Effects of social status, age, and season on androgen and cortisol levels in wild male golden lion tamarins (*Leontopithecus rosalia*)

Karen L. Bales\(^a,b,c,*\), Jeffrey A. French\(^d\), Jennifer McWilliams\(^d\), Reagan A. Lake\(^a\), James M. Dietz\(^a\)

\(^a\)Dept. of Biology, University of Maryland, College Park, MD 20742, USA
\(^b\)Dept. of Psychology, University of California, Davis, CA 95616, USA
\(^c\)California National Primate Research Center, USA
\(^d\)Depts. of Psychology and Biology, University of Nebraska, Omaha, NE 68182, USA

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Abstract

This study examines factors affecting androgen and cortisol levels in wild, male golden lion tamarins (*Leontopithecus rosalia*). Golden lion tamarins are a cooperatively breeding species in which groups often contain two potentially breeding males. Brothers frequently emigrate together and develop a clear dominance hierarchy, but interactions between them are primarily affiliative. Duos in which the males are not related are less stable. In addition, reproductive skew theory predicts that dominant males will be less likely to share reproduction with related subordinates. As such, we predicted that both androgens and cortisol would be higher in subordinate males unrelated to the dominant male. We also predicted that androgens in breeding males would be higher during the mating season than the birth/infant care season, as per Wingfield’s “challenge hypothesis” (1990). Fecal samples were collected from 24 males in 14 social groups and assayed by enzyme immunoassay. Androgen levels were higher in breeding males during the mating season, thus supporting the challenge hypothesis. However, while subordinate males unrelated to the dominant male had significantly lower androgens than any other group, cortisol levels were not correspondingly higher. These results suggest that unrelated subordinate males show measurable reproductive suppression and may use strategies such as infantilization to avert aggression from dominant males.

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Keywords: Androgens; Cortisol; Cooperative breeding; Challenge hypothesis; Tamarin; Reproductive skew

Introduction

The regulation of hormones and fertility by various cues such as social status, age, and season has been studied in several mammalian and avian species. Steroid hormones, principally androgens and glucocorticoids, are of particular interest when studying males because of the dependence of sexual behavior and spermatogenesis on testosterone and other androgens (Bronson, 1989; Nelson, 1995) and the potential of the “stress” hormone cortisol to alter gonadal function, and hence, reproductive potential (Bronson, 1989; Sapolsky, 1985).

Much research on context-specific differences in steroid hormones has focused on predictions derived from two related theoretical positions. The “challenge hypothesis” (Wingfield et al., 1990) predicts that seasonal changes in testosterone will vary based on social system, level of male–male aggression, competition for reproduction, and presence of estrus females. The “trade-off hypothesis” (Ketterson and Nolan, 1999), in turn, proposes that testosterone concentrations in males of biparental species reflect a trade-off between the male reproductive effort directed toward mating vs. parental effort. Together, these hypotheses predict that monogamous, biparental males will show increased testosterone when male–male conflict is
higher and females in estrus are present and decreased testosterone during times when it is unnecessary or incompatible with other behaviors, such as infant care. Although these predictions have been tested widely in avian systems, several mammalian species have also been used to evaluate these hypotheses, including spotted hyenas (Crocuta crocuta: Goymann et al., 2003); ring-tailed lemurs, Lemur catta (Cavigelli and Pereira, 2000); and rhesus macaques (Macaca mulatta: Rilling et al., 2004). Male tufted capuchins (Cebus apella nigritus), in contrast to the above species which showed support for this hypothesis, had peaks in testosterone during times of relatively low aggression (Lynch et al., 2002). Conforming to predictions, testosterone dropped during the expression of paternal care in biparental hamsters (Phodopus campbelli), but there was no similar drop in non-paternal hamsters, P. sungorus (Rebun and Wynne-Edwards, 1999); testosterone was also less likely to share reproduction with relatives, who get indirect fitness benefits and are therefore more likely to stay as helpers. Neither subordinate male high-veld mole-rats (Cryptomys hottentotus pretorius) nor dwarf monogoosees (Helogale parvula) demonstrated physiological suppression of reproduction or rank-related differences in androgens (Creel et al., 1992; van Rensburg et al., 2003); however, in naked mole-rats (Heterocephalus glaber), dominant males had higher testosterone (Clarke and Faulkes, 1998). In captive male cotton-top tamarins (Saguinus oedipus) and common marmosets (Callithrix jacchus), male adults housed in the presence of an older, breeding male showed no physiological signs of reproductive suppression (Baker et al., 1999; Ginther et al., 2002).

