Mating Season Aggression and Fecal Testosterone Levels in Male Ring-Tailed Lemurs (Lemur catta)

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The challenge hypothesis (J. C. Wingfield, R. E. Hegner, B. G. Ball, and A. M. Duffy, 1990, Am. Nat. 136, 829–846) proposes that in birds, reptiles, and fish, “the frequency or intensity of reproductive aggression as an effect of T[estosterone] is strongest in situations of social instability, such as during the formation of dominance relationships, the establishment of territorial boundaries, or challenges by a conspecific male for a territory or access to mates” (p. 833). To determine the extension of this hypothesis to mammalian species, we tested predictions of the hypothesis in a nonpaternal, seasonal breeding, prosimian primate (ring-tailed lemurs, Lemur catta). Semi-free-ranging males were studied during periods of social stability (premating period) and instability (mating period). The annual mating season consists of several days during which males fight for access to promiscuous group females as each individually becomes sexually receptive for 1 day. Male rates of aggression were compared to fecal testosterone levels within premating and mating periods. In the premating period male rate of aggression was not significantly correlated with testosterone level. By contrast, during the mating season testosterone and aggression levels were positively and significantly correlated. However, on days just preceding estrus, male rate of aggression was not significantly correlated with testosterone, but on days of estrus, when aggressive challenges peaked sharply, testosterone and aggression were highly positively correlated. These results suggest that the challenge hypothesis applies to mammals as well as to birds, reptiles, and fish. In addition, elevations in testosterone were tightly circumscribed around days of estrus, suggesting a compromise between costs and benefits of elevated testosterone levels.

Key Words: challenge hypothesis; fecal testosterone; aggression; social instability; lemurs; primates.

Wingfield and colleagues (1990) developed the challenge hypothesis to account for the variability often found in the relationship between androgens and aggression in adult male animals. Based on a review of studies with birds, reptiles, and fish, these researchers hypothesized that androgens and aggression are most closely associated with one another in the context of intense reproductive aggression, for example, during establishment of breeding territories. In particular, they proposed that testosterone levels would be most closely associated with aggression in the context of social instability, as when males challenge one another for access to females. The advantage of this relationship during breeding seasons could be that testosterone helps to support competition over prolonged periods of time (Wingfield, 1994). Studies supporting the hypothesis have been conducted primarily with birds, reptiles, and fish (e.g., Pankhurst and Barnett, 1993; Johnsen, 1998; Klukowski and Nelson, 1998). Few studies have tested the hypothesis with mammals (Creel, Wildt, and Monfort, 1993) and none have tested it with primates, an order in which social and cognitive processes are thought to mask potential relationships between androgens and aggressive behavior (e.g., Dixson, 1980).

The challenge hypothesis, if applicable to primates, would explain why some researchers studying primates in unstable or newly formed social groups have found significant positive correlations between circu-

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lating levels of testosterone and rates of male aggression (e.g., Bernstein, Rose, Gordon, and Grady, 1979; Alberts, Sapolsky, and Altmann, 1992) while others studying stable well-established groups have not found such reliable relationships (e.g., Steklis, Raleigh, Kling, and Tachiki, 1986; Nieuwenhuijzen, de Neef, van der Werff, Bosch, and Slob, 1987; cf. Sapolsky, 1982; Steklis, Brammer, Raleigh, and McGuire, 1985). In this paper we examine whether Wingfield's challenge hypothesis applies to a prosimian primate (ring-tailed lemur, Lemur catta) during the mating season, when social relationships between males are characteristically unstable and male-male challenges frequent.

