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Characterization and Social Correlates of Fecal Testosterone and Cortisol Excretion in Wild Male Moustached Tamarins (*Saguinus mystax*)

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ABSTRACT

Reproductive success in male primates can be influenced by both testosterone (T) and cortisol (C). The aim of this study was to examine these endocrinological parameters in wild *Saguinus mystax* using fecal hormone analysis. Firstly, we wanted to characterize male hormonal status over the course of the year. Further we tested the influence of the reproductive status of the breeding female, social instability, and inter-group encounter rates on T levels, comparing the results with the predictions made by the challenge hypothesis (Wingfield *et al.*, 1990). Finally we tested for inter-individual differences in hormone levels, possibly related to social or breeding status. We collected data during a twelve months study on two groups of moustached tamarins at the Estación Biológica Quebrada Blanco in northeastern Peru. We found fairly similar T and C levels over the course of the year for all males. Yet an elevation of T shortly after the birth of infants, during the phase of ovarian inactivity of the group's breeding female, was evident. Hormone levels were not significantly elevated during a phase of social instability, did not correlate with inter-group encounter rates, and did not differ between breeding and non-breeding males. Our results are in line with the challenge hypothesis (Wingfield *et al.*, 1990). The data suggest that reproductive competition in moustached tamarins is not based on endocrinological, but rather on behavioral mechanisms, possibly combined with sperm competition.

Key words: male *Saguinus mystax*; fecal steroids; testosterone; cortisol; challenge hypothesis

INTRODUCTION

Both androgens and adrenal hormones can influence reproductive success in male vertebrates. Testosterone and other androgens influence morphology like secondary sexual characteristics (e.g. antler growth in red deer, *Cervus elaphus*, stags, Suttie *et al.*, 1984), are important in the control of spermatogenesis (Wickings *et al.*, 1986) and seem to play a role in the expression

of aggressive behavior and the achievement of social status, though this latter relationship might be less pronounced in primates (Bouissou, 1983; Dixson, 1980). On the other side, cortisol and its metabolites are not only indicators of (sustained) environmental or social stress (Sapolsky, 2002) but may also suppress gonadal function, thus modulating testosterone levels (Sapolsky, 1985; 2002) and hence affecting reproductive output.

Apart from the influence of the hypothalamo-pituitary-adrenal (HPA) axis on testicular endocrine function and hence testosterone levels, testicular function can be modulated by various other factors, especially seasonal and social ones. The challenge hypothesis (Wingfield *et al.*, 1990) offers a predictive framework for the relationship between temporal patterns of testosterone and aggression. The hypothesis assumes that the general influence of androgen levels on aggression in the context of reproduction is most pronounced in situations of social instability. Particularly, during times when dominance relationships are established, territorial boundaries are set up, or males compete for access to mates, testosterone levels are predicted to rise above the breeding level that is necessary for the maintenance of the functioning of gonads and accessory organs. This general prediction of the challenge hypothesis was modified by Wingfield and his colleagues insofar as they suspect a trade-off between aggression and paternal behavior (Wingfield *et al.*, 1990). This leads to a set of more specific assumptions that build on the respective degrees of aggressive male-male interactions and of paternal care in a species, with the latter reducing the influence of the former. Although the hypothesis was originally developed on avian species, the authors explicitly encourage testing its applicability in other vertebrate species. Recently, this has been done on some primate species (*Brachyteles arachnoides*: Strier *et al.*, 1999; *Lemur catta*: Cavigelli and Pereira, 2000; *Eulemur fulvus rufus*: Ostner *et al.*, 2002; *Cebus apella nigritus*: Lynch *et al.*, 2002), mainly confirming the predicted relationships between patterns of male T and seasonal and social parameters. These taxa have in common that they are all seasonally breeding, that males mate with several females, that they do not exhibit paternal infant care,

and that with the exception of muriquis (*Brachyteles arachnoides*) they show levels of aggression among males on which a dominance hierarchy can be based.

By contrast, members of the Neotropical family Callitrichidae (marmosets and tamarins) deviate in all named aspects from the pattern described above and thus present a unique opportunity to test the challenge hypothesis in primate species characterized by an unusual set of features. For example, many callitrichids show a variable mating system with polyandry being common (Terborgh and Goldizen, 1985; Sussman and Garber, 1987; for a recent review see Heymann, 2000), and all show high levels of male infant care (see review by Goldizen, 1987; Huck et al., in prep.). Furthermore, reproduction in callitrichids shows only a modest degree of seasonality and, at least for the genus *Saguinus*, the overall level of aggression, even in a reproductive context, between animals of the same group is generally low (see review in Caine, 1993).

