Social factors increase fecal testosterone levels in wild male gray-cheeked mangabeys (*Lophocebus albigena*)

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ABSTRACT

Testosterone can be expected to play a significant role in mediating behavior and life history in social animals, but the number of species with data from the wild is still too small to make generalizations. We investigated the influence of social factors (social status, aggression, and reproductive females) and environmental variation (rainfall and temperature) on fecal testosterone concentrations in wild male gray-cheeked mangabeys (*Lophocebus albigena*) living in five groups in Kibale National Park, Uganda. This species is phylogenetically closely related to baboons, but is arboreal, with multi-male, multi-female groups rarely exceeding 20 individuals. We analyzed 358 fecal samples from 21 adult males. We found that the highest-ranking males had the highest testosterone concentrations while immigrant males had the lowest testosterone concentrations. Aggression was not correlated with testosterone levels. The presence of females with sexual swellings at their most tumescent stage increased testosterone concentrations in all males. Finally, individuals tended to have lower testosterone when the temperature was higher.

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Introduction

Testosterone likely plays a significant role in mediating behavior and life history in social animals. It is an anabolic steroid that is associated with reproductive functions (Balthazart, 1983; Griffin, 1996), the formation of male secondary sexual features (Wickings and Dixson, 1992), muscle mass gain in male vertebrates (Kemnitz et al., 1988; Welle et al., 1992), and the maintenance of musculoskeletal performance (Bribiescas, 2001). In primates there is also a positive correlation between testosterone level and risk-taking behaviors (Booth et al., 2006) necessary in competition for high rank. While high testosterone levels may facilitate reproductive effort by promoting dominant/competitive behaviors and muscle anabolism, high levels may also reduce life expectancy. In particular, risky behaviors can lead to injury or death, and investment in reproductive function may exhaust resources and inhibit immune function in the face of pathogenic challenge or injury (Muehlenbein, 2004). Therefore, males may have been selected to keep testosterone levels low unless short-term benefits can be expected, such as when a male has a good chance of obtaining matings as a result of competition with other males.

While testosterone affects behaviors that can cause stress, stress can also influence testosterone levels. Stress as a state of homeostatic imbalance may have a physical cause, such as bodily injury (common during male–male competition), or a psychological cause, such as intimidation by higher-ranking animals. Studies of wild olive baboons (*Papio anubis*) reveal that the response of testosterone level to stress can vary with rank. For example, testosterone levels declined in stressed lower-ranking males but increased in stressed higher-ranking males (Sapolsky, 1991, 2004).

The outcome of mating competition is often related to position in the dominance hierarchy, with higher-ranking males often achieving greater reproductive success than lower-ranking males (Silk, 1987). Therefore, high social rank and high testosterone concentration are expected to be associated. Indeed, consistent with studies on other mammals (e.g., lagomorphs: Briganti et al., 2003; carnivorans: Holekamp and Smale, 1998; Sands and Creel, 2004; Creel, 2005), studies on a wide range of primates have revealed that higher-ranking males have higher concentrations of testosterone than lower-ranking males, e.g., sifakas (*Propithecus verreauxi*) (Kraus et al., 1999), squirrel monkeys (*Saimiri sciureus*) (Coe et al., 1983; Steklis et al., 1986), mantled howling monkeys (*Alouatta palliata*) (Zucker et al., 1996), tufted capuchin monkeys (*Cebus apella nigritus*) (Lynch et al., 2002), rhesus macaques (*Macaca mulatta*) (Bercovitch, 1993; Rose et al., 1975), talapoin monkeys (*Miopithecus talapoin*) (Eberhart et al., 1985), and chimpanzees (*Pan troglodytes*) (Muehlenbein et al., 2004). However, other studies, including some using the same species as

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those cited above, have failed to demonstrate significant positive correlations between dominance rank and testosterone level, e.g., rhesus macaques (Gordon et al., 1976), Japanese macaques (Barrett et al., 2002), chimpanzees (Seraphin, 2000), and bonobos (*P. paniscus*) (Sannen et al., 2004). Such conflicting results suggest that any association between testosterone concentration and male rank may be sensitive to socioecological context.