Ring-tailed lemurs are a Malagasy, diurnal, non-monogamous, group-living, seasonally breeding prosimian primate in which males do not display parental care and individual females are usually sexually receptive on only 1 day each year (Jolly, 1966; Evans and Goy, 1968; van Horn and Resko, 1977). Estrous cycles are synchronized such that all females within a group usually conceive within a 2-week period. Within that period, however, female cycles are asynchronous such that usually only one female experiences estrus on any given day (Evans and Goy, 1968; Pereira, 1991). This reproductive system, together with female social dominance over males and high female promiscuity, leads to chaotic, agonistic male reproductive competition, entailing frequent social challenges and series of reversals in dominance relations (Jolly, 1966; Koyama, 1988; Sauther, 1991; Pereira and Weiss, 1991).

The contrast between the socially stable premating period and the unstable mating season in this lemurid primate is analogous to the sharp contrast between prebreeding and breeding seasons for many birds. Consequently, this species is an ideal candidate for testing predictions of Wingfield et al.’s (1990) challenge hypothesis. Naturalistic tests can be conducted by comparing the relationship between testosterone and aggression during periods of social stability (prematting) and instability (mating).

We studied semi-free-ranging ring-tailed lemurs at the Duke University Primate Center (DUPC) to test two predictions: (1) that rates of male aggression and testosterone level are more closely correlated during the socially unstable mating period than during the socially stable premating period, and (2) that within the mating season, individual rate of aggression and testosterone level are more closely correlated on days of estrus, when male social relationships are extremely unstable, than on days when no female is in estrus and male-male social relationships are relatively stable. To collect frequent and noninvasive endocrine measures, male testosterone levels were assessed from fecal samples.

**METHODS**

**Subjects**

Two groups of ring-tailed lemurs living adjacent to one another in a large forest enclosure at the DUPC were studied from mid-September to late-December 1994. One group (Lc1 Group) consisted of four adult females, five adult males, and six subadults, and the second group (Lc2 Group) comprised five adult females, eight adult males, and five subadults. The forest enclosure is 9 hectares in size and had been the primary housing for these groups of ring-tailed lemurs since 1983 and 1987, respectively (Pereira and Izard, 1989; Pereira, 1993a). The ring-tails make full use of the range and their daily activity budgets are nearly identical to those of free-ranging ring-tailed lemurs in Madagascar (Pereira, 1996; Cavigelli, 1998). Human intervention in their daily routine was limited to the distribution of a daily food supplement (commercially produced monkey chow), removal and/or treatment of injured or unhealthy animals, and non-invasive research protocols (e.g., behavioral observations). During the study, the ring-tails were also involved in a protocol that involved luring them into holding cages within the forest enclosure on a bi-weekly basis to collect blood samples and morphological measures. Except for our comparison of testosterone levels in blood and feces (see below), data for the current research were not collected on these days. All procedures used in the present study were approved by the Duke University Institutional Animal Care and Use Committee.

**Testosterone Measurement**

Testosterone levels were assessed from concentrations of this hormone in male feces. This method allowed for frequent hormonal measures without disrupting ongoing behavioral processes. Fecal testosterone extraction and assay methods are described below. Individual fecal and serum testosterone levels were compared to test the applicability of the fecal steroid measure for male ring-tailed lemurs.

To compare fecal and serum testosterone measures, blood and fecal samples were collected from nine males during mid-September and mid-October. Blood
samples were collected as follows: on two separate occasions, at approximately 0900 h, lemurs were enticed with fruit into holding cages and then sequentially caught and bled from the femoral vein. All samples were collected between 0900 and 1100 h. Blood samples were collected into Vacutainer SST tubes (Becton-Dickinson, Franklin Lakes, NJ), allowed to clot for over 1 h, and then centrifuged at 1200g for 20 min. Resulting supernatants were decanted and stored at −80°C until analyzed. Fecal samples produced during the blood collection process were collected into Whirl-Pak bags and within 3 h of collection stored at −20°C until analyzed.