Social relationships may also factor into the regulation of the “stress” hormone cortisol. In a meta-analysis of primate studies, Abbott et al. (2003) examined the relationship between dominance status and activity in the hypothalamic–pituitary–adrenal (HPA) axis. Factors identified with higher levels of cortisol for subordinates included higher rates of stressors and lower rates of social support. In contrast, in those species and situations where subordinates experienced lower exposure to potential stressors and had higher levels of social support, subordinates tended to have cortisol levels similar to those of dominant individuals. In general, cooperative breeders demonstrate high levels of social support and low levels of stressors. However, the relationship between rank and cortisol might be expected to differ depending on the relatedness of the subordinate, if this also affects social support and stress levels. If stressors and high levels of glucocorticoids are the mechanism for suppression of reproduction, the relationship between cortisol and social rank might be expected to differ across species, depending on whether subordinate males are reproductively suppressed (Bronson, 1989). There was no difference in cortisol between dominant and subordinate males in Wied’s marmosets (Schaffner and French, 2004), naked mole-rats (Clarke and Faulkes, 1998), rhesus macaques (Bercovitch and Clarke, 1995), cotton-top tamarins (Ginther et al., 2002), or tufted capuchins (Lynch et al., 2002), although male capuchins did display increases in cortisol during the time of highest female sexual activity. In a study of wild chimpanzees, dominant males had higher cortisol (Muller and Wrangham, 2003).

Golden lion tamarins (Leontopithecus rosalia) are arboreal, New World primates of the family Callitrichidae (Mittermeier et al., 1988). Golden lion tamarins and other callitrichid primates are characterized by a cooperatively breeding social system which has been variously characterized as monogamy (Kleiman, 1977, 1978b; Baker et al., 1993, 2002) or cooperative polyandry (Goldizen, 1987; Sussman and Garber, 1987). Golden lion tamarin groups frequently contain two adult males that are unrelated to the breeding female (Baker et al., 2002). While both copulate with her, access to the female during estrus periods is monopolized by the behaviorally dominant male (Baker et al., 1993). Brothers often emigrate together from their natal groups (Baker and Dietz, 1996) and enter a new group as the dominant and subordinate breeding pair. In addition, if a breeding female dies and a new female enters a group, a father and son will often both breed with the new female,
forming a subordinate and dominant duo (Baker et al., 2002). In one study, 75.7% of observed male breeding duos were close relatives (Baker et al., 2002), with the remaining 24.3% of duos consisted of unrelated males. Male duos were stable over time, with observed durations ranging from 1 month to 68 months (Baker et al., 2002). The distinction between male duos that are related vs. those that are unrelated is biologically important for several reasons. Golden lion tamarin duos consisting of unrelated males tended to be less stable (Baker et al., 2002). Most research on captive male callitrichids has found no differences in androgens or cortisol levels between related males of different social statuses. In Wied’s tufted-ear marmosets, levels of urinary testosterone and cortisol did not differ between dominant and subordinate males in multi-male groups or with levels found in single males in monogamous groups (Schaffner and French, 2004). In that study, polyandrous groups containing unrelated males were unsuccessful in breeding due to intrasexual aggression (Schaffner and French, 2004). In male common marmosets, hormone levels did not differ between fathers and sons during short tests, whether they were tested with the mother or an unrelated female present, or whether they were tested individually or together (Baker et al., 1999). However, in groups composed of unrelated adult common marmoset males, subordinate males had lower testosterone (Abbott, 1986, 1993).