Among males, high rank is usually obtained through successful antagonistic interactions (in particular by aggression), and testosterone has been linked to aggression. However, testosterone may not cause aggressive behavior per se but instead may increase the likelihood that it will be expressed (Booth et al., 2006). Testosterone seems to become elevated most consistently in direct competition between males either during times of social instability when positions in the dominance hierarchy are being challenged (Wingfield et al., 1990; McCaul et al., 1992; Sapolsky, 1993; Teichroeb and Sicotte, 2008) or when males are competing for limited resources, such as estrous females (Lynch et al., 2002; Muroyama et al., 2007). Thus, in squirrel monkeys hormonal levels and dominance rank are positively correlated during group formation (Mendoza et al., 1979) and in olive baboons higher-ranking males have higher levels of testosterone than lower-ranking males during unstable periods (Sapolsky, 1983). In tufted capuchins, males show clear elevations of testosterone during the peak of adult female sexual activity (Lynch et al., 2002) and in Japanese macaques males competing for estrous females have increased testosterone levels (Muroyama et al., 2007). Finally, in male golden lion tamarins (*Leontopithecus rosalia*) and ring-tailed lemurs (*Lemur catta*) testosterone levels are higher only during the mating season (Bales et al., 2006; Cavigelli and Pereira, 2000).

It is also known that testosterone levels can vary seasonally, especially for seasonally breeding species such as rhesus macaques, ring-tailed lemurs and squirrel monkeys (Dixon, 1998). Until studies are conducted to rule it out, the possibility exists that environmental factors such as temperature and rainfall influence testosterone levels even in primates that do not breed seasonally (Wolfe-Coote, 2005). Indeed, Gesquiere et al. (2011) report for the first time in non-human primates a negative association between temperature and testosterone in male yellow baboons (*P. cynocephalus*), a non-seasonally breeding species.

Gray-cheeked mangabeys (*Lophocebus albigena*) can provide useful insights into the relationship between testosterone, social factors, and aggressive behavior because they are phylogenetically closely related to the more well-studied baboons and have many similarities, and yet differ from baboons in ways that may result in different hormonal profiles. For instance, both gray-cheeked mangabeys and baboons typically live in multi-male, multi-female social groups, with the number of males varying markedly among groups and within groups over time. However, whereas baboons are highly terrestrial and can live in very large groups gray-cheeked mangabeys are arboreal and live in smaller groups, rarely exceeding 20 individuals (Waser, 1977; Janmaat et al., 2009). Arboreality may create a more challenging environment for males as they compete with one another, and the number of males in groups may also affect the intensity or frequency of competition.

Gray-cheeked mangabeys are typical cercopithecine primates in that females usually remain in their natal groups whereas males disperse from their natal groups around sexual maturity and may transfer to multiple groups during their lifetimes (Olupot, 1999). Male gray-cheeked mangabeys compete for access to females and are more likely to immigrate to other groups when females are in estrus, which can occur throughout the year (Olupot and Waser, 2001; Arlet et al., 2008). Thus, males may be involved in mating competition any time as the opportunity arises.

Here we document fecal testosterone concentrations in male gray-cheeked mangabeys living in five groups with regard to social factors, i.e., rank, length of time in groups, and involvement in aggressive interactions. We test the predictions that testosterone levels are higher 1) in highest-ranking males than in lower-ranking males, 2) in resident than in immigrant males, and 3) when females are in estrus, as indicated by maximal sexual swelling. We also explore the potential for environmental factors, i.e., rainfall and temperature to affect fecal testosterone levels.

Methods

Study area and subjects

The study was conducted for 15 months, from December 2005 to July 2006 and from January to July 2007, in Kibale National Park, Uganda (0°13′–0°41′N and 30°19′–30°32′E), near the Makerere University Biological Field Station at Kanyawara. Kibale (795 km²) is a moist, evergreen, medium altitude forest with a mosaic of swamp, grassland, thicket, and colonizing forest (Struhsaker, 1975).