This pattern applies also to moustached tamarins (*Saguinus mystax*) who, like other species of the family, live in groups of 1-4 adult females and 1-4 adult males together with offspring of up to several litters (e.g. Soini and Soini, 1990). Usually only one female breeds and gives birth to normally one litter of dizygotic twins per year. For non-reproductive females of some callitrichids a form of endocrinological suppression of gonadal function has been proposed (Abbott, 1984; 1993; but see Löttker *et al.*, 2004) and a similar mechanism has been suggested for males as well (Abbott *et al.*, 1992; French and Schaffner, 1995; but see Baker *et al.*, 1999; Ginther *et al.*, 2002). Recent genetic analysis showed that while in some groups one male monopolizes fatherhood, in others paternity is shared either in consecutive years or even within a litter (Huck *et al.*, submitted-a). This suggests at least a potential for male-male reproductive competition, and individual differences in hormone levels between males of the same group might thus be expected. If T levels differ, they are expected to be higher in the "breeding males", i.e. males that monopolize paternity in their respective group, as seen in other species (Brockmann *et al.*, 1998; Kraus *et al.*, 1999; French and Schaffner, 1995).

Yet, with low levels of inter-male aggression combined with a high degree of paternal infant care, moustached tamarins are – according to the challenge hypothesis – expected to show only a slight increase of testosterone (and cortisol) levels at the beginning of the mating season, a prediction we tested in the present study. We further wanted to determine whether hormone excretion correlated with other potentially challenging events such as inter-group encounter rates and the reproductive state of the breeding female. Our prediction was that in times of increased inter-group encounter rates that bear the risk of challenges by intruding males, the hormone levels should rise. Likewise, during periods of highest fertility of the breeding female, competition amongst males should be more intense and thus testosterone levels heightened. Finally, because of the above-mentioned possible influence of cortisol levels on testicular function (Sapolsky, 1985), we also analyzed cortisol levels of different males and compared the results with the predictions made by Abbott and co-workers (Abbott *et al.*, 2003). Their recently published meta-analysis tried to separate the most important factors for predicting cortisol levels in subordinates of different species (Abbott *et al.*, 2003). They identified kinship, the rates of stressors and the opportunities for social support to be the most prominent predictors for an individual's cortisol levels. Using their decision tree, we would expect similar cortisol levels in subordinate and dominant male moustached tamarins.

Against this background, our study had the following four main objectives: Firstly, we designed to characterize endocrine status using fecal testosterone and cortisol levels in adult male moustached tamarins throughout a complete annual reproductive cycle. Secondly, we intended to test the predictions of the challenge hypothesis (Wingfield *et al.*, 1990), with special regard to reproductive status of the breeding female, social instability, and inter-group encounter rates as potentially "challenging" situations. Thirdly, we aimed to examine whether inter-individual differences in testosterone and cortisol levels existed, and finally we wanted to test the prediction made by Abbott et al. (Abbott *et al.*, 2003) concerning the cortisol levels of subordinate vs. dominant males.

METHODS

Study Site, Group Composition, and Group Follows

This study was part of a PhD project of the first two authors on the behavioral and endocrinological mechanisms underlying the mating system of moustached tamarins (*Saguinus mystax*) and their genetic consequences. Data and fecal samples were collected from January to December 2001 at the Estación Biológica Quebrada Blanco (EBQB), situated in primeval Amazonian Lowland forest in northeastern Peru (4°21' S and 73°09' W). For further details of the study site see Heymann (1995).

We studied two groups (W and E) of wild, habituated moustached tamarins that were known individually by natural markings. Both groups were followed daily from the animals' leaving their sleeping tree at dawn (about 6:00 a.m.) to their entering the next sleeping site at about 15:45 a.m., yielding a total of 3004 (group W) and 3257 (group E) contact hours on 330 and 351 days, respectively. These continuous group follows were possible through simultaneous observations of the two groups by the first two authors with the help of four field assistants.

Throughout the major part of the study period, group W comprised seven individuals, including one reproductively active female, three adult males, and three infantile to subadult males, one of which was born in the second month of observation. The female died near the end of the study. Following her death, the group split and a new female immigrated (unpublished data). Group E consisted of nine animals, including one reproductively active female, two non-reproductive adult females, three adult males, and four infantile to subadult offspring, two of which were born in January 2001. Table I gives further details of group compositions. Hereafter we will refer to subadults, juveniles and infants collectively as immatures.