Testosterone was extracted from fecal samples using the extraction procedure described in Wasser, Monfort, Southers, and Wildt (1994), and using 95% ethanol in the boiling step. Extracts and serum samples were analyzed for testosterone concentrations using the microradioimmunoassay method described in Beall, Worthman, Stallings, Strohl, Brittenham, and Barragan (1992) and Brockman, Whitten, Richard, and Schneider (1998). The assay involved modifications to a commercially available RIA kit designed for human serum or plasma testosterone analysis (Equate Testosterone RIA kit, Binax Inc., Portland, ME). The assay had a sensitivity of 1.0 ng/dl and the testosterone antiserum was known to cross-react 1.7% with dihydrotestosterone, 0.8% with 5α-androstane-3α, 17β-diol, and less than 0.06% with androstanedione, estradiol, and progesterone. To prepare samples for assay analysis, fecal extracts were dried and reconstituted in a buffer solution made of 0.1% gelatin phosphate buffered saline (pH 7.4), which was also used to dilute kit standards, antibodies, controls, and serum samples. Kit standards, controls, fecal extracts, and serum samples were diluted 1:10 and run in duplicate in the assays, the first antibody was diluted 1:4 and the second antibody was diluted 1:2. The intraassay coefficient of variance was 5.9% (n = 3) and interassay coefficients of variance for a high and low control were 4.6% (n = 4) and 6.6% (n = 4), respectively. Testosterone concentrations are expressed as nanogram per gram of dry feces.

Aggression Measurement

Behavioral data and fecal samples were collected from the two groups during the socially stable period prior to the mating season (September through late October) and during the socially unstable mating season (late October through December). Observations were alternated daily between the two groups and were timed to occur during the lemurs’ afternoon period of activity. The mating season was defined as beginning on the first observed estrous day (cf. Pereira, 1991). Behavioral data were collected daily for approximately 1.2 h between 14:00 and 18:00, except on days of estrus, when behavioral data were collected for at least 4 h. A total of 73.3 h of behavioral data were collected: 15.6 h during the premating period and 57.7 h during the mating season.

To estimate males’ rates of initiating aggression, receiving aggression, and sexual clamping, agonistic interactions and sexual behavior were recorded using all-occurrence sampling (Altmann, 1974) and agonistic definitions adopted from Pereira and Kappeler (1997). All aggressive and submissive acts among group members were recorded. Aggressive acts included bite, lunge, chase, cuff, and grab. Submissive acts included jump away, flee, cower, and a high-pitch submissive vocalization, spat call, known to be the formal signal of subordination in this species (Pereira and Kappeler, 1997). Intergroup agonistic interactions were also recorded, but these occurred primarily between females; males seldom engaged in extended aggressive interaction between groups. Data were also collected on male sexual behavior (i.e., frequency of clasping receptive females) because male aggression may correlate with frequency of sexual behavior and sexual behavior or proximity to females may affect male testosterone levels. Successive agonistic and sexual acts were considered independent if 5 s elapsed between termination of the first and initiation of the second act.

Relation between Fecal Testosterone Levels and Aggression

Mean individual testosterone levels were compared to mean rate of aggression within and among several periods of the study. We tested the prediction that testosterone and aggression are more closely related during mating season than during premating season by comparing mean fecal testosterone measures for each of 10 males to their mean aggression rates during the two seasons. (Three males were not included because they produced fewer than 3 fecal samples during observations across the two seasons.) From each of the 10 males, a range of 1 to 6 fecal samples was collected during the premating season (X ± SEM: 3.0 ± 0.5) and 4 to 17 samples during the mating season (X ± SEM: 8.6 ± 1.3).

