



## Social correlates of fecal testosterone in male ursine colobus monkeys (*Colobus vellerosus*): The effect of male reproductive competition in aseasonal breeders

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### ABSTRACT

Male testosterone (T) levels are thought to be linked with the mating system, degree of parental care, and male–male aggression in reproductive contexts (The ‘challenge hypothesis’; Wingfield et al., 1990). In many species though, T increases associated with mating behavior cannot be separated from those associated with male–male aggression. We tested the challenge hypothesis on aseasonally breeding ursine colobus (*Colobus vellerosus*), where male–male competition is intense outside of mating contexts. Fecal samples ( $N=109$ ) were collected from >27 subadult and adult males in seven groups during 13-months of research in Ghana in 2004–2005. Fecal T (fT) levels were determined by enzyme immunosorbant assays. Behavioral data was collected using focal-animal and *ad libitum* sampling. The number of receptive females in each group did not positively correlate with male fT. There was a trend for adult males to have higher fT than subadult males; however there was no effect of rank on fT. The level of male–male aggression experienced was positively correlated with fT and individual males showed higher mean fT during ‘challenge’ than during ‘non-challenge’ periods. The number of male incursions experienced positively correlated with fT whereas the number of between-group encounters did not. Males attempt to gain reproductive opportunities during incursions, thus these results support the ‘challenge hypothesis’ in *C. vellerosus*. Outside of mating contexts, higher male fT levels are associated with increased aggression. Male parental investment in the form of infant defense was associated with increased fT, rather than the decline expected from other forms of paternal care.

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### Introduction

For vertebrates, the anabolic steroid testosterone (T), is associated with reproductive functions (Balthazart, 1983; Griffin, 1996), the formation of male secondary sexual characteristics (Wickings and Dixon, 1992; Dixon, 1998), muscle mass gain (Kemnitz et al., 1988; Welle et al., 1992; Bardin, 1996), and the maintenance of musculoskeletal performance (Bribiescas, 2001). In primates, there is a link between high T levels and risk taking and initiative behaviors (Donovan, 1985; Archer, 1991; Booth et al., 2006), but T's effect on behavior is dependent on previous experiences and the social context (Sapolsky, 1993). Thus, T does not cause aggressive or ‘status-maintaining’ behaviors but increases the likelihood that they will be expressed (Booth et al., 2006) and appears most important in direct competition between males when some change in status may occur (Mazur, 1985; Mazur and Booth, 1998; McCaul et al., 1992).

These conditions suggest that male T levels and social rank should be positively correlated, which has been found for several species of primates (e.g. Rhesus macaques, *Macaca mulatta*, Bercovitch, 1983; lesser mouse lemurs, *Microcebus murinus*, Perret, 1992; mountain

gorillas, *Gorilla beringei beringei*, Robbins and Czekala, 1997; Verreaux's sifakas, *Propithecus verreauxi*, Brockman et al., 2001; mandrills, *Mandrillus sphinx*, Setchell and Dixon, 2001; chimpanzees, *Pan troglodytes schweinfurthii*, Muller and Wrangham, 2004; bonobos, *Pan paniscus*, Marshall and Hohmann, 2005). However, a higher number of studies failed to show such a correlation (e.g. Rhesus macaques, *Macaca mulatta*, Gordon et al., 1976; vervet monkeys, *Chlorocebus aethiops sabaeus*, Steklis et al., 1985; stump-tailed macaques, *M. arctoides*, Nieuwenhuijsen et al., 1987; muriquis, *Brachyteles arachnoides*, Strier et al., 1999; red colobus, *Piliocolobus tephrosceles*, Firos, 2000; Japanese macaques, *M. fuscata*, Barrett et al., 2002; tufted capuchins, *Cebus apella nigrurus*, Lynch et al., 2002; redfronted lemurs, *Eulemur fulvus rufus*, Ostner et al., 2002; bonobos, *Pan paniscus*, Sannen et al., 2004). Beyond variation in methodology and social systems that may explain some of the differences, it appears that maintaining high T is costly in terms of immunosuppression (Grossman, 1985; Grossman et al., 1991) and increased time and energy expenditure, which is detrimental to survivability (Marler and Moore, 1988; Muehlenbein and Bribiescas, 2005; Hau, 2007). Thus, rank differences in T levels for some species may only be apparent when there is instability in the hierarchy (Sapolsky, 1993) or when males are contesting for a limiting resource, such as estrus females (e.g. Japanese macaques, *M. fuscata*, Muroyama et al., 2007).