During 375 all-day (0700–1700 h) follows, Arlet and assistants observed 24 adult males in five groups: Lower Camp II (LC2), Mikana (MK), CC, Butanzi (BT1), and Lower Camp (LC). In this study, we used focal data from 21 adult males only. Each group was observed for up to six consecutive days (3–6 days; median: 5, mean: 4.72), approximately every five weeks with the necessary adjustments in response to elephants, lost groups, and heavy rain. These groups have been studied by various researchers since at least 1996 (e.g., Arlet et al., 2007; Olupot and Waser, 2001; Janmaat et al., 2006; Chancellor and Isbell, 2009) and are well habituated. Group sizes ranged from 9 to 22 individuals, with 1–9 adult males per group (Arlet et al., 2009b).

An individual was considered adult when he was able to give whoop-gobble calls, a characteristic call produced only by adult males. Five adult males in this study were individually recognized by uniquely colored collars from a previous study (Olupot, 1999) and 16 by relative size and distinguishing features, e.g., tail shape, scars, and fractures (Arlet et al., 2009a). Estimating the age of adult males in this study was not possible because no visible physical markers of male age are available for this species and most males immigrated to our study groups from non-study groups.

Females were assessed daily for degree of swelling of the perineum as an indication of reproductive condition during independently conducted censuses. Maximal sexual swelling (called peak-swelling here) was characterized by exaggerated turgid swelling and bright pink color in the perineal area (Wallis, 1983).

Observation method

During the all-day follows, we conducted 1157 focal behavioral samples on 24 adult males, including five males who immigrated into the groups. The focal sampling order in each group was opportunistically determined by first sighting of adult males that had not yet been sampled during a particular round (usually a day), while taking care to balance morning and afternoon sampling for each individual. Focal samples were 1 h in the larger groups and 2 h in the smaller groups (total: 2428.2 h, 15–206 h/male; Table 1).

Male dominance and immigrant status

Relative position in the male dominance hierarchy within each group was determined from the outcome of dyadic agonistic interactions, i.e., winners and losers of chases and fights, and supplanters and supplantees during focal samples. This was done for two study periods (December 2005–July 2006, and January–July 2007) separately. Dominance matrices were constructed for each group, with rank-order determined by placing winners above losers while minimizing the number of reversals against the hierarchy (i.e., interactions below the diagonal) (see Arlet and Isbell, 2009 for matrices in 2006) (Fig. 1).

Table 1
Male gray-cheeked mangabey subjects, their groups, residence status, dominance rank, movements between groups, focal observation hrs, and number of fecal samples collected. Abbreviations: R—resident male, IM—immigrant male, HR—high-ranking male, LR—low-ranking male, fT—fecal testosterone. Dash means that the male was not present within any of the studied groups.

Male	Group	Status	Rank	Movement	Focals [h]	fT 2006	fT 2007
LM	BT1	R	HR	No	107	28	4
KK	BT1	R	LR	Out November 2006	29	15	Is in CC
PL	BT1	R	HR/LR	No	76/49	18	4
BG2[a]	BT1	R	LR	In Jan and May 2006	123	26	4
KR	CC	R	HR	Out December 2006	31	4	–
KJ	CC	R	LR	No	67.2	4	4
GR	CC	R	LR	Out March 2006	5.5	7	–
KK	CC	IM	LR	In November 2006	46.5	Is in BT1	4
KA	CC	IM	LR	In March 2007	26.5	–	4
MF[b]	CC	IM/R	LR/HR	in April 2006	20/29/37	5	4
MG	LC	R	HR	No	179	31	4
YM	LC	R	HR	No	192	21	4
R	LC	R	HR	Out October 2006	122.5	34	–
MS	LC	R	LR	In April 2007	14.5	–	4
NK	LC	R	LR	Out October 2006	104	20	–
BW	LC	IM	LR	In March 2007	14.5	–	4
HL[c]	LC	IM	HR	In April, out October 2006	54.5	6	–
BG2[a]	LC	IM	HR	In Jan and May 2006	19	10	–
IB	LC2	R	HR	No	205	25	4
NY	LC2	R	LR	Out January 2007	206	25	–
KY	MK	R	HR	No	163.5	20	4
KC	MK	R	LR	Out December 2006	111	23	–
SH	MK	R	LR	out May 2006	20	7	–

[a] Two values for focal hours and for fecal samples represent BG2 as a low-ranking male in BT1, and as an immigrant in LC in 2006. In 2007, BG2 was low-ranking resident male in BT1.