Sample Collection

Fecal samples were collected from all individuals throughout the entire study period. We intended to collect two samples per adult male per week and at least two samples per month from the immatures. At times of infrequent events in the group, (soon expected birth, consortship behavior, or severe predator attacks), we tried to obtain daily samples from each group member. Due to the arboreal life and the feeding ecology of the species, however, sample collection proved to be rather difficult (see Löttker *et al.*, 2004). On average 1.6 and 1.3 samples per week per adult male were obtained in group W and group E, respectively (range 0-4), yielding a total of 402 suitable samples from adult males and 160 additional samples from immatures. It was not feasible to collect all samples at roughly the same time of day. Samples were therefore collected opportunistically (N=171 before 11:00 a.m., N=276 after 11:00 a.m.). Since the time of collection can influence fecal hormone concentrations (e.g. Sousa and Ziegler, 1998; Ferreira Raminelli *et al.*, 2001; Löttker *et al.*, 2004), we compared T and C levels in samples collected from individual males before and after 11:00 a.m. at the same day using a Wilcoxon Matched Paired test. The results revealed no significant difference (testosterone: N=7, Z=1.18, p=0.24; cortisol: N=4, Z=1.46, p=0.14; see Ferreira Raminelli *et al.*, 2001, for similar results in *Callithrix jacchus*). Furthermore, most correlation coefficients for correlations between log-10-transformed (see below) testosterone and cortisol values and sampling time in individual males were low ($r<0.22$, i.e. $r^2<0.05$) and with a few exceptions not significant (testosterone: EM1: r=-0.25, p=0.02, N=80; WM4: r=-0.371, p=0.03, N=35; cortisol: WM2: r=+0.31, p=0.02, N=54; EM1: r=-0.30, p=0.007, N=80). Since even the significant correlations were rather weak and the correlation pattern for cortisol was not consistent (positive for WM2, negative for EM1), and since the proportions of samples collected in the morning and in the afternoon did not differ significantly between males (G-test, $G_5=8.522$, $p>0.05$) we are confident that variation in hormone levels due to different collection times did not affect the endocrine results seriously. Ferreira Raminelli and

co-workers also did not find effects of time on fecal cortisol levels in male common marmosets, *Callithrix jacchus* (Ferreira Raminelli *et al.*, 2001).

Immediately after an animal was seen defecating, fecal samples were collected in a polypropylene twist-off tube and covered completely with approximately 3ml of 96% ethanol (Wasser *et al.*, 1988). The tubes were labeled (date and time of collection, animal identity, and sample number), sealed with Parafilm®, and kept for up to three weeks at ambient temperature (20-30°C) in the camp. They were then brought to the nearest town and stored in a refrigerator at 4°C until shipment to the laboratory in Germany, where they were kept again at 4°C until hormone analysis.

Hormone Analyses

Hormones were extracted in their original ethanolic solvent according to the method described by Kraus et al. (1999). Briefly, samples were homogenized using a metal spatula. The fecal suspensions were subsequently vortexed for 15 min. After centrifugation at 3000rpm for 10 min, the supernatants were decanted into glass tubes and stored at 4°C. Fecal pellets were then re-extracted with 3ml absolute methanol as described above. Prior to the second centrifugation, seeds larger than about 5mm were removed in order to avoid an interfering influence on fecal weight. The volume of the combined supernatants of both extraction steps was recorded. Approximately 4.5ml extract were transferred to glass tubes and stored at –20°C until hormone analysis. The remaining fecal pellets were vacuum-dried at 50°C, and the dry weight of the samples noted. For analysis, only extracts from samples with a minimum dry weight of 0.02g were used (Löttker *et al.*, 2004). The efficiency of the extraction procedure - determined in a subset of samples (n=40) by monitoring the recovery of ^{3}H-progesterone (^{3}H-P_4, ~30000cpm), added to the samples prior to homogenization - was 90.0±9.0% (mean±SD). Although recovery figures might be slightly different between radioactive progesterone and testosterone or cortisol, we preferred to use the former, given the

potential risk of co-measurement of externally added testosterone/cortisol tracer in the highly sensitive testosterone and cortisol assays used (see below).

Fecal extracts were measured for immunoreactive testosterone (T) and cortisol (C), the measurement of which has been shown to reliably reflect testicular and adrenal function in some primate species (for testosterone e.g.: Brockmann *et al.*, 1998; Barrett *et al.*, 2002; for cortisol: Whitten *et al.*, 1998).

Immunoreactive T was measured by enzyme immunoassay as described by Kraus et al. (1999). In brief, fecal extracts were diluted 1:10 to 1:1600 in assay buffer (0.04 M PBS, pH 7.2) and duplicate 50µl aliquots taken to assay along with 50µl aliquots of reference standard in doubling dilutions over the range of 0.31 to 40pg per well. Cross-reactivity data of the T antibody are given in von Engelhardt et al. (2000). Sensitivity of the assay at 90% binding was 0.3pg. Serial dilutions of fecal extracts gave displacement curves parallel to those obtained with the T standard (t-test for difference in slopes between sample dilution curve and standard curve: t_{18}=0.112, p=0.912). Inter- and intra-assay coefficients of variation, assessed by replicate determination of high- and low value quality controls, were 5.1% (high, N=27) and 11.5% (low, N=27) for inter-assay variation, and 4.7% (high, N=17) and 7.1% (low, N=14) for intra-assay variation, respectively. Since in other New World primate species T metabolites in feces are often excreted as conjugates (Strier *et al.*, 1999; Lynch *et al.*, 2002; Möhle *et al.*, 2002), we hydrolyzed a subset of 30 samples from different males to test if T values measured directly in the fecal extracts (as described above) correlate with those measured after hydrolysis and extraction. We found a highly significant correlation (r=0.98, p<0.001) between the two data sets, indicating that the two measurements provide the same information on relative differences in T levels between certain categories (e.g. males, phases). It is thus unlikely that the results of our study have been affected by the use of a direct T measurement as performed here.