Golden lion tamarins display markedly seasonal breeding (Dietz et al., 1994), with most births occurring in October and November. There is a second, smaller birth peak in February, which consists mostly of second litters, and no births in April–July. Second litters are the result of a fertile post-partum estrus displayed by some females (Bales et al., 2001, Wilson, 1977). Our predictions regarding androgen and cortisol levels are detailed in Table 1.

### Methods

#### Subjects

Subjects were 24 male golden lion tamarins in 14 different social groups, free-ranging in Poço das Antas Biological Reserve (PDA) and outlying reintroduction areas. James Dietz, Andrew Baker, and colleagues have monitored approximately 20 groups in the PDA population since 1983. In all but two cases, the males in the current study were born in the PDA population and therefore were of known age and relationship to other animals. All individuals in the study groups are tattooed and dye-marked and at least one animal in each group was radio-collared to facilitate location.

Five males were descendants of captive-born animals reintroduced from zoos (Beck and Martins, 1998). These males were provisioned three times a week. Descendants of reintroduced monkeys forage independently and behave in many respects like native, wild tamarins (Kierulf et al., 2002; Stoinski, 2000). The males included in the present study did not differ from the PDA males in baseline androgen (n = 24, F_{1,22} = 1.45, P = 0.240) or cortisol levels (n = 24, F_{1,22} = 1.05, P = 0.317), and the two groups were therefore combined for all analyses.

### Table 1

Males were placed in six categories according to group composition and predicted levels of intragroup male–male competition

<table>
<thead>
<tr>
<th>Category</th>
<th>Other males besides offspring present</th>
<th>Adult females present</th>
<th>Predicted level of intragroup male competition</th>
<th>Androgen predictions</th>
<th>Cortisol predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monogamous</td>
<td>Absent</td>
<td>Unrelated female present</td>
<td>None</td>
<td>High during mating season</td>
<td>Low because social support is high, stressors are low</td>
</tr>
<tr>
<td>Dominant</td>
<td>Subordinate male present</td>
<td>Unrelated female present</td>
<td>High (although may depend on relatedness of subordinate)</td>
<td>High during mating season</td>
<td>Low because social support is high, although relatedness of subordinate could affect stress levels</td>
</tr>
<tr>
<td>Subordinate related</td>
<td>Dominant male present (father, brother, or son)</td>
<td>Unrelated female present</td>
<td>High</td>
<td>Low because dominant male should be unwilling to share reproduction</td>
<td>Low because social support is high, stressors are low</td>
</tr>
<tr>
<td>Subordinate unrelated</td>
<td>Dominant male present (unrelated)</td>
<td>Unrelated female present</td>
<td>High</td>
<td>High because dominant male should be willing to share reproduction</td>
<td>High because social support is low, stressors are higher</td>
</tr>
<tr>
<td>Natal</td>
<td>Father present</td>
<td>Mother present (no potential mates)</td>
<td>None</td>
<td>Low because there is no potential mate in group</td>
<td>Low because social support is high, stressors are lower</td>
</tr>
</tbody>
</table>

Hypotheses and predictions for different categories of males were based on the challenge and trade-off hypotheses, reproductive skew theory, and Abbott et al. (2003).
Collection, extraction, and assay of fecal samples

A total of 196 fecal samples were collected with a mean of 8.17 ± 0.91 samples per male. Most samples were collected during four periods: June–July 1996 (preliminary data), June–November 1997, July–December 1998, and February–March 1999. Since cortisol concentrations in feces vary depending on the time of day (Sousa and Ziegler, 1998), we attempted to restrict collection to mornings; 73% of samples were collected by 12 pm. We used only samples that were unambiguous as to the identity of the animal of origin, and we included only one sample per animal per day in the analyses. Numbers of samples differed between males due to circumstances such as deaths or the formation of new study groups. We stored samples at −18°C until analysis.

