Social modulation of testosterone levels in male black howlers (*Alouatta pigra*)

Ariadna Rangel-Negrín [a], Pedro A.D. Dias [b,*], Roberto Chavira [c], Domingo Canales-Espinosa [b]

[a] *Departamento de Biología Animal, Universidad de Barcelona, Gran Via de les Corts Catalanes 585, Cp 08007 Barcelona, Spain*
[b] *Instituto de Neuroetología, Universidad Veracruzana, Av. Dr. Castelazo Ayala S/N, col. Industrial Animas, Ap 566, Cp 91190 Xalapa, Mexico*
[c] *Departamento de Biología de la Reproducción, Instituto de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Colonia Seccion XVI, Tlalpan Cp 14000, México D.F., Mexico*

ARTICLE INFO

ABSTRACT

The influence of social factors on the modulation of male testosterone levels has been demonstrated among several vertebrate species. In addition to sexual activity, parental care and reproductive competition affect testosterone secretion. We examined variations in testosterone levels among male black howlers (*Alouatta pigra*) in various social contexts. Fecal samples were collected from nine males living in five different groups in the Mexican state of Campeche. The potential for intragroup and extragroup competition varied among the groups. The number of resident males living in the groups was the only variable that significantly explained variations in testosterone levels. Males living in unimale groups had higher testosterone levels; the highest testosterone levels were recorded for males that had experienced a shift from multimale to unimale group compositions. In this species, the probability of being challenged by extragroup males and evicted from the group during immigration events increases when males live in unimale groups. Therefore, our results suggest that male black howlers respond to competition for group membership by increasing their testosterone levels. In this context, testosterone secretion represents an anticipatory response to reproductive conflicts. Therefore, although males living in unimale groups have exclusive access to females, they face higher physiological costs associated with sustaining high testosterone levels for extended time periods.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Testosterone (T) is the primary androgen found among male vertebrates, and its secretion is mainly related to the regulation of the reproductive axis (Nelson, 2000). Androgen levels represent a physiological state that is regulated by environmental factors and by the internal state of the individual (Oliveira, 2004). There is evidence suggesting that a cost may be related to maintaining high levels of androgens and that social modulation may help minimize these costs in terms of circulating levels of androgens (Wingfield et al., 2001). Circulating levels of T are affected by a number of social factors, including territorial aggression, mating systems and paternal care. The modulatory effects of these social factors on transitory androgen responsiveness have been modeled by the "challenge hypothesis" (Wingfield et al., 1990). This hypothesis proposes that, at least among male birds, T responsiveness to environmental cues depends on the degree of paternal investment provided by males and on the mating system, such that a monogamous, parental care-giving male will show greater T responsiveness to social challenges than a polygamous, non-parental male (Wingfield et al., 1990). This hypothesis has been corroborated in studies focusing on other vertebrate groups, including reptiles, fish, birds and mammals (e.g., Hirschenhauser and Oliveira, 2006), and has reinforced the idea that the social modulation of male T levels is ultimately related to male–male reproductive competition.

Given the potential costs of high T levels, T secretion should be related to specific social contexts in polygynous male vertebrates, and a number of studies have tested this prediction (Hirschenhauser and Oliveira, 2006). First, the occurrence of sexual behavior is often related to elevated T levels. For example, in the case of several mammals, there is an increase in T during the mating season (e.g., rhesus macaque, *Macaca mulatta*, Herndon et al., 1981; bats, *Pteropus poliocephalus*, McGuckin and Blackshaw, 1991), and there is also evidence that T varies according to the mating system (Wingfield et al., 1990). Among primates, for example, at an interspecific level, monogamous male gibbons (*Hylobates agilis*) have much lower T levels than polygynandrous chimpanzees (*Pan troglodytes*; Coe et al., 1992; Suzuki et al., 2003). However, there is evidence that the relationship between T and sexual behavior is mediated by aggression, as T levels in several species increase when sexual behavior is associated with male–male aggression (Strier et al., 1999; Muller and Wrangham, 2004).

Second, when males provide direct parental care, T levels decrease (e.g., cotton-top tamarins, *Saguinus oedipus*, Ziegler and Snowdon, 2000; rock-pool blenny, *Parablennius sanguinolentus parvicornis*, Oliveira et al., 2001; rodents, Wynne-Edwards and Timonin, 2007). High T may interfere with paternal effort because more time and

energy is allocated to mating. Thus, T levels are adaptively reduced when there is preparation for paternal investment.

Third, direct male–male reproductive competition has been associated with elevated T concentrations (e.g., birds, Wingfield et al., 1987; spiny lizards, *Sceloporus jarrovi*, Marler and Moore, 1989). For instance, there is increasing evidence for a "victory effect" on T concentrations, suggesting that increased T levels following a victory in social conflict prepare individuals for subsequent challenges (e.g., teleost fish, Oliveira et al., 2002; California mice, *Peromyscus californicus*, Oyegible and Marler, 2005; humans, *Homo sapiens*, Archer, 2006). When males reside in permanent groups, T is expected to relate to dominance, if the species participates in frequent aggressive interactions to define rank or during periods of social instability in species in which such competition is not so frequent (e.g., olive baboons, *Papio anubis*, Sapolsky, 1993). Whereas social victories may lead to an increase in T levels in the case of dominant individuals (e.g., squirrel monkeys, *Saimiri sciureus*, Coe et al., 1982; guinea pigs, *Cavia aperia f. porcellus*, Sachser and Pröve, 1986), subordination is associated with lower androgen levels as a consequence of repeated defeats during challenges, reduced sexual activity (e.g., tree shrews, *Tupaia belangeri*, von Holst, 1977; olive baboons, *Papio anubis*, Sapolsky, 1982; rats, *Rattus norvegicus*, Blanchard and Blanchard, 1990), or exposure to non-social stressors (e.g., rats, *Rattus norvegicus*, Bronson, 1973; olive baboons, *Papio anubis*, Sapolsky, 1986).

Therefore, there is ample evidence that the androgen levels of male vertebrates vary as a function of social factors. In primates, social dominance and competition with group mates for females have been found to explain intragroup variations in the T levels of males in species in which male–male aggression is a primary form of male reproductive competition (Anestis, 2010). However, the influence of variation in potential for intragroup and extragroup (i.e., from other groups or solitary individuals) reproductive challenges on male androgen modulation has been rarely assessed (Whitten and Turner, 2004). This question is particularly important because in several primate species, there is significant variation among groups in the number of males per group (Nunn, 1999), in the interindividual differences in access to receptive females (e.g., Hager, 2009), or in the cooperation between resident males for group defense (Kitchen and Beehner, 2007). In this study, we examined variations in T levels among male black howlers (*Alouatta pigra*) in the context of male–male reproductive competition. The variation in social systems (i.e., unimale vs. multimale groups) and potential for extragroup competition (e.g., home range overlap with other groups) exhibited by this species allowed the testing of several predictions concerning the social modulation of T.