The second comparison between testosterone and
aggression was made within the mating season, with the 6 consecutive days encompassing a given female’s estrous day classified as either: (1) preestrus, (2) estrus, or (3) postestrus. Preestrus comprised the 2 days just prior to estrus, estrus comprised the day of and the day following estrus, and postestrus comprised the second and third day following estrus. We tested the prediction that testosterone and aggression are more closely related during estrus (social instability) than during pre- or postestrus (social stability). In the larger group, we observed five estrous periods and collected enough samples from eight males to calculate individual mean testosterone and aggression levels on each of the 2-day blocks described above. For each male, 0 to 3 samples were collected on preestrous days (X ± SEM: 1.4 ± 0.3), 2 to 6 samples on estrous days (X ± SEM: 3.8 ± 0.5), and 1 to 3 samples on postestrous days (X ± SEM: 2.1 ± 0.3). Variability in fecal testosterone within subjects during nonestrous mating season days was low, such that limited sample sizes still provided an informative data base (mean testosterone SEM for eight males from whom samples were collected for 3 or more nonestrous days was 13.7 ng/g). Mean individual fecal testosterone levels were also examined in relation to individual rates of clasping behavior.

In the smaller group, only 2 estrous days were observed and only three adult males exhibited sexual behavior. Because these three males engaged in several aggressive challenges and dominance rank reversals, their levels of testosterone and aggression were followed as a case study of how levels of testosterone relate to aggressive challenge and withdrawal during days of female receptivity.

**Analyses**

Pearson product moment correlation coefficients were calculated to compare individual serum and fecal testosterone levels and to relate individuals’ mean fecal testosterone values and rates of aggression. In the larger group, mean rates of aggression and levels of testosterone were compared across preestrus, estrus, and postestrus using repeated-measures single-factor ANOVAs and planned comparisons with the significance level set at P < 0.05. We compared how estrous-day aggression and sexual behavior (clasping) related to testosterone levels using a multiple regression analysis.

**RESULTS**

**Comparison of Serum and Fecal Testosterone**

Fecal testosterone level correlated significantly and positively with serum testosterone level among 9 adult ring-tailed males (r = 0.870, P < 0.01; Fig. 1). Among 10 males, mean (±SEM) fecal testosterone levels increased significantly from the premating (October) to the mating period (December): 45.6 ± 5.9 vs 76.9 ± 7.9 ng/g (t9 = 3.49, P < 0.01). A limited sample of serum testosterone values (from two pre-mating and three mating season males) suggests that serum levels also increased during this time (from 4.3 ± 0.4 to 18.7 ± 10.5 ng/ml; t3 = 1.37, NS).

**Relation between Fecal Testosterone Levels and Aggression**

**Premating vs Mating Season**

Prior to the mating season the correlation between testosterone level and rate of aggression was not statistically significant (r = 0.467, NS), whereas during the mating season the correlation was positive and significant (r = 0.748, P < 0.01; Fig. 2). These different outcomes may reflect that variability among males increased during the mating season, necessarily increasing the chance of finding significance. A second analysis, using mating season data from only males whose rates of aggression were low (i.e., less than 1.0...
attacks/h; similar to premating season behavior), was also significant ($r = 0.966, P < 0.05$).

**Mating Season**

*larger social group.* On the 5 estrous days observed in the larger study group, two distinct male behavioral types emerged: *fighters* ($n = 4$) and *nonfighters* ($n = 4$). Fighters displayed on average more than five aggressive acts per h (range: 3.5 to 5.5 acts/h), while nonfighters displayed less than one aggressive act per hour (range: 0.0 to 1.9 acts/h). Whereas on estrous days, fighter males were significantly more aggressive than nonfighters ($t_4 = 4.98, P < 0.01$), the two types of males did not differ on either preestrous or postestrous days ($preestrus: t_5 = 2.00, NS; postestrus: t_5 = 1.57, NS; Fig. 3, top panels). Fighter males displayed significantly more aggression on estrous days than on either the 2 days prior to or the 2 days following estrus ($F_{2,4} = 22.19, P < 0.01; preestrus = postestrus < estrus$), while nonfighter aggression levels did not differ significantly across these 2-day blocks.