[b] Three values for focal hours and for fecal samples represent MF as a low-ranking immigrant male (April–September 2006), low-ranking resident male (October–December 2006; no fecal samples collected during this period) and as a high-ranking resident male in 2007 (KR left CC group at the end of 2006).

[c] HL was a low-ranking immigrant male in 2006 (arrived in April 2006, left in October 2006) but was one of the high-ranking resident males in an unstable dominance hierarchy in 2007 (Arlet and Isbell, 2009).

Gray-cheeked mangabey groups often appear to lack clear male dominance hierarchies (Waser, 1977; Olupot and Waser, 2005), perhaps because reduced visibility, the complex three-dimensional structure of the forest canopy, and wide group spread reduce the frequency of interactions between males. In our study, we also documented numerous reversals in dyadic interactions but we were usually able to distinguish relative ranks, sometimes very clearly. We coded males as HR (high ranking) if the male was clearly the highest-ranking (i.e., alpha male) in the group, or when the difference between two or more relatively competitively successful males was unclear (i.e., nearly as many winning as losing interactions between males within dyads). We coded all other adult males as LR (low ranking). In three of the five groups (LC2, MK, and CC) it was clear that one male won more contests with all other males and could be viewed as an alpha male. In two of these (LC2 and MK) there were two adult males in 2006 but only one adult male in 2007 because the lower-ranking males left. In these groups data from 2007 were not used in our model. In BT1 it was unclear which of two males (males LM and PL) held the highest position in 2006, and so they were both coded as HR relative to the other two males in the group, but in 2007 we could confidently rank one male (male LM) as the alpha male. LC had unclear ranks among most males during both observation periods. Four males usually won aggressive encounters with the rest of the males, so we drew the line between HR and LR there.

Immigrants were adult males who joined the group and were seen in two consecutive observation periods (spanning approximately 2 months). After 6 months they were considered resident males based on their glucocorticoid profiles (Arlet et al., 2009b). One of the resident males (BG2) in BT1 group occasionally visited another group (LC) during the first 6 months of the study, and was coded as an 'immigrant' during these visits. All immigrants except one (HL) entered the groups as lower-ranking males. One of the immigrants (MF in CC group) rose in rank from lower-ranking to highest-ranking rapidly after the previous alpha male left CC group. Unfortunately, no fecal samples were collected from him during his 'lower-ranking' period.

Collection of fecal samples

Fecal samples were collected from 21 adult males during focal samples from December 2005 to July 2006, and from January to July 2007. We did not collect fecal samples on the first observation day of each period because we did not know what experiences males had during previous days when they were not under observation. After the first day, when a focal individual defecated, the time was noted and the sample was immediately collected, placed in a sterile scintillation vial, and stored on ice in a cooler. If a focal individual defecated more than once during a focal follow, each fecal sample was collected and stored separately. Our approach for incorporating these repeated observations is described in the Statistical analyses section. At the end of the day, all samples were placed in a −20 °C freezer until hormone solubilization (Strier and Ziegler, 1997; Whitten et al., 1999). In our statistical analysis we used 358 fecal samples (matching behavioral data): 314 from 18 out of 24 adult males in 2006 and 44 from 13 out of 15 adult males in 2007. The median number of fecal samples collected per male over the entire study was 6 (range: 4–40; Table 1).