Black howlers are non-seasonal breeders that live in permanent social groups, usually composed of one to three males and one to three females (Van Belle and Estrada, 2006). Males provide only indirect parental care, such as affiliation and protection against conspecifics and predators (e.g., Bolin, 1981; Treves et al., 2001; Kitchen, 2004). Compared with interactions with extragroup males, social relationships among resident males are characterized by tolerance and mutual avoidance (Van Belle et al., 2008). Single males and coalitions of two to three extragroup males may invade groups (Horwich et al., 2000; Van Belle et al., 2008). Subsequent to immigration, if resident males are not evicted, dyads composed of two remaining or two entering individuals maintain closer proximity and engage in less agonism than dyads composed of one entering and one remaining male (Van Belle et al., 2008). No dominance hierarchies were discerned in the only study that has focused on the social relationships among the males of this species (Van Belle et al., 2008). However, it has been suggested that, in multimale groups, central males may be recognized by their spatial proximity to females (Van Belle et al., 2008) and that central males have priority of access to receptive females, suggesting that multimale groups may be functionally unimale (Van Belle et al., 2009b). Resident males engage in

cooperative howling bouts during dawn choruses and intergroup encounters, but this participation is conditional on the quality of the relationships maintained among group males (Kitchen, 2004). Loud calls play an important role in intergroup contests and interactions with extragroup males, as they provide accurate information about the minimum number of males present in a group, thus indicating both fighting potential and the probability of winning a fight (Kitchen, 2004).

In line with the challenge hypothesis, which predicts that males will have increased T as a result of reproductive competition, Van Belle et al. (2009b) recently found that central black howler males had higher androgen levels than non-central males. These authors proposed that fostering relationships with females (central males spend more time in proximity with females) may represent a nonaggressive, but nonetheless socially challenging, form of male–male competition in this species. However, although one of their two study groups presented very rapid (one change every 1.25 months) changes in its composition through the immigration and emigration of males (e.g., transitions from multimale to unimale), the authors did not find a significant effect of migrations on male androgens. Likewise, androgens were not related to encounters with adjacent groups or extragroup males (Van Belle et al., 2009b). These results are surprising, as extragroup competition and immigration events imply high costs for male black howlers associated with injuries, infanticidal attacks, infanticide, extragroup copulations by resident females and eviction (Brockett et al., 1999; Horwich et al., 2000; Crockett, 2003; Van Belle et al., 2008, 2009a). Conversely, among closely related mantled howlers (*Alouatta palliata*), a species that presents a typical polygynandrous mating system, the T levels of males were found to correlate positively with intruder pressure, suggesting that males respond to challenges arising from outside their groups (Cristóbal-Azkarate et al., 2006). Van Belle et al. (2009b) noted that the frequent changes in male membership in their study groups may have precluded their ability to detect significant effects of reproductive challenges arising from outside the groups on male androgen levels. Therefore, it remains to be tested whether, in this species, the T levels of males vary mainly as a function of intragroup or extragroup reproductive competition.

To answer this question, we studied variations in the T levels of black howler males in a sample of groups that exhibited differences in terms of potential for intragroup and extragroup reproductive competition (Table 1). First, if variation in the T levels of males is mainly determined by intragroup competition, we predicted that: (1) male–male aggression would increase in the context of reproduction, and that we would find an associated rise in T levels when males copulate with receptive females; (2) T levels would correlate positively with male–male aggression in multimale groups; (3) because male centrality may affect T levels, in stable groups, central males should present higher T levels than non-central males (as found by Van Belle et al., 2009a); and, (4) due to increased reproductive competition in terms of access to receptive females, males living in groups with more males should have higher T levels. On the other hand, if extragroup male–male competition is the main factor affecting variation in T levels of black howler males, we predicted that: (1) the number of males per group should affect competitive ability. Therefore, as males living in smaller groups may be more vulnerable to intruder pressure, males living in groups with fewer males should present higher T levels, and males should respond to the loss of group males by increasing their T levels; (2) if loud calls are associated with competition with extragroup males (i.e., solitary males and resident males from other groups), they may, therefore, be used as a proxy for extragroup competition, suggesting that T levels would vary as a function of the participation of males in loud calls; and (3) extragroup competition should be more intense when several groups share portions of their home ranges, so T levels should also correlate positively with the degree of home range overlap.

Table 1
Characteristics of the study groups.

Group	Area (km^2)	Density[a]	Disturbance[b]	Overlap[c]	Males[d]	Females	Immatures	Copulations
Calax	30	2	No	0	1	3	3	No (yes)
Calakmul	1500	15	No	0	3(1)	2(1)	2(3)	No
Álamo	0.96	63	Yes	3	2(1)	2	0	No
Chilar	0.02	100	Yes	1	2	3	3	Yes
Chicbul	0.05	150	Yes	0	1	2	3	No

[a] Ind/km^2.
[b] Disturbance is defined as small area and presence of human activities (e.g., roads, logging, cattle grazing) inside the primates' habitats. T levels did not differ between males living in disturbed and conserved habitats (GLMM $F_{1,36.8} = 0.002$, $p = 0.989$; see Data analyses section for details on GLMM).
[c] Number of groups that overlapped the home ranges of our study groups.
[d] When changes occurred between sampling periods, values for the second period appear in parentheses.

Methods

Study sites and subjects

This study was undertaken between January 2006 and November 2007 in the Mexican state of Campeche. This state is located in the Yucatan Peninsula, and approximately 39.7% of its territory represents protected areas of tropical evergreen, semi-deciduous and dry forests and mangroves (Gobierno del Estado de Campeche, 2010). However, a number of areas have been extremely disturbed by human activities, and many populations of black howlers currently live in forest fragments that vary in size and in vegetation characteristics. The climate is hot and humid (Vidal-Zepeda, 2005), and the average annual rainfall is 1300 mm, with a drier season from November to May (average monthly rainfall = 43.7 ± 25.8 mm), and a wetter period between June and October (average monthly rainfall = 218.9 ± 14.1 mm). The mean annual temperature is 26 °C.