Mean ($\pm$SEM) testosterone levels did not differ significantly between fighter and nonfighter males on the 2 days preceding estrus ($55.5 \pm 9.8$ vs $47.7 \pm 8.8$ ng/g; $t_5 = 0.60, NS$). However, on estrous days, fighter males exhibited significantly higher mean ($\pm$SEM) testosterone levels than nonfighter males ($101.7 \pm 14.2$ vs $45.6 \pm 7.9$ ng/g; $t_6 = 3.45, P < 0.05$; Fig. 3, bottom panels). Fighter male testosterone levels were significantly elevated on estrous days ($F_{2,4} = 14.41, P < 0.05; preestrus = postestrus < estrus$). By contrast, in nonfighter males, testosterone did not increase significantly on estrous days and was significantly lower than that in fighter group mates on the days following estrus ($t_6 = 3.29, P < 0.05$).

Mean rate of aggression correlated positively with mean level of testosterone during estrus ($r = 0.966, P < 0.001$; Fig. 4), but not during preestrus ($r = 0.583, NS$). When estrous day aggression data were limited to only those falling within the range seen for preestrous days (0.0–0.75 aggressive acts/h), the correlation coefficient on estrous days remained high ($r = 0.873, n = 3, NS$).

Frequency of clasping estrous females correlated significantly with levels of aggression ($r = 0.790, P < 0.05$) and testosterone ($r = 0.795, P < 0.05$). When
both aggression and clasping on estrous days were compared to mean testosterone levels on those days, aggression, but not clasping behavior, comprised a significant predictor of testosterone level (see Table 1). When an interaction variable of aggression × clasping was added to the model, it proved less predictive than the aggression variable alone.

**Smaller social group.** In the smaller social group, only two estrous periods were observed and each adult male showed changing patterns of behavior within each day. In other words, these males (SL, LC, HC) could not be classified as fighters or nonfighters. Prior to the mating season, approaches by male HC regularly elicited submissive signals from both SL and LC. On the first day that a female was sexually receptive (Nov. 1), however, SL became highly aggressive and elicited submissive signals from both HC and LC. On the following day, all three males’ testosterone levels were elevated from premating season levels (Fig. 5). During the 4 days following this first estrus, SL continued to elicit submissive signals from the two other males and his testosterone level remained elevated at an average level of 155.3 ng/g, approximately 10 times greater than his testosterone level prior to estrus. By contrast, HC and LC’s levels decreased to less than 55 ng/g over that same period.

On November 6, a second female in the smaller group became sexually receptive. Male LC, who previously responded with submissive signals to approaches by SL, became highly aggressive and on the following days his approaches elicited submissive signals from both SL and HC. The day after this second estrus, LC’s mean testosterone level was above 85 ng/g, as compared to 53 ng/g on the day prior to estrus. One week later LC’s testosterone level was still higher than 65 ng/g. In contrast, SL’s testosterone level was elevated to 132 ng/g on the day after the second estrus and then dropped to below 35 ng/g, and HC’s levels remained below 60 ng/g in this week.

### DISCUSSION

**Fecal Testosterone Measure**

The fecal testosterone measure provided a good index of circulating testosterone levels in semiprovisioned, group-living male ring-tailed lemurs. Results support the use of this measure in behavioral endocrine studies with free-ranging ring-tailed lemurs. Frequent and noninvasive steroid assessment allows researchers to relate day-to-day endocrine variability to

![FIG. 4. Mean fecal testosterone levels relative to aggression rates on estrous days for the four fighter and four nonfighter males in the larger social group.](image)

![FIG. 5. Mean fecal testosterone levels for three adult males in the smaller social group involved in agonistic challenges on two observed days of estrus. On November 1, the first estrous day (the left “E” bar), male SL attacked and elicited submissive signals from both LC and HC. In the following days, SL’s testosterone levels were greater than either LC or HC’s levels. On November 6, the second estrous day, male LC became aggressive and elicited submissive signals from both SL and HC. Subsequently, SL’s testosterone levels declined and LC exhibited the highest levels.](image)