Hormone extraction process in the field

Hormones were solubilized at the field station using the protocol employed for red colobus (Chapman et al., 2006). A fecal sample was removed from the freezer, thawed, and homogenized using a spatula. Then, 0.50 g was solubilized using a 5.0 pH citrate buffer/95% ethanol solution (10 ml, 1:1) that was mixed for 21–27 h. After mixing, samples were spun in a centrifuge for 30 min at 3200 rounds/min to separate the supernatant containing the hormones from the fecal pellet, and then 2 ml of the supernatant was passed through a solid phase extraction cartridge (Alltech maxi-clean filter) for storage and transport to the U.S. (Strier and Ziegler, 2005). The dry weight of each sample was calculated using the percent water of a sample from the same homogenized fecal sample that the hormones were extracted from, by drying these samples to constant weight in the field (Chapman et al., 2006).

Hormone extraction in the laboratory

The samples were sent to the Wisconsin National Primate Research Center's (WNRPC) Assay Services, where the method for measuring testosterone concentration was validated and samples analyzed. An assay comparing serial dilutions of pooled mangabey samples to testosterone standards found no significant difference between the slopes ($t = 1.903$, $df = 26$, $p = 0.07$). Accuracy was determined by superimposing values from pooled mangabey samples on a standard curve. The mean accuracy over eight pooled samples was 109.19% with standard deviation 2.17%. The inter-assay coefficients of variation were 10.6 and 11.7 for the low and high pools, respectively, and the intra-assay coefficients of variation were 3.5 and 1.9 for the low and high pools, respectively.

At the WNPRC, testosterone was extracted from the filters. The cartridges were washed with 1 ml of 20% methanol and the columns were eluted with 2 ml methanol. This methanol was dried, resuspended in 1 ml ethanol, and 50 μl was taken for the enzymeimmunoassay (EIA). The WNPRC lab used the antibody R159, which was developed by Munro and Stabenfeldt (1984) and is well characterized. The antibody

LOSER

GROUP: BT1	LM	PL	BG2	BT
LM	XX	9	6	5
PL	5	XX	7	1
BG2	2	4	XX	4
BT	2	1	3	XX

WINNER (applies to BT1 rows)

LOSER

GROUP: CC	MF	KJ	KA	KK
MF	XX	7	6	4
KJ	3	XX	4	7
KA	2	1	XX	1
KK	0	1	1	XX

WINNER (applies to CC rows)

LOSER

GROUP: LC	MG	YM	MS	MK	BW
MG	XX	7	4	3	5
YM	4	XX	6	3	4
MS	2	4	XX	2	2
MK	3	2	2	XX	2
BW	3	3	1	1	XX

WINNER (applies to LC rows)

Fig. 1. Male dominance matrices for three groups of mangabeys in 2007 (groups LC2 and MK had only one adult male in 2007 and are not included here). For male dominance matrices of mangabeys in 2006, see Arlet and Isbell (2009).

crossreacts 92.4% with dihydrotestosterone, 11.2% with 4-androsten-3beta, 17beta-diol, 5.4% with dehydroandrosterone, 3.4% with androstenediol, 2.1% with androstenedione and less than 1% with other steroids (Ziegler et al., 1995). These analyses provided data on the metabolites of testosterone found in the supernatant. The results validated the use of EIA for fecal testosterone in gray-cheeked mangabeys. The amount of testosterone metabolites in each sample was measured in nanograms per gram of dry feces.

Statistical analyses

Our observational methods incorporated an expected lag in the timing of fecal elimination and sample collection, relative to a male's recent life history events. We used behavioral data on the 21 adult males for which we had fecal samples (Aggression, i.e., involvement in an aggressive encounter; N = 192) and data on presence of females with peak-swelling (N = 202) that were observed 1–3 days prior to the collection of a fecal sample forming a time window that accommodated the entire cycle of behavioral stimulus, hormonal pulse, retention in the gut, and eventual elimination. The mean digestive transit time and mean retention time for gray-cheeked mangabeys were reported as 22.7–38 h (Lambert, 1998). Other variables of interest, such as status and group size, do not vary appreciably on this time scale and do not need to be lagged. It is possible that aggressive interactions occurred during the two days prior to an observation period (we started collecting fecal samples on day two of observation periods with each group). Aggression is recorded as absent for the observations affected by this censoring. If aggression is in fact associated with transient high testosterone levels, we may tend to over-estimate baseline testosterone levels, hence under-estimate the effect of aggression, due to this censoring.