Moreover, a biological validation of the assay by comparing median T values of adult males against median T values measured in immatures revealed significantly higher levels in the former (Mann-Whitney U-test, $N_1=6$, $N_2=6$, $U=0.2$, $p<0.05$). This indicates that the fecal T measurement used reliably reflects testicular endocrine function in the study species.

Immunoreactive C was measured accordingly using an antiserum raised in rabbits against cortisol and biotinylated cortisol as a label (both reagents purchased from Dr. E. Möstl, Vienna). Prior to the assay, samples were diluted 1:20 to 1:2000 in assay buffer. Reference standards ranged from 2.5 to 160pg per well. Cross-reactivity data of the C antibody are provided by Palme & Möstl (1997). Sensitivity of the assay at 90% binding was <2.5pg. Serial dilutions of fecal extracts gave displacement curves parallel to those obtained with the cortisol standard (t-test for difference in slopes between sample dilution curve and standard curve: $t_{11}=0.669$, $p=0.517$). Inter-and intra-assay coefficients of variation were 9.1% (high, N=18) and 21.7% (low, N=18) for inter-assay variation, and 7.7% (high, N=8) and 10.5% (low, N=11) for intra-assay variation, respectively.

All hormone concentrations are given as ng/g dry weight of feces (see Wasser *et al.*, 1993).

It was not possible to conduct an ACTH challenge test to establish the biological validity of the fecal cortisol measurement in reflecting adrenal function in *S. mystax*. However, the assay used has been shown to reliably detect an ACTH response as well as an elevation in glucocorticoid concentrations associated with a stressful process of animal translocation in fecal samples of the related common marmoset (Heistermann, unpublished data). Moreover, the measurement of fecal cortisol has been successfully applied to assess adrenal function in various other primates, including callitrichid species (*Lemur catta*: Cavigelli, 1999; *Cebus apella*: Lynch *et al.*, 2002; *Brachyteles arachnoides*: Strier *et al.*, 1999; *Saguinus oedipus*: Ziegler and Sousa, 2002; *Callithrix jacchus*: Sousa and Ziegler, 1998; Ferreira Raminelli *et al.*, 2001; Albuquerque *et al.*, 2001; *Leontopithecus rosalia*: Bales *et al.*, 2001). We are

therefore confident that our assay is suitable for assessing adrenal activity in moustached tamarins, too.

Data Analyses

Values of hormone concentrations were not normally distributed and therefore log-10-transformed (Fowler and Cohen, 1990). Henceforward, we always refer to the transformed values when mentioning hormonal levels in any statistics if not stated otherwise. If applicable, we conducted parametric tests (ANOVA, t-test for independent samples using the Statistica 6.0 software package). In case ANOVA detected a significant difference or trend in the data set, post-hoc analyses were performed using the Scheffé test.

We used ANOVA to examine whether adult males within a group differed in T or C concentrations. In order to evaluate whether fecal samples from consecutive days could be pseudo-replicates we calculated the absolute value of the difference of hormone concentrations of samples from consecutive days and on the other hand of samples that were collected at least 28 days apart. The values were then tested for differences between the means using t-tests. They were not significantly different for all males for both testosterone and cortisol with the exception of cortisol in EM1 (t_{83}=-2.2; p=0.03). A comparison of the absolute value of the difference of cortisol concentrations between samples from EM1 collected two days apart with samples collected four weeks apart yielded no difference. Hence, in further analyses concerning cortisol we omitted one of the two samples collected on consecutive days for this male. We feel confident that all remaining values can be viewed as independent data points.

An ANOVA was performed in order to examine possible seasonal influences on hormone concentrations by comparing monthly means (using each male's mean T respectively C value as one data point). Autocorrelations were performed for mean monthly values (mean of the means of the six males) for T and C with lags between one and eight.

In order to analyze whether males exhibited different hormone levels during different phases of the reproductive cycle of the group's breeding female, we compared mean values for each male during times when the respective female was pregnant, showed ovarian activity, or was reproductively inactive. The female's reproductive phases were determined according to fecal progestogen and estrogen profiles and are defined in detail by Löttker et al. (2004). In brief, pregnancy was determined by calculating backwards 150 days from parturition. The ovarian inactive phase started directly after parturition and was indicated by a marked drop of the females' hormone levels to baseline concentrations similar to those found in a juvenile female which were maintained for >60 days. The resumption of post-partum ovarian activity was indicated by a significant rise in both progestogen and estrogen levels and a cyclic fluctuation thereafter. Since the transition between the end of the ovarian activity phase and the onset of pregnancy could not be clearly separated by the female endocrine pattern, samples collected during this period were not included in the analysis. In order to evaluate whether a general relationship between male hormone concentration and female reproductive phase exists, Repeated Measures ANOVAs were conducted using the mean hormone concentration during a given phase of each of the six adult males as one data point.