We allowed fecal samples to thaw at room temperature and placed 0.125 g fractions of each sample in a round-bottom 20-ml test tube. We removed seeds, leafy material, and insect parts from the fecal sample prior to weighing. For the androgen assay, we solubilized the feces with a 5.0 ml volume of ethanol. We then reconstituted the sample with 1.0 ml of ethanol and placed the tubes on a shaker rack overnight (8–12 h). We pelletted the particulate matter in the resulting slurry by centrifugation for 15 min at 2500 rpm, poured off the ethanol into clean tubes, and evaporated the ethanol. We next reconstituted the sample with 1.0 ml of phosphate-buffered saline (PBS) and froze until assay. For the cortisol assay, we added a 5.0 ml volume of solubilizer (40% methanol:60% PBS) and placed the tubes on a shaker rack overnight (12–16 h). We pelletted the particulate matter in the resulting slurry by centrifugation for 15 min at 2000×g and separated the supernatant into a clean test-tube. We refoze the extracted samples and stored them prior to assay.

Androgen assay

We measured fecal androgens using an enzyme immunoassay previously described (Dloniak et al., 2004; Nunes et al., 2000) and validated for the golden lion tamarin. The assay utilized a testosterone antibody (R156/7) and a testosterone conjugate (horseradish peroxidase; HRP) provided by William Lasley and Coralie Munro (University of California, Davis). Assay quality control was monitored by assaying a lion tamarin fecal extract pool sample on each plate. The inter-assay coefficient of variation (CV) was 9.0%, and the intra-assay CV was 3.7%.

Cortisol assay

We measured fecal cortisol using an assay previously characterized (Smith and French, 1997) and validated for the golden lion tamarin. Intra- and inter-assay coefficients of variation for a fecal quality control pool were 7.1% and 16.4%, respectively. Cortisol standards and serial dilutions of extracted feces collected from females at different times during pregnancy produced parallel displacement curves. We determined the accuracy of the assay by spiking the supernatant of a fecal sample with 100 pg of cortisol standard. The recovery of cortisol standard was 101 ± 2.0% (n = 6).

Data analyses

Data were analyzed by mixed-model ANOVA (Littell et al., 1996) in Proc Mixed in SAS 8.0 (SAS Institute, Cary, NC), with post-hoc analysis by least-squared means.

Analysis 1

In the first analysis, fecal androgens and cortisol were predicted based on social status, with age of the animal as a covariate (Table 2). Data were log-transformed in order to meet assumptions of parametric tests. Because 6 animals had samples in more than one social status, the animal identity was included as a random factor but was eliminated when it was not significant. The one male sampled who was dominant to an unrelated male was combined in all analyses with males dominant to related males.

Behavioral dominance was assessed based on archwalking, mounts, and chases (Baker et al., 1993, 2002). Males were grouped into five categories (see Table 1). The analysis for each hormone was carried out a second time excluding former dominant males who had become subordinate on the basis that they might have started out with higher steroid levels than other males.

Analysis 2

For analysis of androgens and cortisol by season, males not in breeding positions (i.e., natal males) were excluded from the analysis. Samples from males in breeding positions were categorized into mating season (mid-May through late July) or birth/infant care season (early September through late November) samples. Due to the small sample size available during these relatively short periods, dominant/monogamous males were combined and subordinate related males were combined. Data were then analyzed by mixed-model ANOVA with season, social status, and a season by social status interaction.