### TABLE 1

<table>
<thead>
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<th>Predictor</th>
<th>Coefficient</th>
<th>t</th>
<th>P</th>
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<td>Aggression</td>
<td>12.58</td>
<td>4.72</td>
<td>0.005</td>
</tr>
<tr>
<td>Clasping behavior</td>
<td>3.41</td>
<td>0.45</td>
<td>0.669</td>
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Adjusted \( R^2 = 0.905 \)

\( F_{2,5} = 34.51 \)

\( P < 0.01 \)
daily behavioral variations, providing for fine-tuned analyses of hormone-behavior relationships in the natural context. In addition, the measure allows investigators to characterize the temporal dynamics of hormonal responses, an important aspect of endocrine responses (e.g., Koolhaas, Meerlo, de Boer, Strubbe, and Bohus, 1997). Future refinement of methods will allow estimation of mean daily levels of circulating testosterone based on fecal measures by illuminating the time delay between changes in circulating and fecal levels. The current limitation to the fecal measure is that it cannot be used to assess very short-term temporal patterns in circulating androgens (i.e., time intervals shorter than fecal production intervals). On the other hand, the measure does successfully describe reproductive condition in free-ranging males (see also Brockman et al., 1998).

Support for the Challenge Hypothesis in Male Primates

In two groups of semi-free-ranging ring-tailed lemurs, adult male fecal testosterone levels were more closely associated with rates of aggression during the mating season, when social challenges were frequent, than during the more stable period preceding mating. In addition, testosterone level on the day of and the day after estrus correlated with rate of aggression during those days, whereas testosterone and aggression were not correlated on the 2 days preceding estrus. As the social context became progressively less stable for males (i.e., premating season, to mating season, to specific days of estrus), the correlation between aggression and testosterone level increased. These findings support Wingfield et al.’s (1990) challenge hypothesis suggesting that it explains temporal associations between testosterone level and male aggression in this prosimian primate. Fewer fecal samples were collected during periods when correlation values were lower (i.e., premating period and days preceding estrus). Fewer data allow for greater error in measurement and therefore reduced chances of demonstrating significant correlations. Artificial manipulations of testosterone and social stability would likely confirm our findings; however, male ring-tailed lemurs conduct their own natural experiments during each mating season (Jolly, 1966).

The challenge hypothesis appears to extend beyond birds, reptiles, and fish to at least some primates. Comparability of results from ring-tailed lemurs may be due to the fact that mating system dynamics in this species resemble those of many nonmonogamous birds. Relations between testosterone and aggression in primates with less distinct mating seasons and more stable dominance relations may be more complex. The literature, however, indicates that the challenge hypothesis may indeed be applicable to primates other than lemurs (e.g., Michael and Zumpe, 1993). Many studies have suggested a positive relationship between these two variables whereas others indicated only a weak or no relationship (Dixson, 1980; Sapolsky, 1993a). Dixson (1980) noted that androgens probably play a more important role in primate aggressive behavior when males are in an unstable social context. Studies that show a significant positive relationship between testosterone and aggression are often those conducted with relatively novel or unstable social groups (e.g., macaques, Rose, Holaday, and Bernstein, 1971; Rose, Gordon, and Bernstein, 1972; Bernstein et al., 1979; Bernstein, Gordon, and Rose, 1983; marmosets, Dixson, 1993) and studies with no such correlation are often those conducted with more stable groups (e.g., macaques, Nieuwenhuijzen et al., 1987; humans, Campbell, Muncer, and Odber, 1997).

Further studies with primates that take social stability into consideration are needed. As has been suggested by Moore and Marler (1987), Gwinner and colleagues (1994), and Wingfield (1994), the proximate mechanisms of aggression differ from season to season or from one context to the next. Intruder and staged-fight protocols used to test the association between testosterone and aggression in primates, rodents, and other mammals (e.g., Rose et al., 1972; Eppe, 1981; Lumia, Thorner, and McGinnis, 1994) can inadvertently overemphasize the relationship between these two variables. The context of social unfamiliarity, when animals must establish their status, is likely to be the one context in which testosterone and aggression are most closely associated (e.g., Zumpe and Michael, 1996). This context occurs naturally during the mating season in many species, when social dominance is frequently tested and challenged. Testosterone may be associated with aggression particularly during this time when elevated testosterone can prepare an organism for success in the extended agonistic interaction that characterizes mating seasons (Wingfield, 1994).