To test our predictions, we selected five groups of black howlers that showed variations in terms of ecological and demographic attributes (Table 1). Additionally, to analyze the effects of temporal changes in behavior and group characteristics on T, each group was sampled repeatedly during two different sampling periods. First, we observed a group in a forest patch inside a silvicultural farm (18° 48′ N, 90° 58′ W). We sampled this group (called Alamo hereafter) in June and November of 2007. Second, we observed a group (called Calakmul hereafter) in the southern core-area of the Reserva de la Biósfera Calakmul (18° 27′ N, 89° 54′ W) in January and September 2007. Third, in April and July 2007, we sampled a group (called Calax hereafter) in the buffer-zone of the Área de Protección Especial Laguna de Términos (18° 51′ N, 91° 18′ W). Fourth, we observed a group (called Chicbul hereafter) near the village of Chicbul living in a forest patch (18° 46′ N, 90° 56′ W), in December 2006 and again in May 2007. Finally, we observed a group in an area of trees scattered in a chili/maize/pumpkin agricultural field at the Nuevo Ontario settlement (18° 54′ N, 90° 53′ W). This group (called Chilar hereafter) was sampled in April and June 2006.

Data collection

Behavioral data were collected from each group for a total of 8 weeks, divided into two sampling periods (dates provided in the preceding section). During each week, groups were sampled for a 3-day period. A total of 1200 h of observations were analyzed (240 h per group and 30 h per week). We recorded all occurrences (Altmann, 1974) of (1) male–male aggressive interactions (Dias et al., 2009), (2) copulations, and (3) loud calls (Dias et al., 2010). These recordings were performed during full-day follows (10-h sessions from 7:00 to 17:00). During our samplings, we recorded ad libitum the presence of other groups in areas that were used by our study groups. Accordingly, the Álamo and Chilar groups had three and one neighboring groups, respectively, whereas the Calakmul, Calax and Chicbul groups did not overlap with other groups.

To determine male centrality, we studied the spatial proximity of males to females within each multimale group. We used 60-min focal animal samples with instantaneous recordings repeated at 10-min intervals (Altmann, 1974). For each instantaneous recording, we noted the presence of females within a 5-m radius of the focal male. Focal observations were evenly distributed across all males in each multimale group, and all individuals could be identified by facial features, scars, broken fingers, and genital morphology. In all multimale groups, there were significant differences between males in terms of the proportion of instantaneous samplings spent in proximity (i.e., 0–5 m) to females (χ^2 tests for similar expected frequencies: Álamo (first sampling period): $\chi^2 = 7.817$, $n = 2$, $p = 0.005$; Calakmul (first sampling period): $\chi^2 = 213.5$, $n = 3$, $p < 0.001$; Chilar: $\chi^2 = 88.19$, $n = 2$, $p < 0.001$). Therefore, we classified as central those males in the group that spent more time closer to females.

Fecal sample collection and testosterone analyses

Fecal samples were collected opportunistically whenever they could be matched with individuals: fresh samples uncontaminated by urine were collected from the forest floor and deposited in polyethylene bags labeled with the identity of each individual. The bags were kept in a cooler with frozen gel packs while in the field and stored at the end of the day in a freezer at −20 °C at the Sabancuy field station of the Universidad Veracruzana until steroid extraction was performed. Samples were stored for 3 to 5 months until the extractions were conducted. The freezing procedure used for storing the samples has been reported to have a weak time-storage effect on fecal hormone metabolites (Khan et al., 2002). We analyzed 93 fecal samples, with an average ($\pm$ SD) of 10.33 ± 3.67 samples per male, 2.09 ± 0.9 samples per week per male, and 18.6 ± 5.68 samples per group.

Testosterone was extracted following a modification of the method described in Wasser et al. (2000) for the extraction of steroids. Briefly, 0.6 g of homogenized, lyophilized and pulverized feces was shaken for 20 h in 4.0-ml of analytical-grade methanol. Extracts were then centrifuged (460g for 20 min), and the supernatant containing the steroids was recovered. After complete evaporation of the solvent, pellets were reconstituted with a phosphate assay buffer free of steroids (Siemens, Los Angeles, CA, USA) and used for the T analyses.

Steroid assays were conducted at the Instituto de Ciencias Médicas y Nutrición Salvador Zubirán, in Mexico City. Fecal T metabolite levels were measured using a solid-phase chemiluminescent enzyme immunoassay method (Wood et al., 1984), a commercial T kit (Immulite Total Testosterone, Siemens, Los Angeles, CA, USA; sensitivity = 0.5 nmol/l; calibration range = 0.7–55 nmol/l) and an automated analyzer (Immulite, Siemens, Los Angeles, CA, USA).

No captive animals were available for gonadotropin-releasing hormone (GnRH) challenge tests to validate the androgen response of this species (Van Belle et al., 2009b). Therefore, to assess whether our

assay was measuring T metabolites in feces, we first performed partition chromatography on Celite columns and collected fractions for subsequent assays, as described by Abraham et al. (1977). Four fecal samples were prepared following the extraction method described. Fractions of the principal androgens [Δ4 androstenedione (3.5 ml isooctane), 5α dihydrotestosterone (3.5 ml 5% ethyl acetate in isooctane) and T (3.5 ml 15% ethyl acetate in isooctane)] were identified. Although the Immulite Total Testosterone kit (Siemens, Los Angeles, CA, USA) that we used for T analysis reports 0.4% cross-reactivity with Δ4 androstenedione and 2.4% cross-reactivity with 5α dihydrotestosterone, we found levels of 0.006% and 0.76%, respectively, in black howler feces.

As a noninvasive biological validation of our assay, we also compared T levels between eight female and eight male fecal samples. The average ($\pm$SE) T levels in male samples (4485.6 ± 234.3 ng/ml) were nearly 580% higher than those in female samples (659.1 ± 206.7 ng/ml) ($t = 24.49$, $p < 0.001$). Together, these tests indicate that our assay reliably detected T output in the feces of male black howler monkeys.

Howler monkeys' pooled fecal extracts, when added to the standard curve points, exhibited an accuracy of $R^2 = 0.98$ ($n = 4$, $p < 0.001$), and serial dilutions of a fecal pool from male black howlers yielded results that ran parallel to the T standards ($R^2 = 0.99$, $n = 5$, $p < 0.001$). Serum pools (CON6, Siemens, Los Angeles, CA, USA) with three different total T levels (low, medium and high) were used as quality controls. A total of 48 assays were run for each T level, and the intra- and inter-assay coefficients of variation were 7.65% and 7.98%, respectively. The mean recovery of T extracted from fecal samples, as described above, was 59.7% ($n = 6$). All samples were run in duplicate, and mean T values are reported as ng/g dry feces.