Furthermore, in group W, we compared for each male T concentrations (mean and 95% upper boundary) of samples collected while female WF1 was still alive against the mean concentration of samples collected after the new female WF3 had immigrated.

Additionally, occurrences of inter-group encounters were noted. A daily rate per month was calculated by dividing the number of encounters by the number of days in the particular month on which encounters had been observed. If the study group met with more than one different neighboring group, all incidences were counted separately. This ratio was then correlated with mean monthly hormone levels of individual males.

RESULTS

Variation in Hormone Levels According to Season, Female Reproductive Status and Inter-group Encounter Rates

The temporal pattern of fecal T and C excretion followed a similar course in all focal males, but concentrations of neither T nor C differed significantly over the months of the study period (ANOVA: T: $F_{11, 60}$=1.56, p=0.13; C: $F_{11, 60}$=0.74, p=0.70, Fig. 1). None of the autocorrelation coefficient (ACC) was significant (T: between ACC_{lag1}=-0.008, p=0.97, and ACC_{lag5}=0.29, p=0.50; C: between ACC_{lag1}=0.06, p=0.80, and ACC_{lag6}=-0.36, p=0.06), confirming the absence of a seasonal pattern in hormone levels.

A Repeated Measures ANOVA showed a significant difference in mean T levels over the three different reproductive phases of the group's respective breeding female ($F_{2,10}$=12.16 p<0.003; Fig. 2a). The post-hoc Scheffé test indicated that samples of males collected during the female's post-partum ovarian inactive phase (mean of the untransformed values, weighed for each male: 3655ng/g fecal dry weight) exhibited significantly higher T concentrations than those collected during her pregnancy (mean: 2670ng/g fecal dry weight; df=10, p<0.005) and during the phase of ovarian activity (mean: 3093ng/g fecal dry weight; df=10, p<0.008). No significant difference between T levels of samples collected during the phase of ovarian activity and pregnancy was apparent (p=0.93). In contrast, the Repeated Measures ANOVA indicated no significant influence of the female's reproductive phase on C levels ($F_{2,10}$=0.53, p=0.60; Fig. 2b).

When comparing the mean T and C levels of adult males in group W during the tenure of female WF1 with those after she had died and the new female (WF3) had immigrated, only the T level of WM3 was significantly elevated in the latter period (t-test: $t_{66,5}$=-2.46, p=0.02, Table II). Nonetheless, after WF1's death mean hormone levels of all males fell well below the upper 95% boundary of the values before WF1 died (Table II).

As shown in Table III, both study groups showed high daily rates of inter-group encounters for each month of the study period. Group W was involved in a total of 256 encounters on 306 days, group E in 180 encounters on 310 days. The frequency of inter-group encounters showed no correlation with hormone levels except for T in WM1 (Table IV) and in most individuals, levels tended to be lower with an increasing number of encounters per month.

Individual Differences in Hormone Levels

There were no significant differences in mean T levels between the adult males in either group W or group E (Fig. 3). In the latter group, however, a clear trend towards lower T values in male EM2 was found. For mean C levels we found significant differences between males in group W and a trend in group E (Fig. 4). The post-hoc test indicated WM3 to have significantly higher C levels than WM1 (Scheffé post-hoc test: df=211, p=0.016) while EM2 tended to have a lower mean C level than EM3 (Scheffé post-hoc test: df=174, p=0.098).

DISCUSSION

This is the first study combining behavioral observations over the period of a whole year with hormone analyses in wild male moustached tamarins. Testosterone and cortisol levels did not change significantly over the course of the year for all males. Yet an elevation of testosterone was evident shortly after the birth of infants, during the phase of ovarian inactivity of the group's breeding female. Hormone levels were not significantly higher during a phase of social instability, did not correlate with inter-group encounter rates, and did not differ between males.