Model building

The distribution of testosterone concentration was compared for samples taken in the morning and in the afternoon and no significant difference was found (Kolmogorov–Smirnov D = 0.10, p = 0.48). We also found no significant difference in concentration of testosterone between samples from 2005 to 2006 and those from 2007 (Kolmogorov–Smirnov D = 0.11, p = 0.69). Therefore, we did not consider these factors in the model.

Model selection was conducted using F-tests for marginal significance tests of fixed effects and Akaike information criterion (AIC) for other model parameters, i.e., random effects and correlation structure (as advised in Section 2.4 of Pinheiro and Bates, 2000). Modeling was carried out using the package nlme (Pinheiro et al., 2009) for R. The starting point was a model that included only the intercept and an exponential correlation structure with nugget (as given in Section 5.3 of Pinheiro and Bates, 2000) to account for the positive dependence of observations of the same male that are taken

close in time. This model is referenced as a null model. Our null model thus assumes that the correlation decays over time exponentially (for the same male) with the range parameter giving the rate of decay while the nugget parameter describes the correlation of two hypothetical observations (of the same male) that are taken at the same time. More specifically, this correlation is unity minus the nugget.

Initially, male-specific and group-specific random effects were considered (i.e., a different baseline for each male or each group) to improve the null model but in both cases the respective variance component was estimated to be zero and therefore these random effects were not retained in the model. The following predictors were then considered to improve the null model: 1) Aggression (coded as Present if the male was observed to be involved in an aggressive interaction 1–3 days prior to the fecal sample collection, and Absent otherwise), 2) Dominance rank (the male's rank at the time of sample collection (2006 or 2007), 3) Status—resident (R) or immigrant male (IM), 4) Group Size (the total number of adults in the group at the time of sample collection), 5) Number of Males (the total number of adult males in the group at the time of sample collection), 6) Number of Peak-swelling Females (number of females in the group who reached peak swelling 1–3 days prior to sample collection), 7) Three-day Average Rainfall (calculated by averaging rainfall amounts in millimeters, measured by a rain gage placed in a fixed location at the study site, over the three days prior to sample collection) and 8) Three-day Average Air Temperature. We view Rainfall and Temperature as predictors that could potentially capture effects of environmental variation on testosterone.

The order of inclusion of the possible predictors in the model was determined by the maximal decrease in AIC, but for each variable to be retained in the model, the respective marginal F-test needed to reject the null hypothesis. Finally, all possible interactions of the variables were taken as possible candidates to be included.

In all of the models considered, the natural logarithm of testosterone was used as the response variable to symmetrize the distribution and stabilize the variance. In other words, the model parameters also describe the average levels of the logarithm of testosterone but, where possible, results are interpreted on the original (non-logarithmic) scale. All of the presented confidence intervals (CI) have a 95% chance of covering the real parameter value. Because the logarithm of testosterone has been modeled, the parameter estimates should be considered as multiplicative and have to be exponentiated to interpret them on the original scale (ng/g). Interpretation is then possible when other conditions are kept fixed.

Finally, it should be noted that when starting with a null model that includes the male-specific random effect but not the correlation structure, *another* possible final model can be obtained. This model includes more predictors and, while theoretically one might expect that including all the important covariates in the model would guarantee that there is no autocorrelation left in the residuals, this is not actually the case and as such the model assumptions are violated.

Results

Model selection

In the final model fixed effects of Rank, Status (Immigrant vs. Resident), Number of Peak-swelling Females, and Three-day Average Air Temperature were retained whereas Aggression, and Three-day Average Rainfall were not retained. Together with the correlation structure parameters, the four included predictors made up the model as neither of the random effects improved the value of AIC. There were insufficient data to consider group effects based on both Number of Males and Group Size simultaneously in the model.