The ‘challenge hypothesis’ was developed from research on birds and states that T levels in adult males are closely linked with the

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mating system, degree of parental care, and male–male aggression in reproductive contexts (Wingfield et al., 1990). It predicts that, in the absence of mating behavior, males should maintain only the low baseline T level (Level A) required for the feedback regulation of GnRH and gonadotrophin release. For seasonally breeding species, environmental cues such as increased day length should lead to an androgen response in males sufficient for spermatogenesis and the expression of reproductive behaviors (Level B). Increases in T beyond Level B to Level C should be seen facultatively when males compete in reproductive contexts or during interactions with receptive females (Goymann et al., 2007). High levels of T (at Level C) seem to interfere with parental behavior, so the challenge hypothesis predicts a decrease in T levels when males are required to show a high degree of parental care. When no male parental care is required, males should maintain a high level of T throughout the breeding season (Level B–C), while if only some male parental care is needed a compromise between these two androgen patterns should be seen (Wingfield et al., 1990; Goymann et al., 2007).

The challenge hypothesis has been supported in many bird species (Wingfield et al., 2000) but for most nonavian vertebrates the results have been equivocal (Hirschenhauser and Oliveira, 2006). Several recent primate studies have found that the hypothesis generally appears valid for this group of animals (ring-tailed lemurs, *Lemur catta*, Cavigelli and Pereira, 2000; Gould and Ziegler, 2007; tufted capuchins, *Cebus apella nigitus*, Lynch et al., 2002; redfronted lemurs, *Eulemur fulvus rufus*, Ostner et al., 2002; chimpanzees, *Pan troglodytes schweinfurthii*, Muller and Wrangham, 2004; moustached tamarins, *Saguinus mystax*, Huck et al., 2005; bonobos, *P. pansicus*, Marshall and Hohmann, 2005; golden lion tamarins, *Leontopithecus rosalia*, Bales et al., 2006; chacma baboons, *Papio hamadryas ursinus*, Beehner et al., 2006; mantled howlers, *Alouatta palliata*, Cristóbal-Azkarate et al., 2006). However, for many of these studies it has been difficult to examine increases in T due to male aggression without the confounding increases that are caused by courtship and mating behaviors, an association often due to seasonal breeding (e.g. Cavigelli and Pereira, 2000; Lynch et al., 2002; Ostner et al., 2002; Gould and Ziegler, 2007).

Ursine black-and-white colobus monkeys (*Colobus vellerosus*) provide the opportunity to examine the correlation between social factors and T excretion under conditions where male–male aggression and mating behavior often occur separately. We therefore tested the challenge hypothesis on this species at the Boabeng–Fiema Monkey Sanctuary in Ghana to determine the social situations associated with increased T levels for males. Variation in male T levels has never been investigated in a black-and-white colobus species and has rarely been studied in colobines (but see: Firos, 2000; Wich et al., 2003; Gao et al., 2003; Ren et al., 2003). *Colobus vellerosus* shows aseasonal mating behavior but male competition for group membership and high dominance rank (both of which increase mating possibilities in the future) is intense. Interactions with extra-group males (during between-group encounters and male incursions) may threaten resident male(s)' reproductive investment in their group because extra-group copulations, attacks on immatures, and take-over attempts may occur (Sicotte and MacIntosh, 2004; Teichroeb et al., 2005; JAT, unpubl.data). Indeed, the immigration of new males often leads to takeovers and infanticide (Saj and Sicotte, 2005; Sicotte et al., 2007; Teichroeb and Sicotte, 2008). High-ranking males tend to monopolize receptive females in multi-male groups through mate-guarding (males stay in close proximity to receptive females and prevent lower-ranking males from mating with them), thus dominance relationships between males appear to mediate access to fertile females in this species (JAT, unpubl. data). A similar effect of dominance is reported in many primate species (reviewed in: Smuts, 1987) and may skew paternity in favor of dominant males (reviewed in: Campbell, 2007). We therefore looked at fecal T (fT) levels for individual males in relation to the number of receptive females in the group, age, rank, the number of between-group encounters and male incursions, and the

overall level of male–male aggression experienced to test the challenge hypothesis. We predicted that fT would be higher in adult males compared to subadults and that males would show an increase in fT when their aggression rates were high (during times of 'challenge') and when encounter rates with extra-group males were frequent. In the absence of challenges, we did not expect that dominant males would maintain higher fT than subordinates.

## Methods

### Study site and subjects

Research on ursine colobus monkeys (*C. vellerosus*) has been conducted at the Boabeng–Fiema Monkey Sanctuary (BFMS), central Ghana (7° 43'N and 1° 42'W) under the direction of PS since 2000. This is a dry semi-deciduous forest fragment, 191.6 ha in size, located 350 m above sea level in the Nkoranza district of the Brong–Ahafo Region. The vegetation is a mosaic of primary forest, regenerating farmland (secondary forest), and woodland (Fargey, 1991; Saj et al., 2005). Annually, there are two rainy seasons and two dry seasons. Rainfall was monitored daily during this study in a rain gauge located <1 km from the study groups' home-ranges. The annual rainfall at BFMS during this time (July 2004–June 2005) was 1329 ml (monthly range: 0.4 to 227.6 ml). Nineteen bisexual groups of *C. vellerosus* reside at the site with a growing population of Campbell's mona monkeys (*Cercopithecus campbelli lowei*) (Kankam, unpub. data). Group sizes vary considerably (range: 9–38, mean: 15.0,  $N=15$ ; Wong and Sicotte, 2006) and group composition is multi-male and uni-male, with the presence of all-male bands (Teichroeb et al., 2003; Saj and Sicotte, 2005). *Colobus vellerosus* at BFMS is mainly folivorous, with leaves representing 79–89% of the diet.