In our study, no seasonal influences on mean monthly T and C values were apparent (in line with findings for plasma T levels in captive *Callithrix jacchus*, Kholkute, 1984). Contrastingly, in seasonally breeding species like some lemurs or tufted capuchins, T levels are often about two- to four times higher at the beginning of the breeding season compared to

non-breeding times (Kraus *et al.*, 1999; von Engelhardt *et al.*, 2000; Ostner *et al.*, 2002; Lynch *et al.*, 2002). However, breeding in moustached tamarins is not strictly seasonal. Even near the equator conditions in some months are somewhat more favorable than in others but possibly they do not always drop to critically low levels where breeding attempts would never pay like in temperate zones. Under optimal conditions shorter interbirth intervals might sometimes be achievable. While moustached tamarins in this population show a birth peak around the beginning of the rainy season (November to February), births did occur throughout the year (unpublished data; see also Snowdon and Soini, 1988; Soini and Soini, 1990). Our breeding females showed a long period of ovarian activity before conceiving, and the non-breeding females seem to have had the potential to conceive throughout the year (Löttker *et al.*, 2004). In contrast to more strictly seasonally breeding species, male moustached tamarins therefore should be prepared year-round to mate with fertile females (perhaps also in extra-group copulations with neighbors). This might explain that T levels do not drop to a non-breeding-season value as in many birds (see references in Wingfield *et al.*, 1990) or sifakas, *Propithecus verreauxi*, and redfronted lemurs, *Eulemur fulvus*, (Kraus *et al.*, 1999; Ostner *et al.*, 2002), where T levels are nearly two (redfronted lemurs) respectively three times (sifakas) lower during the pre-mating season, and testis are markedly smaller.

While seasonal influences on hormone levels could not be found, the males' T levels seem to be related to the reproductive status of the breeding female. In all six males, the highest T values were found during the female’s post-partum ovarian inactive phase, while in four males the levels during her pregnancy were lowest. These differences, though significant if compared over all males, are not very marked (less than 50% increase from the lowest to the highest value). Our finding of only a slight elevation in T levels before the start of the tamarins' breeding season (as indicated by the phase of ovarian activity following an approximately two-month post-partum ovarian inactivity phase) fits with the prediction of the challenge hypothesis. This hypothesis states that in species with low levels of aggressive

male-male interactions and a high degree of paternal infant care no or only minor elevations of T values before the onset of breeding are to be expected (Wingfield *et al.*, 1990, their fig. 4 vi & p. 836). Also consistent with the challenge hypothesis are findings in a study on the related cotton-top tamarin, where males showed increased androgen levels within three days before the female's post-partum ovulation (Ziegler *et al.*, 2003). These results, however, contrast in two aspects with our findings: Firstly, the females in our study group showed an extended period of postpartum ovarian inactivity (Löttker *et al.*, 2004). The androgen levels in our males were thus elevated over a longer time. Secondly, the first ovulation of wild moustached tamarin females after a birth is not likely to be the conceptive ones, since birth is given usually only once a year (see above, cf. Löttker et al., 2004). In addition to possible species differences, the variation might be partly related to the less constrained conditions in captivity where female apparently can afford to experience an ovulation shortly after parturition and are able to give birth twice a year regularly.

The rise of T levels in males of this study did not only precede the main breeding season but coincided with the time of highest need of parental care: In group W the phase of ovarian inactivity of the female ended on April 18 and carrying behavior declined after April 19, in group E the period of ovarian inactivity terminated around March 25 to April 12 and infants were carried much less after April 1 (Huck *et al.*, submitted-b). Wingfield and colleagues (1990) argue that T levels above the breeding baseline can be attributed solely to aggression and that a trade-off exists between male-male aggression (positively related to T levels) and paternal care (often negatively correlated with aggression and T levels). Species that depend highly on paternal care should therefore not forfeit their reproductive success by high aggression rates that might risk the brood. Therefore, if T levels indeed inhibited paternal care, rather a lower value was to be expected for our species in this period. The evidence for a negative influence of testosterone on parental behavior, however, is controversial. While this relationship was found for a variety of avian species (e.g. *Sturnus unicolor*: Moreno *et al.*,

1999, and references therein), some other species, for example the California mouse, *Peromyscus californicus*, show a positive influence of T on paternal behavior (Trainor and Marler, 2001). In cotton-top tamarins (*Saguinus oedipus*) only experienced males showed an increase in T shortly after parturition (Ziegler *et al.*, 2000). All of our adult males were likewise experienced. Thus, we envisage that elevated T levels during the postpartum unovulatory phase of the breeding female are rather related to demands of infant care in tamarins than to preparation of a loosely defined mating season. It should be borne in mind, however, that our finding contrasts to that in black tufted-ear marmosets (*Callithrix kuhli*) that showed a significant decline in urinary T levels after the infants' birth (Nunes *et al.*, 2000). The relative importance of male helpers as compared to the mother differs between callitrichid taxa (see review by Heymann, 2003), and likewise the relationship of T and paternal care in this family seems to be heterogeneous. Further studies on possible influences of certain behavioral and environmental patterns (e.g. degree of male infant care, occurrence of postpartum estrus, captive or wild population) on this link in different species are thus needed to better understand this issue.

The "challenge hypothesis" (Wingfield *et al.*, 1990) further predicts that in times of social instability, T levels should rise. Such a case of instability was probably given in group W after the death of the only female WF1 and the subsequent immigration of WF3. These events gave rise to a couple of further demographic changes in this group, including the eviction of one subadult male (WM4) and the emigration of the adult male WM1 (unpublished data). Although T levels were rather higher than mean values recorded before the change in group composition, they still lay within the 95% boundary of all former values. Given the small sample size, the lack of evidence for a consistent relationship between T levels and social group changes as described here may not be surprising. We therefore consider it to be premature to draw final conclusions.