Data analyses

There were no seasonal ($F_{1, 91} = 0.622$, dry $n = 50$, wet $n = 43$, $p = 0.433$) or circadian ($F_{1, 91} = 0.622$, morning $n = 50$, afternoon $n = 43$, $p = 0.433$) differences in the T levels of males; therefore, we analyzed hormone concentrations without reference to these variables. Hormonal and behavioral data were averaged for each week of observations.

To test the prediction that T varies as a function of intragroup competition, we performed four analyses. First, to analyze if T would increase when males engage in sexual behavior, we compared T concentrations between males that copulated and males that did not copulate. For this test, we used a generalized linear mixed model (GLMM), with male identity and sampling weeks nested within groups as random factors to control for intragroup variation and repeated measurements of T for each male. Copulations were observed only in the case of one multimale group (Chilar) and one unimale group (Calax). Therefore, in order to analyze the relationship of sexual behavior, male–male aggression and T, we compared rates of aggression and T between weeks when copulations occurred and weeks when no copulations occurred in the Chilar group using Mann–Whitney tests. Then, for each male, we related weekly rates of copulations and aggression by applying Spearman correlations. Second, to analyze whether T increases in response to male–male aggression, we related the weekly T levels and aggression rates of all males and, also, of only central males belonging to multimale groups (i.e., Álamo (first sampling period), Calakmul (first sampling period) and Chilar) using Spearman correlations. Third, we compared T concentrations between central and non-central males living in multimale groups using a GLMM that controlled for the random factors described above. Fourth, we compared T levels among all males using the number of males in each group (i.e., one, two or three) as a predictor variable in a GLMM with the random factors mentioned, followed by Tukey HSD *post-hoc* tests.

To test the prediction that T varies as a function of extragroup competition, we performed three tests. First, we compared T levels among males, using the number of males per group as a predictor variable to verify the prediction that groups with fewer males are likely to be targeted more frequently by extragroup males and, thus, are likely to have higher T levels. Second, to corroborate further this prediction, we focused on two groups (Álamo and Calakmul) that both converted from multimale to unimale group compositions between sampling periods. Specifically, we compared T between sampling periods for the remaining males in these groups using Mann–Whitney tests. To exclude the possibility that changes in the T levels of these males could be associated with other social or ecological factors that varied among sampling periods, we also compared T between sampling periods for males that lived in groups in which male composition was not altered (i.e., Calax, Chicbul and Chilar), also using Mann–Whitney tests. Third, we related weekly T concentrations and loud call rates for all males, as well as for each group, by applying Spearman correlations to test the prediction that T levels may vary as a function of male participation in loud calls. Furthermore, as loud calling rates may vary according to male centrality, we compared the rates of loud calls between central and non-central males of multimale groups that howled (i.e., Álamo (first sampling period) and Chilar) using Mann–Whitney tests. We then related the T levels and loud call rates of central males by applying Spearman correlations. Fourth, we compared T levels among males in terms of the number of groups with home ranges that overlapped with those of our study groups (i.e., none, one and three) as a predictive variable in a GLMM, with male identity and sampling weeks nested within groups as random factors.

In all GLMMs, T levels were log10-transformed to normalize their distribution and equalize their variances.

Our research complied with all laws of the Estados Unidos Mexicanos, and protocols were approved by SEMARNAT (SGPA/DGVS/01273/06 and 04949/07).

Results

Intra-group competition

We observed copulations in two groups, Calax and Chilar. In the first group, copulations were recorded at a rate of 0.008/h, and only occurred during the wet season, whereas in the second group, the rate was 0.04/h, and copulations were observed during both seasons. The average ($\pm$SE) T levels across the five groups did not differ between males that copulated (3262.1 ± 921.6 ng/g) and those that did not copulate (2567.8 ± 385.1 ng/g; GLMM $F_{1,36.8} = 0.002$, $p = 0.989$). In the case of the Chilar group (the only multimale group where copulations were observed), both males copulated with receptive females during both sampling periods. In this group, although male–male aggression was higher during the weeks when males copulated (with copulations: 0.36 ± 0.395/h; without copulations: 0.24 ± 0.312/h), this difference did not reach statistical significance ($Z = 0.746$, $p = 0.455$). Likewise, the T levels were not significantly higher in weeks with copulations than in weeks without copulations ($Z = 0.348$, $p = 0.728$), and no relationship was found between weekly rates of copulations and aggression in both males (M1: $r_s = 0.30$, $n = 8$, $p = 0.322$; M2: $r_s = 0.24$, $n = 8$, $p = 0.518$).

In multimale groups, male–male aggressive interactions occurred at a mean ($\pm$SD) rate of 0.04 ± 0.02 interactions/individual/hour and were not associated with variations in T levels among all males ($r_s = -0.18$, $n = 32$, $p = 0.191$) or central males (Álamo: $r_s = 0.40$, $n = 8$, $p = 0.121$; Calakmul: $r_s = 0.21$, $n = 8$, $p = 0.076$; Chilar: $r_s = 0.02$, $n = 16$, $p = 0.783$). In these multimale groups, non-central males showed higher (2213.9 ± 236.99 ng/g) T levels than central males (2075.5 ± 89.36 ng/g), but this difference was not significant (GLMM $F_{1,24.19} = 2.271$, $p = 0.145$). Males living in larger groups did

not have higher T levels and, in fact, had lower T levels than males in smaller groups (one male $= 3381.4 \pm 220.94$ ng/g; two males $= 2418.3 \pm 131.06$ ng/g, three males $= 1936.7 \pm 250.55$ ng/g; GLMM $F_{2,41.06} = 11.065$, $p < 0.001$).

*Extragroup competition*

In the context of extragroup competition, the results support the prediction that males living in groups with fewer males will present higher T levels. *Post-hoc* analyses revealed significant differences in T levels between single-male groups and groups with either two or three males (Tukey's tests, $p < 0.05$; Fig. 1).

