$, $N = 27$; Fig. 2).

The higher rates of aggression stimulated by the presence of parous females in estrus were related to male testosterone levels in predictable ways. Male testosterone was

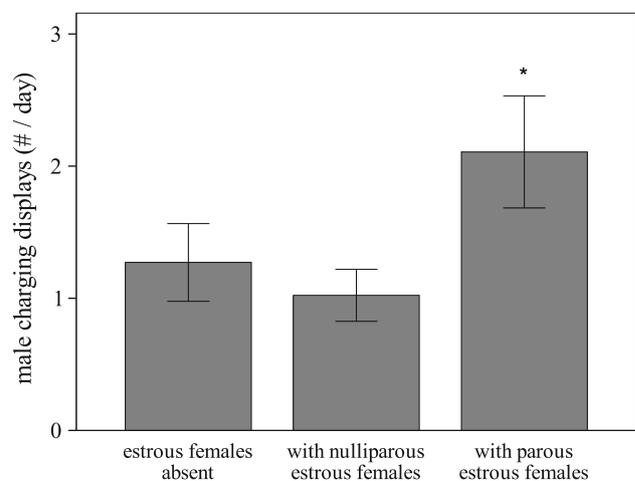


Fig. 1 Male chimpanzee aggression varies as a function of the presence and absence of estrous females. Rates of aggression, assayed by charging displays, when estrous females, either nulliparous or parous individuals, were present are compared with those when estrous females were absent. Displayed are means of individual male means ± 1 SE. $N = 27$ males. * $p < 0.001$ for comparisons between parous estrous females and estrous females absent and between parous estrous females and nulliparous estrous females

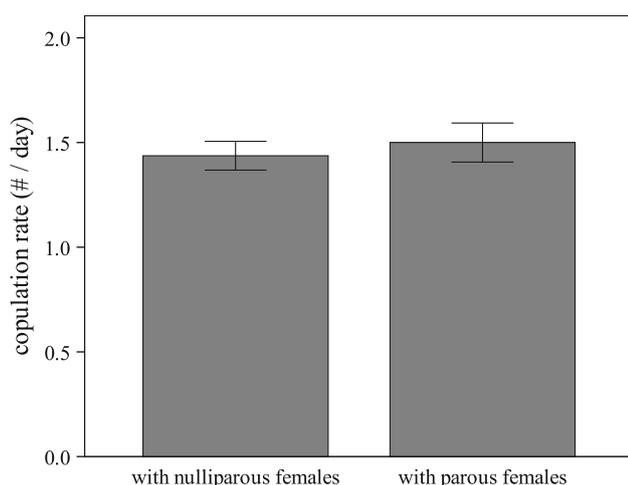


Fig. 2 Female parity does not affect male chimpanzee copulation rates. Male copulation rates with nulliparous and parous females are shown. Displayed are means of individual male means \pm 1 SE. $N = 27$ males

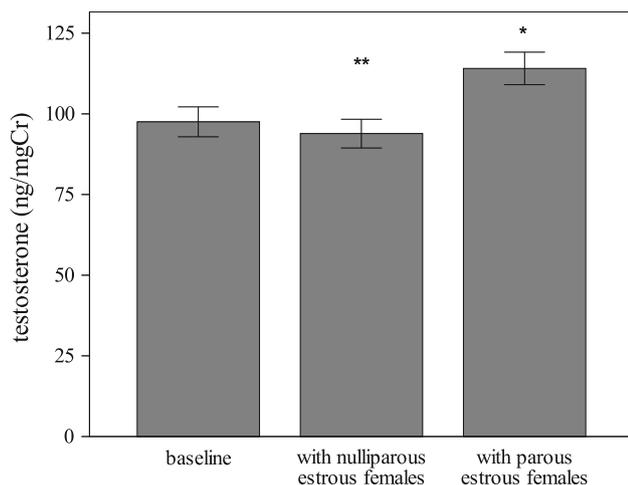


Fig. 3 Male chimpanzee testosterone levels vary as a function of female parity. Male baseline testosterone concentrations are compared with their concentrations when they accompanied nulliparous estrous females and parous estrous females. Displayed are means of individual male means \pm 1 SE. $N = 26$ males. * $p < 0.001$ for the comparison of male baseline concentrations and those when they followed parous estrous females. ** $p < 0.01$ for the comparison between nulliparous estrous females and parous estrous females

elevated above baseline levels on days when parous females were present (Wilcoxon test: $Z = 4.32$, $p < 0.01$, $N = 26$; Fig. 3). In contrast, testosterone concentrations did not differ from baseline on days that males accompanied nulliparous females in estrus (Wilcoxon test: $Z = 0.70$, $p > 0.45$, $N = 26$; Fig. 3). As a result, male urinary testosterone concentrations were also higher when they followed parous estrous females compared to when they accompanied nulliparous estrous females (Wilcoxon test: $Z = 3.98$, $p < 0.001$, $N = 26$; Fig. 3).

The rise in testosterone experienced by males while following parous estrous females could not be attributed to the fact that they formed large parties on these days; male testosterone levels did not exceed baseline on days that they formed large parties in the absence of estrous females (Wilcoxon test: $Z = 1.01$, $p > 0.30$, $N = 25$). In addition, urinary testosterone concentrations of males who followed parous estrous females did not vary as a function of the presence or absence of the alpha male (Wilcoxon test: $Z = 0.03$, $p > 0.95$, $N = 23$). Male testosterone levels were also high on days that they followed parous estrous females in the absence of the alpha male; their values on these days consistently exceeded baseline (Wilcoxon test: $Z = 2.03$, $p < 0.05$, $N = 24$).