Testosterone Costs and Benefits

Testosterone provides a variety of benefits. One highlighted by Sapolsky (1987, 1991) for male baboons is enhancement of protein synthesis and muscle mass. Testosterone can also help sustain directed attention
through remote blood collection techniques, might analyses of aggression and testosterone, perhaps status during the next estrus. Finer-tuned temporal the first estrus and quickly lost his new dominance and antebrachial scent glands (Andriamandrana and Rumpler, 1968) used during male-male aggression and sexual interactions (Jolly, 1966).

Negative consequences of elevated testosterone potentially include increased conspicuousness to predators due to increased activity (e.g., Wingfield et al., 1990) and suppression of immune function (e.g., Schuurs and Verheul, 1990; Folstad and Karter, 1992; Wichmann, Ayala, and Chaudry, 1997; cf. Klein, Hairston, De Vries, and Nelson, 1997). Some males’ abilities to prevail over others during periods of social instability may reflect that they can acutely elevate circulating testosterone levels to maintain the energy, attention, and olfactory secretions that promote success during fighting (e.g., Sapolsky, 1993b). The most successful males could thus take advantage of testosterone’s benefits and minimize its negative aspects by sharply circumscribing periods of testosterone elevation (e.g., Sapolsky, 1982).

Fighter males in our study exhibited short elevations of testosterone (1 to 2 days during estrus), something not predicted by the challenge hypothesis for non-paternal species like ring-tailed lemurs. This pattern suggests a cost associated with long-term elevations, other than decreased paternal behavior. In particular, elevated testosterone could waste physiological resources and compromise immune function, two very negative consequences in the face of impending estrus, when energy requirements are high and physical injury common for males. Additionally, for ring-tailed lemurs, the mating season is a time when crucial fasting occurs, just prior to the extended food limitations experienced annually during the 7- to 8-month dry season (Pereira, Strohecker, Cavigelli, Hughes, and Pearson, 1999). Long-term elevation of testosterone during the mating period potentially would inhibit this important fasting process (e.g., Wade and Gray, 1979; Björntorp, 1997). The idea that chronic testosterone elevation confers significant costs to males is anecdotally supported by the case of male SL, who maintained high testosterone levels for several days after the first estrus and quickly lost his new dominance status during the next estrus. Finer-tuned temporal analyses of aggression and testosterone, perhaps through remote blood collection techniques, might help to illuminate costs and benefits of acute and chronic circulating testosterone elevations. Advantages and disadvantages will need to be evaluated in accord with species-typical life history strategies (Pereira 1993b; Pereira et al., 1999).

Our study shows that male testosterone level prior to days of severe male agonistic competition is not predictive of individual rates of aggression during eventual challenges. Males that expressed high rates of aggression during estrus did not exhibit higher fecal testosterone measures on previous days than males that did not become aggressive. To understand this temporal pattern fully we need to illuminate the time delay between blood and fecal testosterone levels. But even without this information, it is clear that not long before estrous days, fighter and nonfighter males had similar testosterone levels. Similarly, individual testosterone levels prior to formation of new social groups of rhesus macaques and squirrel monkeys do not predict eventual success in attainment of dominance status (Mendoza, Coe, Lowe, and Levine, 1979; Clarke, Harrison, and Didier, 1996). All such results further indicate that significant costs are associated with maintaining high testosterone levels during periods of social stability.

Further work is required with other primate and nonprimate mammals to determine how widely the challenge hypothesis explains the relationship between androgens and aggression across taxa and to determine the costs and benefits of different temporal secretion patterns.

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