The Calakmul and Álamo groups lost two males and one male, respectively, between the first and the second sampling periods, converting these groups from multimale to unimale groups. The T levels of the remaining males (that were central males in the previous sampling period) increased significantly in both cases between the first period (Álamo: 1966.5 ± 656.28 ng/g; Calakmul: 2007.2 ± 362.96 ng/g) and the second (Álamo: 3172.2 ± 498.93 ng/g; Calakmul: 3376.9 ± 452.02 ng/g) period (Álamo: $Z = 2.816$, $p = 0.037$; Calakmul: $Z = 4.761$, $p = 0.012$; Fig. 2). The T levels of males living in groups that did not change their composition did not vary significantly between sampling periods (Calax: $Z = 0.244$, $p = 0.876$; Chicbul: $Z = 0.439$, $p = 0.724$; M1 Chilar: $Z = 1.001$, $p = 0.329$; M2 Chilar: $Z = 1.002$, $p = 0.366$).

The average ($\pm$ SD) rate of howling per week across the study groups was 0.215 (± 0.26/h). The Chilar (0.625 ± 0.26/h) and Álamo (0.317 ± 0.07/h) groups (which were the only groups with home ranges that overlapped with those of other groups) registered the highest rates, followed by the Chicbul (0.083 ± 0.043/h) and Calax groups (0.05 ± 0.06/h). Males from the Calakmul group never engaged in loud calls. Weekly T levels and rates of loud calling were not related across groups ($r_s = 0.08$, $n = 48$, $p = 0.671$) or within a particular group (Álamo: $r_s = 0.34$, $n = 12$, $p = 0.521$; Calax: $r_s = 0.44$, $n = 8$, $p = 0.284$; Chicbul: $r_s = -0.56$, $n = 8$, $p = 0.092$; Chilar: $r_s = 0.32$, $n = 16$, $p = 0.298$). In the two multimale groups that howled (Chilar in both sampling periods and Álamo in the first period), central males had significantly higher loud calling rates than non-central males (Chilar: $Z = 2.883$, n central $= n$ non-central $= 8$, $p = 0.003$; Álamo: $Z = 2.247$, n central $= n$ non-central $= 4$, $p = 0.028$). Testosterone levels and rates of loud calling by central males were not related in either group (Chilar: $r_s = 0.59$, $n = 8$, $p = 0.279$; Álamo: $r_s = 0.33$, $n = 4$, $p = 0.376$).

Finally, males living in groups with no overlap with other groups had the highest T levels (2653.9 ± 340.06 ng/g), followed by males with overlap with one group (2582.9 ± 330.32 ng/g) and then by males
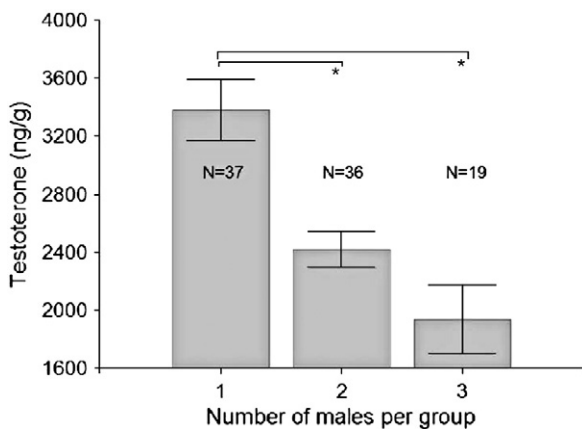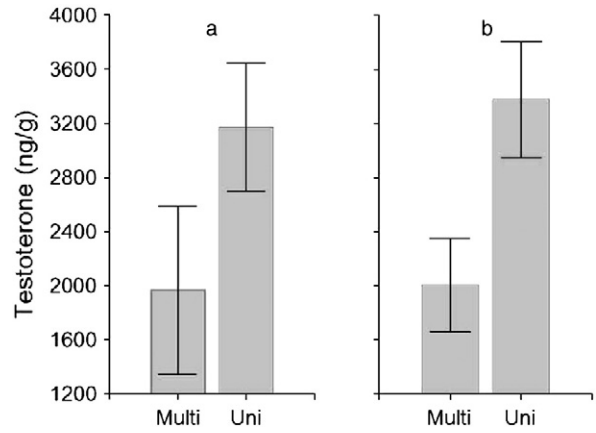
Fig. 2. Mean ($\pm$ SE) T levels of male black howlers living in the two groups that changed their composition from multi- to unimale from the first to the second sampling period: (a) Álamo, (b) Calakmul ($n = 6$ fecal samples per period).

with overlap with three groups (2481.4 ± 447.96 ng/g). However, these differences were not statistically significant (GLMM $F_{2,38.4} = 0.116$, $p = 0.891$).

Discussion

Overall, our results corroborate the notion that T secretion can rarely be explained by simple cause–effect relationships (Bergman et al., 2006). In mammals, sexual behavior by adult males is promoted by circulating T and/or its metabolites, estradiol and dihydrotestosterone, and, likewise, T levels are elevated in this context (Hull and Rodríguez-Manzo, 2009). The absence of a direct relationship between sexual activity and T in black howler males may indicate that it is the interaction between mating behavior and other variables that exerts a significant effect on the hormone profiles of males. Nevertheless, the number of males in groups was shown to relate significantly with the T levels of male black howlers, suggesting that, in this species, androgen secretion is mainly linked to male–male reproductive competition.

The fact that males living in unimale groups had higher T levels supports the prediction that, in this species, T is mainly related to extragroup competition. As has been observed for other howler monkey species (mantled howlers, *Alouatta palliata*, Cristóbal-Azkarate et al., 2006) and other primates (e.g., guerezas, *Colobus guereza*, Fashing, 2001), it is possible that reproductive challenges for male black howlers mainly come from outside the group, either from dispersing males or as a result of interaction with other groups. Extragroup encounters may impose fitness costs on males due to physical injury, infanticide, or extragroup copulations on the part of group females (Crockett, 2003; Van Belle et al., 2008, 2009a). However, immigrations may have a greater negative impact on the future reproductive performance of males, as resident males are frequently evicted from groups (Horwich et al., 2000; Van Belle et al., 2008). In this species, both single males and coalitions of males have been observed to immigrate either to unimale or multimale groups. Nevertheless, the probability of the eviction of resident males increases in unimale groups because these groups may be taken over by single males or coalitions of males, whereas in multimale groups, males are evicted only if groups are invaded by coalitions of two or more males (Horwich et al., 2000; Van Belle et al., 2008). Therefore, it would appear that unimale groups are more attractive to dispersing males because they are more vulnerable, and resident males may experience challenges more frequently, suggesting that they manifest increased T levels in response to possible confrontations with extragroup males and the increased risk of being ousted from their groups.