Testosterone levels in relation to model selection

Parameter nugget suggests that the correlation between two observations of log-testosterone of the same male taken (hypothetically) at the same time and conditions is, $1-0.33 = 0.67$ with CI (0.56, 0.77). The residual correlation (after accounting for the model) of two observations of log-testosterone taken d days apart from the same male is calculated to be $0.672 \exp(-d/36.579)$. This means that the residual correlation after two days is estimated to be 0.63 with CI (0.50, 0.75).

On average, low-ranking males had testosterone levels that were 21% lower $(\exp(-0.231) = 0.79$ with CI (0.67, 0.93)) than high-ranking males. Immigrant males had 36% lower testosterone levels than residents, on average $(\exp(-0.454) = 0.64$ with CI (0.51, 0.79)) (Table 2).

This pattern among males was also corroborated for the two males that changed status during the study: testosterone levels of BG2 were lower when he was an immigrant and marginally higher when he was a low-ranking resident male (not a significant difference). Immigrant male MF (in CC group), showed a significant increase in testosterone level when his status changed from (low-ranking) immigrant to high-ranking resident male (Fig. 3).

The model shows that each additional peak-swollen female increased average testosterone level for all males in the groups by $\exp(0.089) = 1.09$ times with CI (1.03, 1.16) (Fig. 2).

Maximal average temperature in the sample differed from minimal average temperature by 6 °C (19.1 °C and 14.3 °C respectively). The effect of temperature was negative (a 7% decrease in testosterone for each additional degree compared with a global average with CI (0.88, 0.98)) i.e., hotter days were associated with lower testosterone levels.

Discussion

Our study shows that fecal testosterone concentrations in male gray-cheeked mangabeys 1) are higher in males very high in the dominance hierarchy than in low-ranking males, 2) are lower in immigrant males than in resident males, and 3) increase in all males when more females are at the peak of sexual swelling within a given group. These findings are consistent with the predictions outlined in the Introduction and with the majority of studies of other male primates.

We interpret higher testosterone levels in males of very high rank as the result of their effort to maintain their ranks, which often involves aggressive interactions. Previous studies have shown that in gray-cheeked mangabey groups the highest-ranking males received the highest proportion of sexual presentations and gained the highest mating success (Arlet et al., 2007, 2008). High levels of testosterone may be required for such males to maintain a competitive edge and

Table 2
Parameter estimates for the extended linear models of log-Testosterone.

AIC	Final model			Null model		
	339			376		
	Estimate	SE	p	Estimate	SE	p
Intercept	3.822	0.106	<0.001	4.167	0.056	<0.001
No. peak-swelling females	0.089	0.032	0.006			
Status = R	0.454	0.110	<0.001			
Rank = LR	−0.231	0.083	0.005			
Temp	−0.072	0.026	0.006			
Nugget	0.328			0.318		
Range	36.579			38.495		
Residual SE	0.424			0.525		

AIC is measured when parameters have been estimated using Maximum Likelihood (ML), and parameter estimates have been obtained using Restricted Maximum Likelihood (REML).
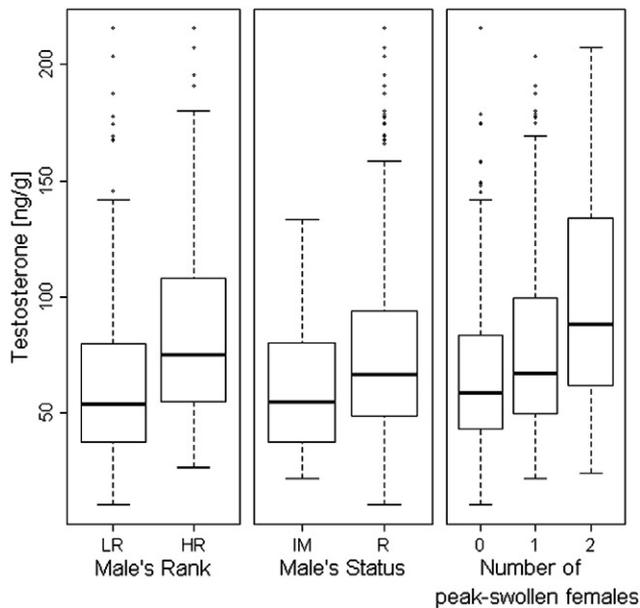
Fig. 2. Boxplots of fecal testosterone concentrations: by male rank—LR: low-ranking male (158 samples) and HR: alpha and other very high-ranking males (200 samples), by male residency status—IM: immigrant males (31 samples); R: resident males (327 samples), and with different numbers of peak-swelling females in the groups. Boxplots represent median and 1st and 3rd quartiles with the whiskers extending to the most extreme data point which is no more than 1.5 times the interquartile range away from the box.