Inter-group encounters do not seem to cause rises in T levels either, similar to findings in *Eulemur fulvus* and *Cebus apella* (Ostner *et al.*, 2002; Lynch *et al.*, 2002). Encounter rates probably are consistently high and not dissimilar enough in different months, so that they do not pose unpredictable, threatening events.

While inter-group encounters are often tumultuous, the overall level of aggression between animals of the same group is generally low (see review in Caine, 1993). This fits well with the finding that in our study groups no significant differences of hormone levels between individual males were found- regardless of male breeding status. On the contrary, the two breeders were the ones with highest (EM3) and lowest (WM1) C values of all six males. Both showed intermediate T values. Thus, no endocrinological reproductive inhibition of males was apparent. Likewise, in stable groups of olive baboons, tufted capuchins, redfronted lemurs, and cotton-top tamarins the T levels of high and low ranking individuals did not differ (*Papio anubis*: Sapolsky, 1982; *Cebus apella*: Lynch *et al.*, 2002; *Eulemur fulvus*: Ostner *et al.*, 2002; *Saguinus oedipus*: Ginther *et al.*, 2001). In contrast, many other primate species show not only pronounced rank hierarchies but these are also reflected strongly in differing hormone levels. For example, testosterone concentrations in feces of the highest-ranking male Verreaux' Sifaka were fivefold higher than those of subordinates, and black tufted-ear marmoset fathers exhibited higher T levels than their adult sons (*Propithecus verreauxi*: Brockmann *et al.*, 1998; Kraus *et al.*, 1999; *Callithrix kuhli*: French and Schaffner, 1995). Findings of other studies on physiological suppression in male callitrichids, even within the same species, are not always consistent. One study of common marmosets (*Callithrix jacchus*) found nearly twofold higher plasma levels of T in dominant versus subordinate males (Abbott *et al.*, 1992) while an earlier one obtained no evidence for endocrinological differences between males but only for (incomplete) behavioral suppression (Abbott, 1984). Similarly, Baker et al. (1999) did not find indications for a physiological suppression of adult sons in this species, but only for avoidance of mating between females and their sons. In our

study groups, a complete behavioral inhibition did not take place, since all males but EM1, the son of group E's breeding female, mated with the respective breeding female.

In line with the findings for T, cortisol levels did not correspond consistently to male breeding status (for similar findings in *Callithrix jacchus* see Ferreira Raminelli *et al.*, 2001). It also did not show any relation to the reproductive phase of the breeding female (also no elevated C levels were found postpartum in *Saguinus oedipus*: Ziegler *et al.*, 1996), nor to the inter-group encounter rate. This fits well with the fact that using the decision tree of the meta-analysis by Abbott and co-authors (Abbott *et al.*, 2003) one would predict the cortisol level of subordinates to be 98% of that of dominant individuals, i.e. hardly detectable differences (note that we interpret the social system of moustached tamarins in a slightly different way than was done for captive cotton-top tamarins). Abbott et al's considerations refer to the species level but it might be worthwhile to adapt it for individual circumstances, for example unrelated individuals.

In summary, our results conform well to predictions made by the challenge hypothesis. However, since testosterone levels are very similar in different males and gonadal function is apparently not suppressed by elevated adrenal hormone production as suggested by Sapolsky (1985) we conclude that differential reproductive success in moustached tamarins seems not to stem from endocrine mechanisms. We rather envisage behavioral mechanisms (e.g. mate-guarding) and perhaps (as suggested by their relatively large testis size, see Harcourt, 1995) sperm competition to be the major factors influencing a male's reproductive output.

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Fig 1: Mean monthly log-10 transformed testosterone (closed circles, line) and cortisol (open squares, broken line) levels of all males. Vertical bars denote 95 % confidence intervals. Untransformed values are in ng/g dry weight.

ANOVA: T: $F_{11,60} = 1.56$, $p = 0.13$; C: $F_{11,60} = 0.74$, $p = 0.70$

Fig 2: Mean log-10 transformed hormone levels of each male in different reproductive phases of group's breeding female. Untransformed values are in ng/g dry weight.