Some females may undergo a post-partum estrus in the 2 weeks following a birth. This is a period characterized by high infant dependency. Only a limited number of samples

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Independent variables included in models used in each analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis 1</td>
<td>Status (monogamous, dominant, subordinate/unrelated, natal) Age</td>
</tr>
<tr>
<td>Analysis 2</td>
<td>Status (monogamous/dominant vs. subordinate) Season (mating vs. birth) Season by status interaction</td>
</tr>
</tbody>
</table>
Results

Androgens

Analysis 1: social status, relatedness, and age

Fecal androgen values were significantly different between categories of different social status ($F_{2,25} = 3.92, R^2 = 0.44, P = 0.0092$; Fig. 1). Age was non-significant and therefore dropped from the final model. In post-hoc testing, we found that males subordinate to an unrelated male had significantly lower androgen levels than males in all other categories, while natal males also had marginally lower androgens than males subordinate to a related male ($t = -1.83, P = 0.079$). Removal of former breeding males who then became subordinates (and therefore might be expected to start out with higher androgens than a subordinate male which had never been in a breeding position) also did not change the results, with a new mean for subordinate-related males of $156.34 \pm 70.1$ ng/g feces.

Analysis 2: social status, season, and status/season interactions

Both social status (monogamous/dominant vs. subordinate) and the status/season interaction were non-significant and therefore dropped from the final model. Androgens did vary significantly by reproductive season, with higher levels of fecal androgens during the mating season than during the birth/infant care season ($F_{1,24} = 6.80, R^2 = 0.22, P = 0.015$; Fig. 2).

Seven males had samples available from both seasons, and these were also compared within individual males using a paired test. Six out of seven individual males for which both mating and birth/infant care values were available had higher fecal androgens during the mating season (Wilcoxon signed-rank, $S = 13.0, P = 0.031$).

Cortisol

Analysis 1: social status, relatedness, and age

Mean levels of fecal cortisol differed among the categories of males (Fig. 3), with the highest levels exhibited by natal males. However, the overall ANOVA for cortisol values showed no significant difference among categories, as well as no difference due to age (overall model: $F_{6,22} = 1.33, R^2 = 0.27, P = 0.285$). Removal of former breeding males that were now subordinates did not change the results, with a new mean for subordinate-related males of $374.5 \pm 181.4$ ng/g feces. A paired test on the five males who moved from their natal groups to breeding positions revealed higher concentrations in the natal category than in the breeding category (natal: $758.06 \pm 178.91$ ng/g; breeding: $114.78 \pm 27.12$ ng/g), a difference that was marginally significant (Wilcoxon signed-rank, $S = 7.5, P = 0.062$).

Analysis 2: social status, reproductive season, and status/season interactions

Both status (monogamous/dominant vs. subordinate) and the status/season interaction were non-significant and therefore dropped from the final model. There was a non-significant trend in all breeding males combined for cortisol to be higher during the birth/infant care season than during the mating season ($F_{1,24} = 3.68, R^2 = 0.133, P = 0.067$; Fig. 2). Three out of seven individual males for which both mating and birth/infant care season values were available had higher cortisol during the mating season, and four had higher cortisol during the birth season (Wilcoxon signed-rank, $S = -4.0, P = 0.578$).

Discussion

The results of this study add to the growing literature indicating that gonadal function in cooperatively breeding mammals is exquisitely sensitive to subtle aspects of group demography. Androgens in golden lion tamarin males varied...
with social status, reproductive season, and relatedness. Only subordinate males unrelated to the dominant male of their group showed androgen levels lower than those of dominant males; subordinate males that were related to the dominant male had levels of fecal androgens that were statistically indistinguishable from those of the dominant male.

Based on studies of captive marmosets and tamarins (Möhle et al., 2002; Ziegler et al., 2000), it is likely that these androgens were primarily of gonadal rather than adrenal origin. It is also notable that, while fecal cortisol trends up in the birth/infant care season, androgens are significantly lower, suggesting a dissociation between the secretory products associated with the hypothalamic–pituitary–gonadal axis and the hypothalamic–pituitary–adrenal axis. In addition, cortisol did not show significant variation according to male status, suggesting that stress- or metabolic-induced changes in the hypothalamic–pituitary–adrenal axis do not influence the production of androgens in subordinate, unrelated males under these conditions. There are, however, a number of potential factors (social, environmental, and physiological) affecting cortisol that we were unable to address in this study. Our one finding with regard to cortisol is that within individual males there was a trend for cortisol to drop when a male moved from his natal group to a breeding position. In mammals that form pair-bonds, it is common for the formation of a pair-bond to be associated with a short- or long-term drop in stress hormones (Carter, 1998; Ginther et al., 2001; Hoffman, 1998; Mendoza et al., 2001; Reburn and Wynne-Edwards, 1999; Schaffner and French, 2004). It is interesting that this drop in cortisol when entering a breeding position is apparent even when the male is entering into a subordinate position and presumably engaging in less sexual behavior with the female (Baker et al., 1993; Kleiman, 1978a).