### Behavioral observations and sample collection

Behavioral observations and fecal sample collection were done on four groups of *C. vellerosus* (WW, DA, B2 and RT) and an all-male band (AMB) during 13-months (July–November 2004, January–August 2005). Additional fecal samples were also taken from the single adult male of two additional groups (OD and SP), although no behavioral data was collected on them. The size and composition of the groups and the number of hours of data collection are provided in Table 1. All males except those in the AMB were individually recognized by features of the face and tail. Two-hundred and eleven full-day follows were conducted for the four study groups and the AMB. The four focal groups were followed for two, two-day periods per month from dawn to dusk (6:00 am to 6:00 pm) by JAT with the help of a research assistant. Behavioral observations were recorded during 10-min focal samples that were alternated among adult and subadult individuals. *Ad libitum* datum collection was employed to record aggressive events and copulations that occurred outside of focal-samples (Altmann, 1974).

Male age was sometimes known from previous contact with that individual but in most cases, it was estimated from the size of the individual relative to individuals of known age. Subadult males were either smaller or the same size as adult females and ranged in age from approximately 3–6 years old. Males were defined as adult when they had achieved full body size (i.e. were larger than adult females) and regularly participated in loud call bouts with the other adult males (at  $\geq 7$  years of age). Male dominance relationships were determined from the direction of aggressive displacements and submissive avoids and pant-grunts during focal samples and contact hours. Dominance relationships within each group were linear and males could be assigned a number ranking. The intensity of male–male aggressive events was taken from both focal-animal and *ad libitum* data and was categorized daily for each male in three levels: 0=no aggression observed; 1=low-intensity aggression: threats (aggressive stiff-legs and jump displays (Oates, 1977)) were given or received by the focal-male; and 2=high-intensity aggression: chases or contact aggression was observed between the focal-male and other males.

Between group encounters and male incursions occurred whenever individuals from two groups came within 50 m of one another (Oates, 1977). They differed in that

**Table 1**  
Study group composition, contact and focal hours

Name	Group size	Adults		Subadults		Juveniles/ infants	Contact hours <sup>a</sup>	Focal hours
		M	F	M	F			
RT	13	1	5	1	1	5	567.5	106.5
B2	13–17	1–3	4	2–4	0–1	4–5	574.5	102
DA	21–31	3–8	9–10	3–5	1–3	4–5	574	99.8
WW	28–33	6–10	10–11	2–6	2–3	2–5	690	125
AMB	4–10	0–3	0–1	4	0–2	0	101	1
OD	18	1	8	0	4	5	20	0
SP	7	1	4	0	0	2	20	0
Totals							2547	434.3

<sup>a</sup> Including JAT and research assistants.

between group encounters ( $N=163$ ) involved whole groups and both sexes coming into proximity whereas male incursions ( $N=85$ ) involved only male(s) from another group approaching and often chasing repeatedly individuals in a group (Sicotte and MacIntosh, 2004). Encounters separated by at least one h were considered distinct.

The fT values of individual males were compared when the males were going through periods of 'challenge' versus 'non-challenge'. 'Challenge' periods were when the focal male was: 1) a new immigrant to the group; 2) attempting to rise in rank; 3) being evicted from the group by other male(s); and 4) attempting to evict other male(s). These events tend to be associated with infanticidal attacks (Teichroeb and Sicotte, 2008), which leads to two further contexts for challenge periods; when male(s) were: 5) performing infanticidal attacks; or 6) defending infants during attacks by other male(s). 'Non-challenge' periods were times when none of the above situations were occurring for the focal male. Increased male–male aggression was seen during periods of 'challenge' compared with 'non-challenge' periods.

Fecal samples were collected opportunistically from individually recognized males in the six bisexual groups (RT, B2, DA, WV, OD and SP) and five samples were taken from unrecognized males in the AMB. In all, 109 fecal samples were collected representing at least 27 males (20 adult males,  $\geq 7$  years old and 10 subadult males, 3–6 years old) (Table 2). (Three males are represented in both categories because they changed from subadults to adults during the study; for analyses of age and fT levels these males were only included in the category they were in the longest – and only samples from those time periods were used.) We collected a mean of 4 ( $\pm 3$ ) and a median of 3 (range: 13) samples per male. Immediately after defecation, fecal samples were stored in glass vials in 70% ethanol, they were transported to a freezer as soon as possible (usually within 30 days) and stored at  $-40$  °C until analyses began.