Fig. 1. Mean ($\pm$ SE) T levels of male black howlers according to number of males per group ($n = 60$ weeks). Significant differences of pair-wise Tukey HSD tests are indicated (*$p < 0.05$).

It has been hypothesized that, in this case, as among red howlers (*Alouatta seniculus*: Pope, 1990), kinship support among male black howlers may be important for group defense (Kitchen, 2004), which implies that a single male lacking this support may manifest increased T levels in preparation for challenges from dispersing males. In the Calakmul group, for instance, observational data indicate that, during the first sampling period, the three resident males consisted of a central male that had lived in the group for at least 6 years and two young adult males, aged approximately 4 and a half years, which were his putative sons. In our sample, the highest T levels corresponded to males that experienced a change from a multimale to a unimale group composition, indicating that grouping patterns have a strong modulatory effect on T among black howlers, associated with competition over group membership. This result concurs with previous research concerning primates. Among vervet monkeys (*Chlorocebus aethiops*), for example, males living in unimale groups have higher T levels and higher T responsiveness, compared with males in multimale groups (Whitten and Turner, 2004), and in sifakas (*Propithecus verreauxi*), resident males exhibit elevated T levels following encounters with extragroup males (Brockman et al., 1998). However, loud calls and T levels did not relate, countering our prediction that vocal communication with extragroup males could be used as a proxy of extragroup competition. A more definitive examination of this hypothesis requires an improved assessment of takeover risks, as loud calling rates are not always related to interactions with extragroup individuals.

Contrary to our predictions, we found no evidence indicating a modulation in the T levels of male black howlers associated with intragroup competition. This result is surprising, as the number of males co-residing in groups affects the potential for monopolizing reproduction and the intensity of male–male competition (Emlen and Oring, 1977). In primates, when there are few males in groups, monopolization increases, power differentials are more likely to emerge and there is a positive relationship between dominance and reproductive success (Cowlishaw and Dunbar, 1991). Black howler groups usually include between one and three males, and reproduction is nonseasonal. Although it has been suggested that a single male has priority access to receptive females in multimale groups (Horwich et al., 2000; Van Belle et al., 2009a), in this species, male reproductive success decreases both in relation to group size and according to the number of males per group (Van Belle and Estrada, 2008), indicating that this access is not exclusive. Thus, males co-residing with more males were predicted to have higher T levels as a result of increased reproductive challenges, and these T levels should also correlate positively with rank, as observed in the case of other primates (e.g., chimpanzees, *Pan troglodytes*, Muller and Wrangham, 2004). However, we did not find evidence that central and non-central males differed in their T levels, which would be predicted by the challenge hypothesis. A recent study by Van Belle et al. (2009b), however, did uncover such a difference, as the centrality of male black howlers significantly influenced androgen secretion. The two groups studied by Van Belle et al. (2009b) experienced frequent changes in terms of their male composition, and the authors recognized that the unusually rapid changes in male membership in one of the study groups may have distorted their assessment concerning the influence of immigration on male androgen levels. When immigrant and resident males both remain in groups, the process of establishing and reorganizing power relationships during the period after takeovers usually leads to increased competition and aggression (e.g., *Alouatta palliata*, Dias et al., 2010) and is associated with elevated androgen and glucocorticoid levels (e.g., *Papio ursinus*, Beehner et al., 2005, 2006). In our sample, elevated T levels among males living alone in groups may indicate that they anticipate future danger during challenges, whereas Van Belle et al. (2009b) report that central males experience increased T levels as a result of frequent instability in male–male relationships and as a result of their efforts to maintain reproductive supremacy. Therefore, these studies concur

with regard to the influence of social challenges, either perceived (i.e., unimale groups) or actual (i.e., multimale groups that were recently invaded), on the androgen levels of male black howlers. It remains to be determined whether males living in unimale groups sustain elevated T levels for extended time periods, but if they do, they may incur a number of fitness costs. Males with elevated T may increase their visibility to predators (e.g., lizards, *Sceloporus jarrovi*, Marler and Moore, 1988), increase their energy expenditure (e.g., arctic ground squirrels, *Spermophilus parryii*, Buck and Barnes, 1999) and experience diminished immune function caused by T (e.g., salmonid fish, Slater and Schreck, 1997).

In the future, we need to establish whether cooperation between related males is frequent among black howlers, as there is evidence that kinship may affect social relationships and control over reproduction in other howler species (e.g., *A. palliata*, Dias and Rodríguez-Luna, 2006; *A. seniculus*, Pope, 1990), which, in turn, may be related to T secretion. If it is common for related males to group in this species, a concessions model (Clutton-Brock, 1998) may help explain why male T levels are lower in multimale groups. Central males would receive fewer challenges and more support from subordinates, providing group defense at the cost of minimal staying incentives, as subordinates would gain inclusive fitness benefits from helping their relatives (Vehrencamp, 1983). To address these questions, we are currently defining genetic kinship relationships in our study groups.

In conclusion, these analyses demonstrate that, among male black howlers, T secretion is affected by social challenges. The number of males per group probably determines invasion probabilities, suggesting that, even if males living in unimale groups benefit from exclusive access to females, they will face increased intruder pressure and a higher risk of being evicted in the case of immigration of new males. In this scenario, male black howlers increase their T levels in preparation for challenges from extragroup males. Therefore, group membership is an important factor conditioning male reproductive strategies among male black howlers. At present, it is unclear how the interaction among grouping patterns, partitioning of reproduction within groups and the physiological costs of the hormonal responses of males to intrasexual competition affect the reproductive success of male primates. On one hand, in larger groups, males may benefit from cooperation with other males to defend groups and, therefore, may have longer tenures and lower physiological costs related to group protection. However, they will probably have to share reproduction with other group males. On the other hand, males living in unimale groups face higher intruder pressures and higher physiological constraints associated with maintaining high androgen levels but have exclusive access to the females in their group. Future testing of these interactions in species such as howlers, in which social groups vary in the number of males and, potentially, in the reproductive skew among these males, will improve our understanding of the costs and benefits associated with different male reproductive strategies.

References

Abraham, G.E., Malimos, F.S., Garza, R., 1977. Radioimmunoassay of steroids. In: Abraham, G.E. (Ed.), Handbook of radioimmunoassay. Marcel Dekker Inc., New York, pp. 591–656.

Altmann, J., 1974. Observational study of behavior: sampling methods. Behaviour 49, 227–267.

Anestis, S.F., 2010. Hormones and social behavior in primates. Evol. Anthropol. 19, 66–78.