Discussion

Prior research has revealed that dominance rank affects testosterone in male chimpanzees. High-ranking males display relatively high testosterone levels compared with lower ranking individuals (Muehlenbein et al. 2004; Muller and Wrangham 2004). In contrast, there was no relationship between these two variables among males in this study. Age is a likely factor that might account for the failure to document a relationship between male rank and testosterone at Ngogo (Table 1). Testosterone decreases with age in human males (Bribiescas 2001), and some high and middle ranking male chimpanzees in our sample were old. Further research will be necessary to investigate this possibility.

Additional analyses indicate that male chimpanzees at Ngogo compete for mating opportunities for females, but that they do so primarily for parous females. Male aggression increased on days these females were present compared to days when males followed nulliparous females and on days when estrous females were absent. Testosterone is associated with the heightened aggression over more attractive parous females, as concentrations in the presence of these females were higher than baseline levels and those when males accompanied nulliparous females. The elevation in male testosterone is directly attributable to reproductive competition for females and not associated with increased mating activity per se because males copulated with parous females as often as they did with nulliparous females. Large group size cannot explain these differences because urinary testosterone concentrations did not vary from baseline on days when males formed large parties in the absence of estrous females. In addition, the elevated testosterone levels that we documented in males as they competed for parous estrous females could not be attributed to the presence of the alpha male; male testosterone concentrations did not

differ in the presence or absence of the alpha, and they continued to exceed baseline when parous estrous females were present and he was not there. Taken together, our results are consistent with the Challenge Hypothesis, which proposes that the production of testosterone will be associated with aggression directly related to reproduction.

The fact that testosterone did not increase in the presence of estrous females, who have not yet given birth, may be surprising. Nulliparas, however, are invariably recent immigrants who experience a prolonged period of infertility after moving into their new communities. As a result, these females cycle consistently, sometimes for several years, before giving birth for the first time (Goodall 1986; Boesch and Boesch-Achermann 2000; Nishida et al. 2003; Sugiyama 2004). Because many cycles do not represent legitimate reproductive opportunities, males do not compete for nulliparas as vigorously as they do for older, parous females (Muller et al. 2006).

Our findings are consistent with the only previous study that has tested the Challenge Hypothesis in wild chimpanzees. In the Kanyawara chimpanzee community, also located in the Kibale National Park, Muller and Wrangham (2004) found that males there selectively increased their rates of aggression in the presence of parous estrous females. Elevated testosterone levels were also displayed by males who accompanied parous estrous females compared with those who followed nulliparous estrous females or those who were alone in the absence of any estrous females. Because males failed to mate parous females more frequently than they did nulliparas, Muller and Wrangham (2004) concluded, like us, that the increase in testosterone could be attributed to heightened aggression around parous estrous females in general, rather than mating activity alone. One limitation of this prior study was that data were collected from a relatively small number of males ($N = 8-11$), nulliparous females ($N = 2$), and parous females ($N = 3$), with observations focused on a single parous estrous female during a single estrous cycle. These small samples raise the possibility that the findings of this previous study cannot be generalized to chimpanzees in other communities and as a whole. The results presented here were based on much larger samples of males ($N = 26$), parous females ($N = 8$), nulliparous females ($N = 11$), and reproductive cycles of females (parous females: $N = 12$; nulliparous: $N = 20$). Our findings support those derived from studies of other vertebrates (Wingfield 2005) and suggest that the Challenge Hypothesis is likely to be broadly applicable to chimpanzees.

This study also adds to our understanding of how social interactions influence male testosterone in a species that does not typically display pronounced seasonal variation in aggressive behavior. Most previous tests of the Challenge Hypothesis have been conducted with seasonally breeding

taxa that show large fluctuations in aggressive behavior over time (reviews in Oliveira 2004; Hirschenhauser and Oliveira 2006). Few data exist regarding non-seasonally breeding species because such changes in aggression are less obvious, making it difficult to relate changes in testosterone to changes in behavior. The Challenge Hypothesis, however, proposes that social interactions that have particularly significant fitness consequences will lead to transient changes in androgen levels irrespective of the time of year, and as a consequence, it should apply to seasonally-breeding and non-seasonally breeding species alike. Because many primates mate year-round, they provide a model taxon to investigate in this regard. Obtaining additional data from these animals represents an important area for future research.

Acknowledgments Our fieldwork was sponsored by the Uganda Wildlife Authority, Uganda National Council for Science and Technology, and Makerere University Biological Field Station. We thank A. Magoba, G. Mbabazi, L. Ndagizi, and A. Tumusiime for field assistance, J. Lwanga for logistic aid, and J. Beehner, K. Hosaka, and an anonymous reviewer for comments on the manuscript. N. Presley and S. Putnam provided help in the laboratory with hormone analyses. This research was supported by grants from the L.S.B. Leakey Foundation and the U.S. National Science Foundation (IOB-0516644 and BCS-0752637). This research was conducted in compliance with all legal requirements of Uganda and was approved by the University Committee on the Use and Care of Animals (UCUCA) at the University of Michigan (Protocol #1092).

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