#### Hormone analyses

Fecal samples were thawed and a portion was dried and ground to a fine powder before being shipped to the Wisconsin National Primate Research Centre for analyses of fT levels using enzyme immunoassay (EIA). Celite chromatography was used to separate fT from other androgens in the samples according to the System 1 technique by Abraham et al. (1972) with modifications by Ziegler et al. (1996). Between 0.1 and 0.2 g of the dried feces were mixed with 5 ml of 50:50 (EtOH: H<sub>2</sub>O) as this method recovers over 90% of the T in samples. After vortexing, the supernatant was re-extracted with 5 ml of ethyl-acetate and dried. One ml of 90:10 (Isooctane:Ethyl Acetate) was then added and samples were vortexed for 1 min and placed in a warm sonicator until added to their respective chromatography column. Eluted T was dried and re-suspended with 1 ml of EtOH (dilution of the samples varied from 1:25 to 1:50). Samples were diluted with 300  $\mu$ l of testosterone horseradish peroxidase (T:HRP, supplied by C. Munro, University of California, Davis) and plated in 100  $\mu$ l duplicates using the T enzyme immunoassay procedure laid out by Ginther et al. (2001). Assay plates were coated with T-antibody (R156, supplied by C. Munro, University of California, Davis) at a concentration of 1:16,000 (Standards purchased from Sigma (T-1500)). Absorbance for each well was read on a Spectromax 340 (Molecular Devices Corporation, Sunnyvale, California). The sensitivity of the assay was 8.6 ng/g and the antibody showed a 92.4% cross-reactivity with dihydrotestosterone. Results were obtained using weighted least squares regression (Rodbard and Lewald, 1970). The fT EIAs were parallel to the standard curve with no difference in slopes ( $N=6$ ,  $t=-1.11$ ,  $df=24$ ,  $p>0.05$ ; Brownlee, 1960). The accuracy was 109.74 ( $\pm 2.74$ ) and the intra- and inter-assay coefficients of variation for fT were 18 and 4.7% for a low pool and 6.8 and 1.3% for a high pool.

All fT values are presented as ng/g of dry feces. There is some lag time for steroids to appear in the feces of animals because of the time it takes for them to be processed by the liver and reabsorbed by the gut (Ziegler and Wittwer, 2005). Although a study of ring-tailed lemurs showed a positive correlation between serum T and fT (Lemur catta, Cavigelli and Pereira, 2000), colobus monkeys have specialized guts that are long and require slow food passage rates (Dasilva, 1992), thus the T in feces is probably representative of the T that male experienced on the day before the fecal sample was collected. Indeed, when the monkeys in this study were seen to eat soil, it took 15–17 h for their feces to show the red colour associated with the soil. Therefore, when we attempted to correlate behavior to fT values, we used behavioral values from the day before the sample was collected.

Although the fT appears less subject to diurnal variation than T in urine samples (Muehlenbein et al., 2004), some studies have found higher T values in morning samples compared to afternoon samples (urinary T, mountain gorillas, Gorilla beringei beringei, Czekala et al., 1994; serum T, humans, Homo sapiens, van Cauter, 1990; fT, tufted capuchins, Cebus apella nigratus, Lynch et al., 2002; urinary T, chimpanzees, Pan troglodytes schweinfurthii, Muller and Wrangham, 2004). Thus, we compared morning samples (those before 10:00 am) and afternoon samples (those after 10:00 am) in this study. No diurnal variation was found (Wilcoxon signed-rank test:  $N=10$ ,  $z=-0.84$ ,  $p=0.4$ ). Indeed, on the five occasions when we collected fecal samples from the same male on the same day, fT values increased slightly for samples later in the day in four cases and in one case, values decreased. We therefore used samples from throughout the day for our analyses. Wich et al. (2003) also found no diurnal variation in fT levels for another colobine (Thomas langurs, Presbytis thomasi).

#### Data analyses

To determine if fT levels for adult males were related to the number of receptive females, a Spearman correlation was run for each group to see if individual adult male fT values correlated with the number of receptive females on the previous day. Only adult male samples were used because subadult males rarely copulate. A Mann–Whitney U test