Archer, J., 2006. Testosterone and human aggression: an evaluation of the challenge hypothesis. Neurosci. Biobehav., R 30, 319–345.

Bergman, T.J., Beehner, J.C., Cheney, D.L., Seyfarth, R.M., Whitten, P.L., 2006. Interactions in male baboons: the importance of both males' testosterone. Behav. Ecol. Sociobiol. 59, 480–489.

Beehner, J.C., Bergman, T.J., Cheney, D.L., Seyfarth, R.M., Whitten, P.L., 2005. The effect of new alpha males on female stress in free-ranging baboons. Anim. Behav. 69, 1211–1221.

Beehner, J.C., Bergman, T.J., Cheney, D.L., Seyfarth, R.M., Whitten, P.L., 2006. Testosterone predicts future dominance rank and mating activity among male chacma baboons. Behav. Ecol. Sociobiol. 59, 469–479.

Blanchard, D.C., Blanchard, R.J., 1990. Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. Neurosci. Biobehav., R 14, 455–462.

Bolin, I., 1981. Male parental behaviour in black howler monkeys (*Alouatta palliata pigra*) in Belize and Guatemala. Primates 22, 349–360.

Brockett, R.C., Horwich, R.H., Jones, C.B., 1999. Disappearance of infants following male takeovers in the Belizean black howler monkey (*Alouatta pigra*). Neotrop. Primates 7, 86–88.

Brockman, D.K., Whitten, P.L., Richard, A.F., Schneider, A., 1998. Reproduction in free-ranging male *Propithecus verreaxi*: the hormonal correlates of mating and aggression. Am. J. Phys. Anthropol. 105, 137–151.

Bronson, F.H., 1973. Establishment of social rank among grouped male mice: relative effects on circulating FSH, LH, and corticosterone. Physiol. Behav. 10, 947–951.

Buck, C.L., Barnes, B.M., 1999. Annual cycle of body composition and hibernation in free-living arctic ground squirrels. J. Mammal. 80, 430–442.

Clutton-Brock, T.H., 1998. Reproductive skew, concessions and limited control. TREE 13, 288–292.

Coe, C.L., Franklin, D., Smith, E.R., Levine, S., 1982. Hormonal responses accompanying fear and agitation in the squirrel monkey. Physiol. Behav. 29, 1051–1057.

Coe, C.L., Savage, A., Bromley, L.J., 1992. Phylogenetic influences on hormone levels across the primate order. Am. J. Primatol. 28, 81–100.

Cowlishaw, G., Dunbar, R.I.M., 1991. Dominance rank and mating success in male primates. Anim. Behav. 41, 1045–1056.

Cristóbal-Azkarate, J., Chavira, R., Boeck, L., Rodríguez-Luna, E., Veà, J.J., 2006. Testosterone levels of free-ranging resident mantled howler monkey males in relation to the number and density of solitary males: a test of the challenge hypothesis. Horm. Behav. 49, 261–267.

Crockett, C.M., 2003. Re-evaluating the sexual selection hypothesis for infanticide by *Alouatta* males. In: Jones, C.B. (Ed.), Sexual selection and reproductive competition in primates: new perspectives and directions. : American Society of Primatology: Special Topics in Primatology, 3. The American Society of Primatologists, Norman, Oklahoma, pp. 327–365.

Dias, P.A.D., Rodríguez-Luna, E., 2006. Seasonal changes in associative behavior and subgrouping patterns of mantled howler monkey males living on an island. Int. J. Primatol. 27, 1635–1651.

Dias, P.A.D., Rangel-Negrín, A., Coyohua-Fuentes, A., Canales-Espinosa, D., 2009. Behaviour accumulation curves: a method to study the completeness of behavioural repertoires. Anim. Behav. 77, 1551–1553.

Dias, P.A.D., Rangel-Negrín, A., Veà, J., Canales-Espinosa, D., 2010. Coalitions and male–male behavior in *Alouatta palliata*. Primates 51, 91–94.

Emlen, S.T., Oring, L.W., 1977. Ecology, sexual selection and the evolution of mating systems. Science 197, 215–223.

Fashing, P.K., 2001. Male and female strategies during intergroup encounters in guerezas (*Colobus guereza*): evidence for resource defense mediated through males and a comparison with other primates. Behav. Ecol. Sociobiol. 50, 219–230.

Gobierno del Estado de Campeche, 2010. Campeche. <www.portal.camp.gob.mx> Accessed on August 20, 2010.

Hager, R., 2009. Explaining variation in reproductive skew among male langurs: effects of future mating prospects and ecological factors. In: Hager, R., Jones, C.B. (Eds.), Reproductive Skew in Vertebrates: Proximate and Ultimate Causes. Cambridge University Press, Cambridge, pp. 134–164.

Herndon, J.G., Turner, J.J., Collins, D.C., 1981. Ejaculation is important for mating-induced testosterone increases in male rhesus macaque. Physiol. Behav. 27, 873–877.

Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. Anim. Behav. 71, 265–277.

Horwich, R.H., Brockett, R.C., Jones, C.B., 2000. Alternative male reproductive behaviors in the Belizean black howler monkey (*Alouatta pigra*). Neotrop. Primates 8, 95–98.

Hull, E.M., Rodríguez-Manzo, G., 2009. Male sexual behavior. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), Hormones, brain and behavior. Academic Press, San Diego, pp. 5–66.

Khan, M.Z., Altmann, J., Isani, S.S., Yu, J., 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. Gen. Comp. Endocrinol. 128, 57–64.

Kitchen, D.M., 2004. Alpha male black howler monkey responses to loud calls. Anim. Behav. 67, 125–139.

Kitchen, D.M., Beehner, J.C., 2007. Factors affecting individual participation in group-level aggression among non-human primates. Behaviour 144, 1551–1581.

Marler, C.A., Moore, M.C., 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. Behav. Ecol. Sociobiol. 23, 21–26.

Marler, C.A., Moore, M.C., 1989. Time and energy costs of aggression in testosterone-implanted male mountain spiny lizards (*Sceloporus jarrovi*). Physiol. Zool. 62, 1334–1350.

McGuckin, M.A., Blackshaw, A.W., 1991. Mating-associated peak in plasma testosterone concentration in wild male grey-headed flying foxes (*Pteropus poliocephalus*). J. Reprod. Fertil. 92, 347–352.

Muller, M.N., Wrangham, R.W., 2004. Dominance, aggression and testosterone in wild chimpanzees: a test of the "challenge hypothesis". Anim. Behav. 67, 113–123.