Androgens also varied according to the predictions of the challenge and trade-off hypotheses, being higher during the mating season (a period characterized by high levels of mate-guarding and male–male interaction, as well as access to breeding females). Also as predicted, androgen levels were lower during the season of high paternal care. These data are consistent with those from captive Wied’s marmosets (Nunes et al., 2000, 2001), in which urinary testosterone dropped following post-partum mating, and males who carried infants more had lower testosterone. Similar results have been found in biparental hamsters (Reburn and Wynne-Edwards, 1999) and Mongolian gerbils, Meriones unguiculatus (Clark et al., 2004). Nunes et al. (2001) also found a negative relationship between cortisol and carrying behavior. While we did not find lower cortisol in the birth/infant care season, here, we did not consider individual variation in hormone levels and infant carrying. This would be a valuable analysis to undertake in the future when additional samples become available. In addition, species have a post-partum estrus during which mating and infant care might be expected to produce ‘‘competing’’ endocrine responses. Another avenue for future research might involve following up on the observation that, in this study, male androgen levels tended to be lower during the females’ post-partum estrus.

The lower androgens in the subordinate of unrelated male–male duos might not be unexpected if one predicts that high androgens in a subordinate might lead to behavior viewed as a challenge by the dominant male. However, this finding does run contrary to predictions derived from reproductive skew theory (Emlen, 1995). According to this body of theory, subordinates to whom the dominant male is most closely related receive the largest benefit from helping raise the dominant’s offspring due to indirect fitness benefits. The dominant should therefore be less obliged to share reproduction with them in an effort to entice them to stay in the group. Our results are clearly in opposition to this prediction, showing lower reproductive suppression of related males.

Several clues suggest that the relationship between dominant and unrelated subordinate males is more tenuous than that between dominants and related subordinates. Not only do unrelated male duos tend to persist for less time in this study population of golden lion tamarins (Baker et al., 1993; Kleiman, 1978a).
02002), but the formation of unrelated male duos in this and other callichrid species in captivity has been difficult (Kleiman, 1978a; Schaffner and French, 2004). Unrelated subordinate males may also participate less in certain daily activities of the social group, although more field data may be necessary to determine this. In a study of captive golden lion tamarins, the male that sexually monopolized the female also interacted more with her in non-sexual contexts (Kleiman, 1978a). Male infant transport may vary according to the relatedness of the subordinate; in the one male duo in Baker's study in which the subordinate and dominant were known to be unrelated, the subordinate carried less than half as much as the dominant (Baker et al., 1993). As infant transport varied according to relatedness to the infant in natal males (Baker, 1991), it is possible that the same is true in breeding males. Studies of wild moustached tamarins (Saguinus mystax) and common marmosets also documented large disparities in some groups in the extent to which each male carried the infants (Garber, 1997). In addition, subordinate males in three out of seven groups observed were not seen to copulate at all with the dominant female (all dominant males copulated) (Baker et al., 1993), although once again it is unclear if relatedness of the subordinate was the key difference.

The overall picture suggests tolerance of unrelated subordinate males but less than full integration into the group. Animals seeking admission into a group (Bales, pers. obs.) and subordinate males following an aggressive encounter (Baker et al., 1993) often give infanticiled calls, probably to avert aggression. Suppression of androgens in this context could also be meant to avert aggression and could be either a cause or a consequence of infantilization.

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