**Table 2**  
Fecal sample distribution and testosterone values for males

Group	Male	Age	Rank <sup>a</sup>	# Samples	Mean T (SD in brackets)	Range	Social changes <sup>b</sup>
RT	Hu	SAM	2	8	51.2 ( $\pm 15.8$ )	22.2–70.9	C
	St	AM	1	14	53.7 ( $\pm 21.3$ )	31.6–115.3	C
B2	Gi	SAM	5	3	34.9 ( $\pm 9.9$ )	26.4–48.8	–
	Go	SAM	6	3	33.1 ( $\pm 13.6$ )	21.8–52.2	–
	Li	SAM	6–4	10	60.1 ( $\pm 14.1$ )	36.7–77.3	R
	Fi	SAM/AM	3	4	95.7 ( $\pm 68.3$ )	51.1–213.9	M
	Lo	SAM/AM	4–1	6	45.2 ( $\pm 15.1$ )	27.3–72.2	R, M
	Lx	AM	1	2	47.4 ( $\pm 2.3$ )	45.1–49.7	–
DA	Wo	AM	1–2	6	81.7 ( $\pm 35.8$ )	38.6–133.1	I, R
	Jf	SAM	6	1	59.8	–	–
	Js	SAM	8	1	48	–	–
	Sh	SAM/AM	3	2	51.4 ( $\pm 7.1$ )	44.3–58.5	M
	Ma	AM	2	2	55.9 ( $\pm 0.05$ )	55.8–55.9	–
	Cy	AM	2	1	48.1	–	–
	Do	AM	2–1	2	70.4 ( $\pm 39.2$ )	31.2–109.5	R
	Mc	AM	2	1	68.8	–	–
	Cl	AM	2–5	4	60.3 ( $\pm 13.7$ )	40.8–75.6	R
	Er	AM	4	1	21.4	–	–
WW	Ha	AM	1	6	75.5 ( $\pm 26.4$ )	24.4–123.6	I, C
	Jr	AM	3–4	9	67.2 ( $\pm 26.1$ )	41.1–128.7	C, R
	Nr	AM	7	1	100.5	–	I
	Pe	AM	1	1	55.6	–	–
	Q	AM	2–3	7	44.1 ( $\pm 17.1$ )	19.3–74.2	R
	Ru	AM	6	1	39.7	–	–
OD	Wa	AM	1	3	74.3 ( $\pm 28.6$ )	53.2–114.7	–
SP	Ed	AM	1	5	75.0 ( $\pm 10.6$ )	63.8–93.5	–
AMB	?	SAM and AM		5			Identities unknown
Total	>27			109			

<sup>a</sup> Rank when samples were taken.

<sup>b</sup> Social changes are represented by: C=challenged, I=immigration, M=maturation from subadult to adult, R=rank change.

was done to compare mean fT for adult ( $\geq 7$  years old) versus subadult (3–6 years old) males by lumping males from the six study groups and the AMB. However, the two categories of males were not represented in similar proportions in these groups (subadult male samples were predominantly from group B2 and the adult male samples were predominantly from WW group). This introduces the possibility that differences in the two age categories could instead be due to group membership. We thus present the reader with the mean fT for subadult and adult males in each group where they were available, although no statistical comparison could be done within each group because of small sample sizes. A Mann–Whitney U test was also done to compare the mean fT of alpha versus non-alpha males. Alpha males were those who ranked highest in their individual groups, non-alpha males ranked two to seven. Wilcoxon signed-rank tests were done to compare mean fT for individual males when they occupied higher ranks compared with lower ranks and to compare mean fT during 'challenge' periods and 'non-challenge' periods. The alpha level set at 0.05 for these tests. To examine the association between individual fT level and male–male aggression, three Pearson correlations were run between fT and the previous days' aggression level, number of between group encounters, and number of male incursions (both given and received by the group). We performed a Bonferroni correction for these tests, lowering the alpha level to 0.017, because we reused male fT values. Statistical tests were all two-tailed and done were using SPSS 14 and by hand.

## Results

The mean fT for all samples ( $N=109$ ) was 61.0 ng/g ( $\pm 30.3$ ) and the median was 54.6 ng/g (min. = 19.3, max. = 213.9).

### Mating and female receptivity

Copulations ( $N=81$ ) were recorded in all study months (median per mo.: 6, range per mo.: 2–12,  $N=13$  months). There is the possibility that some of these copulations happened during infertile periods for females as non-conceptive mating has been observed at BFMS (Teichroeb and Sicotte, 2008). We cannot determine whether these non-conceptive matings show a seasonal pattern but births over 17 months in four groups did not show a seasonal pattern (Fig. 1). Births may occur at the same time for several females in one given group due to a pattern of male takeover and infanticide that

results in several females losing infants simultaneously. Those females then become receptive at the same time (Teichroeb and Sicotte, 2008; see below).

Fig. 2 shows the mean fT for males in the four focal groups (RT, B2, DA, and WW) and the mean number of receptive females per group; neither variable shows a seasonal pattern. The rise in the number of receptive females in January–February 2005 was due to the infanticide of three infants in WW group (Teichroeb and Sicotte, 2008) and the weaning of five infants in RT group that resulted from a male takeover and three cases of infanticide in that group in 2003 (Sicotte et al., 2007). The fT values for adult males in three groups were not correlated with the number of receptive females on the previous day (RT:  $N=5$ ,  $r_s=0.22$ ,  $p>0.05$ ; DA:  $N=5$ ,  $r_s=0.71$ ,  $p>0.05$ ; WW:  $N=16$ ,  $r_s=-0.29$ ,  $p=0.28$ ). The last group showed a negative correlation between adult male fT values and the number of receptive females (B2:  $N=8$ ,  $r_s=-0.87$ ,  $p\leq 0.05$ ). This is the opposite relationship to what would be expected if mating behavior were related to increased fT. Thus, it appears that males maintain breeding levels of fT year-round. We did not have enough samples to determine if individual males had elevated fT directly on the day after they copulated, an element that would need to be tested to reach a more definitive conclusion.