Nelson, R.J., 2000. An introduction to behavioural endocrinology. Sinauer, Sunderland.

Nunn, C.L., 1999. The number of males in primate social groups: a comparative test of the socioecological model. Behav. Ecol. Sociobiol. 46, 1–13.

Oliveira, R.F., 2004. Social modulation of androgens in vertebrates: mechanisms and function. In: Slater, P.J., Rosenblatt, J., Roper, T.J., Snowdon, C.T., Brockmannm, H.J., Naguib, Marc (Eds.), Advances in the Study of Behavior. Vol. 34. Academic Press, San Diego, pp. 165–239.

Oliveira, R.F., Canario, A.V.M., Grober, M.S., Serrão Santos, R., 2001. Endocrine correlates of male polymorphism and alternative reproductive tactics in the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis*. Gen. Comp. Endocrinol. 121, 278–288.

Oliveira, R.F., Hirschenhauser, K., Carneiro, L.A., Canario, A.V.M., 2002. Social modulation of androgens in male teleost fish. Comp. Biochem. Physiol. 132B, 203–215.

Oyegible, T.O., Marler, C.A., 2005. Winning fights elevates testosterone levels in California mice and enhance future ability to win fights. Horm. Behav. 48, 259–267.

Pope, T.R., 1990. The reproductive consequences of the male cooperation in the red howler monkey: paternity exclusion in multimale and single-male troops using genetic markers. Behav. Ecol. Sociobiol. 27, 439–446.

Sachser, N., Pröve, E., 1986. Social status and plasma testosterone-titers in male guinea pigs (*Cavia aperia f. porcellus*). Ethology 71, 103–114.

Sapolsky, R.M., 1982. The endocrine stress-response and social status in the wild baboon. Horm. Behav. 16, 279–292.

Sapolsky, R.M., 1986. Stress-induced elevation of testosterone concentrations in high ranking baboons: role of catecholamines. Endocrinology 118, 1630–1635.

Sapolsky, R.M., 1993. The physiology of dominance in stable versus unstable social hierarchies. In: Mason, W.A., Mendoza, S. (Eds.), Primate social conflict. Suny, Albany, pp. 171–204.

Slater, C.H., Schreck, C.B., 1997. Physiological levels of testosterone kill salmonid leucocytes in vitro. Gen. Comp. Endocrinol. 106, 113–119.

Strier, K.B., Ziegler, T.E., Wittwer, D.J., 1999. Seasonal and social correlates of fecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoides*). Horm. Behav. 35, 125–134.

Suzuki, J., Kato, A., Maeda, N., Hashimoto, C., Uchikoshi, M., Mizutani, T., Doke, C., Matsuzawa, T., 2003. Plasma insulin-like growth factor-I, testosterone and morphological changes in the growth of captive agile gibbons (*Hylobates agilis*) from birth to adolescence. Primates 44, 273–280.

Treves, A., Drescher, A., Ingrisano, N., 2001. Vigilance and agregation in black howler monkeys (*Alouatta pigra*). Behav. Ecol. Sociobiol. 50, 90–95.

Van Belle, S., Estrada, A., 2006. Demographic features of *Alouatta pigra* populations in extensive and fragmented forests. In: Estrada, A., Garber, P.A., Pavelka, M., Luecke, L. (Eds.), New perspectives in the study of Mesoamerican primates: distribution, ecology and conservation. Springer, New York, pp. 121–142.

Van Belle, S., Estrada, A., 2008. Group size and composition influence male and female reproductive success in black howler monkeys (*Alouatta pigra*). Am. J. Primatol. 70, 613–619.

Van Belle, S., Estrada, A., Strier, K.B., 2008. Social relationships among male *Alouatta pigra*. Int. J. Primatol. 29, 1481–1498.

Van Belle, S., Estrada, A., Ziegler, T.E., Strier, K.B., 2009a. Social and hormonal mechanisms underlying male reproductive strategies in black howler monkeys (*Alouatta pigra*). Horm. Behav. 56, 355–363.

Van Belle, S., Estrada, A., Ziegler, T.E., Strier, K.B., 2009b. Sexual behavior across ovarian cycles in wild black howler monkeys (*Alouatta pigra*): male mate guarding and female mate choice. Am. J. Primatol. 71, 153–164.

Vehrencamp, S.L., 1983. Optimal skew in cooperative societies. Am. Zool. 23, 327–355.

Vidal-Zepeda, R., 2005. Las regiones climáticas de México, I.2.2. Instituto de Geografía, UNAM, México DF.

von Holst, D.V., 1977. Social stress in tree shrews: problems, results and goals. J. Comp. Physiol. Psychol. 120, 71–86.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120, 260–275.

Whitten, P.L., Turner, T.R., 2004. Male residence and the patterning of serum testosterone in vervet monkeys (*Cercopithecus aethiops*). Behav. Ecol. Sociobiol. 56, 565–578.

Wingfield, J.C., Ball, G.F., Dufty Jr., A.M., Hegner, R.E., Ramenofsky, M., 1987. Testosterone and aggression in birds. Am. Sci. 75, 602–608.

Wingfield, J.C., Hegner, R.E., Dufty Jr., A.M., Ball, G.F., 1990. The 'Challenge Hypothesis': theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am. Nat. 136, 829–846.

Wingfield, J.C., Lynn, S.E., Soma, K.K., 2001. Avoiding the "costs" of testosterone: ecological bases of hormone–behavior interactions. Brain Behav. Evol. 57, 239–251.

Wood, W.G., Fricke, H., Haritz, J., Gadow, A., Krausz, H.S., Tode, B., Strasburger, C.J., Scriba, P.C., 1984. An evaluation of four different immunoassay systems: CELIA (chemiluminescent immunoassay), SPALT (solid-phase antigen luminescence technique), ILMA (immunoluminometric assay) and ILSA (immunoluminometric labelled second antibody). A critical study of macro solid phases for use in immunoassay systems, Part III. J. Clin. Chem. Clin. Biochem. 22, 349–356.

Wynne-Edwards, K.E., Timonin, M.E., 2007. Paternal care in rodents: weakening support of hormonal regulation of the transition to behavioral fatherhood in rodent animal models of biparental care. Horm. Behav. 52, 114–121.

Ziegler, T.E., Snowdon, C.T., 2000. Preparental hormone levels and parenting experience in male cotton-top tamarins, *Saguinus oedipus*. Horm. Behav. 38, 159–167.