retain their positions. Although the cause-and-effect relationship between high status and elevated testosterone is somewhat unclear, it is reasonable to conclude that for alpha males and other very high-ranking males the reproductive benefits of elevated testosterone outweigh the costs of increased risk of mortality, disability, or disease from male–male competition or high testosterone.

In contrast, because the chances of low-ranking males of succeeding in matings are poor (Arlet et al., 2008), and high testosterone levels may exhaust resources and inhibit immune function, such males may benefit from lower testosterone levels because they have little chance of obtaining matings. Our findings of a relationship between rank and testosterone in gray-cheeked mangabeys are also consistent with findings in other primates (e.g. Rose et al., 1975; Coe et al., 1983;
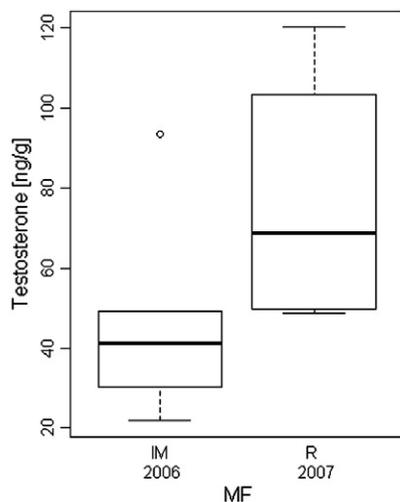
Fig. 3. Boxplots of fecal testosterone concentrations of MF as an immigrant (IM) in CC, and a year later as a highest-ranking resident male (HR) in that same group. Boxplots represent median and 1st and 3rd quartiles with the whiskers extending to the most extreme data point which is no more than 1.5 times the interquartile range away from the box.

Eberhart et al., 1985; Steklis et al., 1986; Bercovitch, 1993; Kraus et al., 1999; Lynch et al., 2002; Muehlenbein et al., 2004).

Our results also showed that immigrant males had lower testosterone concentrations than resident males. Previously, we found that immigrants also had the highest levels of fecal glucocorticoids (Arlet et al., 2009b). We suggest that their high stress levels as new males in groups negatively affected their testosterone levels. A negative correlation between stress and testosterone has also been found in baboons (Sapolsky, 1993), to which mangabeys are closely related phylogenetically.

The function of competition among males for the alpha position is most likely to ultimately enable access to receptive females for mating. We previously showed that aggressive interactions were more common as the number of females with peak swellings increased (Arlet et al., 2009b). In the current analysis, we found that male testosterone levels were higher when more swollen females were present in the group, but surprisingly, we did not find an association between aggression and testosterone. Associations between receptive females and elevated testosterone in males have been reported in long-tailed macaques (*M. fascicularis*) (Girard-Buttoz et al., 2009) and in one study of chimpanzees (Muller and Wrangham, 2004). Another study of chimpanzees, however, reported no such effect (Muehlenbein et al., 2004).

Finally, our results showed that temperature was negatively correlated with testosterone concentrations. The same pattern has been found in yellow baboons (Gesquiere et al., 2011), human males (Celac et al., 2009), and domestic mammals (Hansen, 2009). Whether this becomes problematic for gray-cheeked mangabey reproduction as the world becomes hotter remains to be seen.

This study contributes to a broadening knowledge of male gray-cheeked mangabeys. Future research should consider how testosterone and glucocorticoids are influenced by characteristics of males beyond rank, e.g., personality, as has been found in baboons (Sapolsky, 2004). Recognition that males vary considerably in their spatial associations within groups (Olupot and Waser, 2005) suggests that this may be an area of fruitful discovery.

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