#### Age

Overall, mean fT increased with age (subadult males: 48.9 ng/g,  $N=9$ ; adult males: 61.2 ng/g,  $N=19$ ), and there was a trend for adult males to have higher fT values than subadult males ( $N_{adults}=19$ ,  $N_{subadults}=9$ ,  $U=46$ ,  $p=0.055$ ). In the three groups that had both adult and subadult males, adult males had higher mean fT than subadults (RT:  $mean_{adults}=53.7$  ( $\pm 21.3$ ),  $N=1$ ,  $mean_{subadults}=51.2$  ( $\pm 15.8$ ),  $N=1$ ; B2:  $mean_{adults}=55.6$  ( $\pm 40.5$ ),  $N=4$ ,  $mean_{subadults}=42.7$  ( $\pm 12.5$ ),  $N=3$ ; DA:  $mean_{adults}=60.8$  ( $\pm 39.3$ ),  $N=4$ ,  $mean_{subadults}=50.7$  ( $\pm 1.6$ ),  $N=3$ ).

#### Dominance rank

There was no difference in the mean fT values of alpha males compared with non-alpha males ( $N_{alpha}=8$ ,  $N_{non-alpha}=8$ ,  $U=30$ ,  $p=0.87$ ). In addition, when individual males changed rank, there was no difference in their mean fT values when occupying higher ranks compared with lower ones ( $N=7$ ,  $T=11$ ,  $p>0.05$ ).

#### Male–male aggression

Mean fT levels increased with the amount of male–male aggression individuals experienced on the previous day. At an aggression level of 0, mean fT was 43.3 ng/g ( $\pm 10.9$ ,  $N=19$ ), at a level of 1, mean fT was 53.7 ng/g ( $\pm 26.5$ ,  $N=18$ ), and at a level of 2, mean fT was 87.8 ng/g ( $\pm 31.9$ ,  $N=8$ ; Fig. 3). The level of male–male aggression experienced by individuals the previous day was positively correlated with fT levels ( $N=45$ ,  $r^2=0.30$ ,  $p<0.0001$ ). The number of male incursions experi-

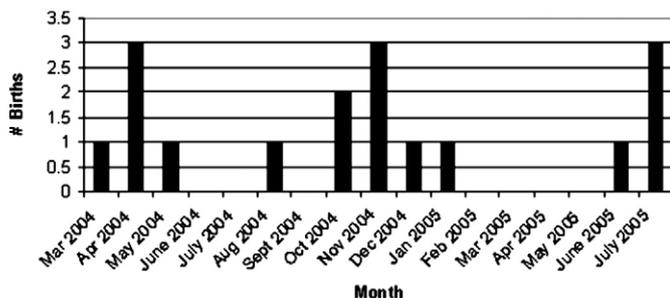


Fig. 1. Births ( $N=17$ ) that occurred over 17 months (March 2004 to July 2005) in four groups (RT, B2, DA, and WW) of *Colobus vellerosus* at BFMS. Wet months ( $>85$  ml of rain) showed 0.92 births/month and dry months ( $\leq 85$  ml of rain) showed 1.0 births/month.

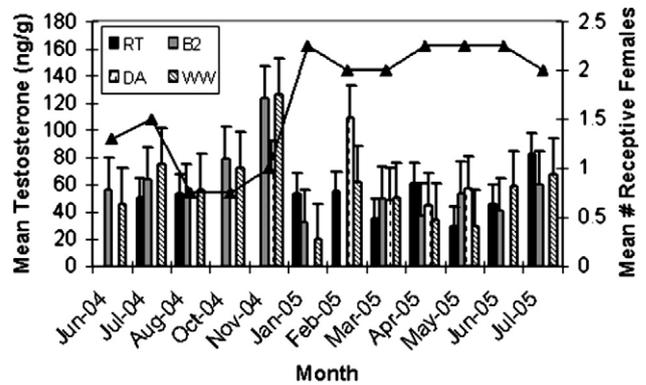


Fig. 2. Mean testosterone for males in each group and the mean number of receptive females per group (females observed mating) during each month of the study. The higher fT values for B2 and WW in November and DA in February are associated with changes in the alpha male in these groups at these times.

enced by the group on the previous day was also positively correlated with male fT values ( $N=46$ ,  $r^2=0.32$ ,  $p=0.015$ ) whereas the number between-group encounters was not ( $N=46$ ,  $r^2=-0.08$ ,  $p=0.31$ ). Since male–male aggression is correlated with fT, this finding is not surprising because 81.2% of male incursions involved level 2 aggression compared with only 48.5% of between group encounters. Males that underwent ‘challenge periods’ (Table 3) had higher mean fT than in ‘non-challenge periods’ ( $N=6$ ,  $T=0$ ,  $p=0.05$ ).

#### Discussion

We did not manipulate male T levels in this study so we cannot draw conclusions about the direction of a causal relationship between fT levels and male–male aggression for *C. vellerosus* at BFMS. However our study supports the notion of a strong link between these two variables. While the number of receptive females was not associated with increased fT levels, male competition for group residency, high rank, and during infant defense was related to peaks in fT for males.

#### The challenge hypothesis

Patterns of T secretion in feces were found to support the challenge hypothesis, which predicts that T levels should rise during male–male aggression in reproductive contexts (Wingfield et al., 1990). Mating is not seasonal in *C. vellerosus* but male rank positively influences access to receptive females in multi-male groups in this species (JAT unpubl. data). We report an increase in fT for males during times of instability in male relationships, such as

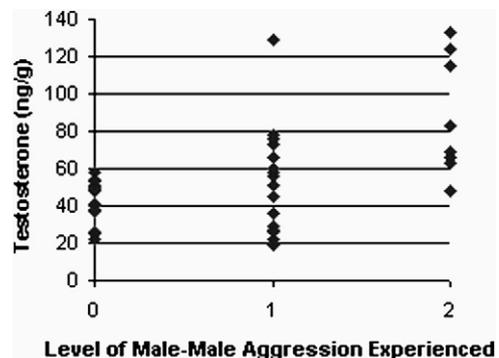
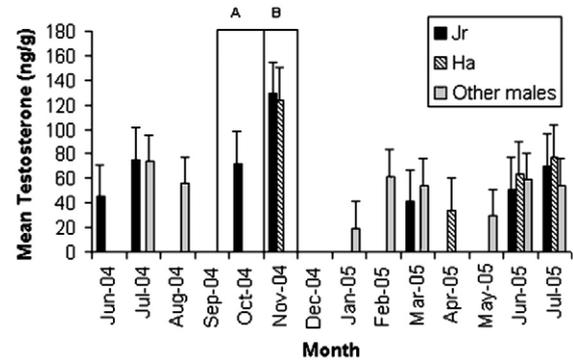


Fig. 3. Testosterone levels present in male fecal samples on the day after experiencing male–male aggression defined as: 0=no aggression (mean=43.3 $\pm$ 10.9,  $N=19$ ), 1=threats between males (mean=53.7 $\pm$ 26.5,  $N=18$ ), 2=chases and contact aggression between males (mean=87.8 $\pm$ 31.9,  $N=8$ ).

after new males immigrated, when evictions were taking place, or when males were attempting increase their rank. Individual males showed higher mean fT levels during these times of ‘challenge’ compared to ‘non-challenge’ periods. The number of male incursions given or received positively correlated with fT levels, whereas the number of between group encounters did not. This is congruent with the fact that male incursions involve more high-intensity aggression than between group encounters and the intensity of male–male aggression experienced by individuals in this study was significantly correlated with fT levels. Male incursions are more of a challenge between males than between group encounters because attempts at male immigrations, take-overs, extra-group copulations, and infanticidal attacks occur during these forays (Sicotte and MacIntosh, 2004). Thus male incursions provide extra-group males with potential reproductive opportunities and allow them to assess the strength of the resident male(s) of neighbouring groups and they represent a real threat for the resident males’ reproductive investment in their group (Sicotte and MacIntosh, 2004; JAT unpubl. data). It is not surprising then that male incursions seem to elicit more of a hormonal response among males than between group encounters. Since mating can occur throughout the year in *C. vellerosus* groups, males seem to maintain breeding levels (equivalent to Level B, Goymann et al., 2007) of T with increases to Level C during times of challenge from conspecific males.

One aspect of the challenge hypothesis that may differ for non-Callitrichid primates when compared with other animals (Hirschenhauser and Oliveira, 2006) is Wingfield et al.’s (1990) prediction that T should drop to baseline levels for males while they are providing parental care. Most primate males reside with their offspring but provide a low level of parental care that may only involve putative sires protecting infants when infanticidal males or predators are present (Borries et al., 1999; Palombit et al., 2000; Weingrill, 2000; Saj and Sicotte, 2005; Teichroeb and Sicotte, 2008). The guarding of offspring in this context is generally considered paternal investment (Ridley, 1978) but it does not involve affiliation between the infant and the putative sire. Infant protection rather involves direct male–male aggression in a reproductive context and thus is predicted to lead to a T increase rather than a decrease (Wingfield et al., 1990). This was indeed the case in this study, with male *Ha* and male *Jr* in WW group. Aggression between these males was associated with increases in fT (Table 3) when male *Ha* attacked four infants and male *Jr* (a likely sire) defended these infants (Teichroeb and Sicotte, 2008). Male *Jr* had higher fT at the point where he was defending these infants than in



**Fig. 4.** Mean testosterone per month for all males in WW group. Period A represents the period during which male *Ha* immigrated and established himself as the new alpha male. (No sample is available for male *Ha* during this period.) Period B represents the period where four infants were born that were attacked by male *Ha* and defended by male *Jr* before they were killed. No other male actively defended the infants (Teichroeb and Sicotte, 2008).

the previous month when male *Ha* immigrated into the group and established himself as the new alpha male (Fig. 4). No other male was seen to defend these infants.

*Testosterone levels and dominance rank*

We found no correlation between fT levels and dominance rank in male *C. vellerosus*, which suggests that when the hierarchy is relatively stable, males do not maintain high T. This appears to be the case in many primate species because male aggression rates are generally low in stable hierarchies (Sapolsky, 1993). It is possible however that the social systems of some species vary from this typical situation. Indeed, males in species that show fission–fusion social organization may interact aggressively despite having relatively stable hierarchies, so that T levels show rank-related differences (chimpanzees, *Pan troglodytes schweinfurthii*; Muller and Wrangham, 2004; Muehlenbein et al., 2004). Since maintaining high T is costly for males (Grossman, 1985; Marler and Moore, 1988; Grossman et al., 1991), over the long-term, high levels could be harmful. The best strategy for males is to maintain baseline levels of T but quickly respond with an androgen increase when they need a short-term competitive advantage (Zuk and McKean, 1996; Whitten and Turner, 2004).

*Age differences in testosterone levels*

Our results show that fT values in *C. vellerosus* males increase with age. This is not surprising since a rise in this hormone at puberty for mammals is related to increases in weight and testicular volume (reviewed in: Anestis, 2006). In chacma baboons (*Papio hamadryas ursinus*) it has been noted that young males that have just reached adulthood have a peak in fT levels, which then decrease steadily as males age (Beehner et al., 2006). A similar pattern has been noted for serum T in captive chimpanzee males (*Pan troglodytes*, Young et al., 1993). Unfortunately, we did not know the ages of the males in our ‘adult male’ category and could not determine if variation in fT values was due to declining values for older males. *Colobus vellerosus* may be similar to baboons though, since one of the males in this study (male *Fi*) showed the highest concentration of fT detected (213.9 ng/g) at the point where he transitioned from subadult to adult.

*Testosterone research in colobines*

This is the first study to specifically test the challenge hypothesis on a colobine species, however T levels have been previously investigated in three other species of colobines. Generally the results of this study fit well with what has previously been reported for other male colobines.

**Table 3**  
Testosterone levels for non-challenge periods vs. challenge periods

Group	Male	Mean T: non-challenge periods (N)	Mean T: challenge periods (N)	Type of challenge
RT	<i>St</i>	46.1±9.5 (12)	99.4±22.5 (2)	Male <i>St</i> is attempting to evict male <i>Hu</i> , with chases and displays, male <i>Hu</i> begins sleeping separate from the group
	<i>Hu</i>	48.6±16.8 (5)	66.5±6.2 (2)	
B2	<i>Wo</i>	58.4±19.7 (4)	128.2±7 (2)	Male <i>Wo</i> takes over B2, evicting three resident adult males and one subadult male
WW	<i>Ha</i>	65.8±18.8 (5)	123.6 (1)	Male <i>Ha</i> enters WW, he becomes alpha and kills four infants, resident male <i>Jr</i> is the main aggressor and infant defender
	<i>Jr</i>	57.7±16.8 (7)	100.4±40 (2)	
	<i>Cl</i>	55.1±14.8 (3)	75.6 (1)	Male <i>Cl</i> immigrates into WW and fights his way to the beta position

Firos (2000) found that dominance was not correlated to urinary T levels for male red colobus (*Piliocolobus tephrosceles*) at Kibale National Park, Uganda. For Thomas langurs (*Presbytis thomasi*) in Sumatra, Wich et al. (2003) found that males in all-male bands had lower fT levels than males in bisexual groups, who showed no differences for several life-phases. They attributed higher fT in males in bisexual groups compared with males in all-male bands to the social changes associated with being a single male with a group of females. Captive Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*) showed seasonal variation in urinary T levels that peaked with reproductive behaviors (Gao et al., 2003), however peaks for males were also seen outside of the mating season in association with short-term increases in aggression between males (Ren et al., 2003).

#### Directions for future research on testosterone in primates

Future research on fT levels in primates should include an increased number of samples per male, so that the duration of peaks in fT can be more accurately measured and it can be determined whether there are temporal changes in fT due to copulations in aseasonal breeders. Longer studies may also establish whether fT is important in determining rank changes and the 'winners' of male-male interactions. For instance, in chacma baboons (*Papio hamadryas ursinus*), males ascending the hierarchy had higher fT values than males descending the hierarchy (Beehner et al., 2006) and fT predicted the outcome of male-male interactions better than dominance rank alone (Bergman et al., 2006). It might also be worthwhile to capture males to extract blood, so that a comparison of serum T with fT could give a more accurate assessment of the time it takes for T to be excreted in the feces (e.g. Cavigelli and Pereira, 2000), especially with the specialized digestive system of colobines.

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