Repeated Measures ANOVA: a) testosterone: $F_{2, 10} = 12.16$ *:post-hoc comparision: $p < 0.01$

b) cortisol: $F_{2, 10} = 0.53$, $p = 0.60$

Fig 3: Mean log-10 transformed testosterone levels of adult males in a) group W and b) group E. Vertical bars denote 95 % confidence intervals. Untransformed values are in ng/g dry weight.

a) ANOVA: $F_{2, 213}=1.38$, $p=0.25$ b) ANOVA: $F_{2, 183}=2.86$, $p=.06$

Fig 4: Mean log-10 transformed cortisol levels of adult males in a) group W and b) group E. Vertical bars denote 95 % confidence intervals. Untransformed values are in ng/g dry weight.

a) ANOVA: $F_{2, 211} = 4.35$, *: $p < 0.05$ b) ANOVA: $F_{2, 174} = 2.88$, $p = 0.06$

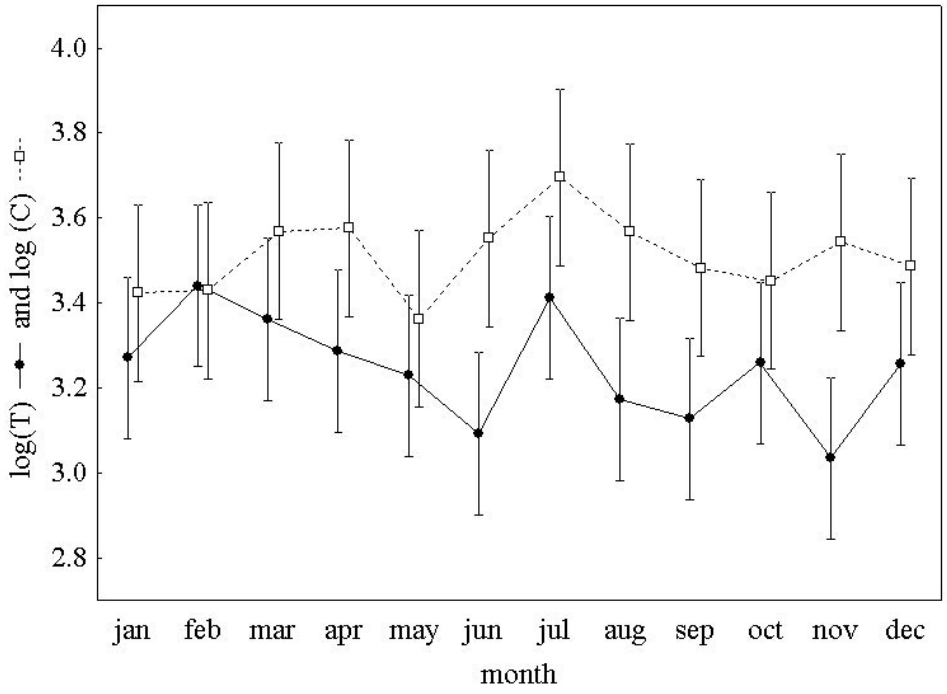
4.0
3.8
3.6
3.4
3.2
3.0
2.8
log(T) and log (C)
jan
feb
mar
apr
may
jun
jul
aug
sep
oct
nov
dec
month

fig 1

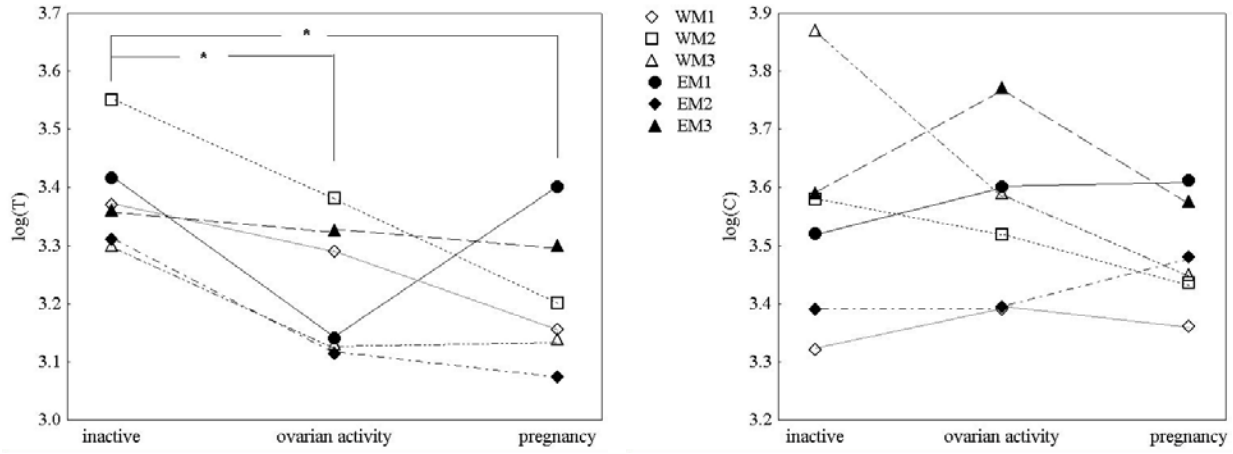
log(T)
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
*
*
inactive
ovarian activity
pregnancy
WM1
WM2
WM3
EM1
EM2
EM3
log(C)
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
inactive
ovarian activity
pregnancy

fig 2 a & b

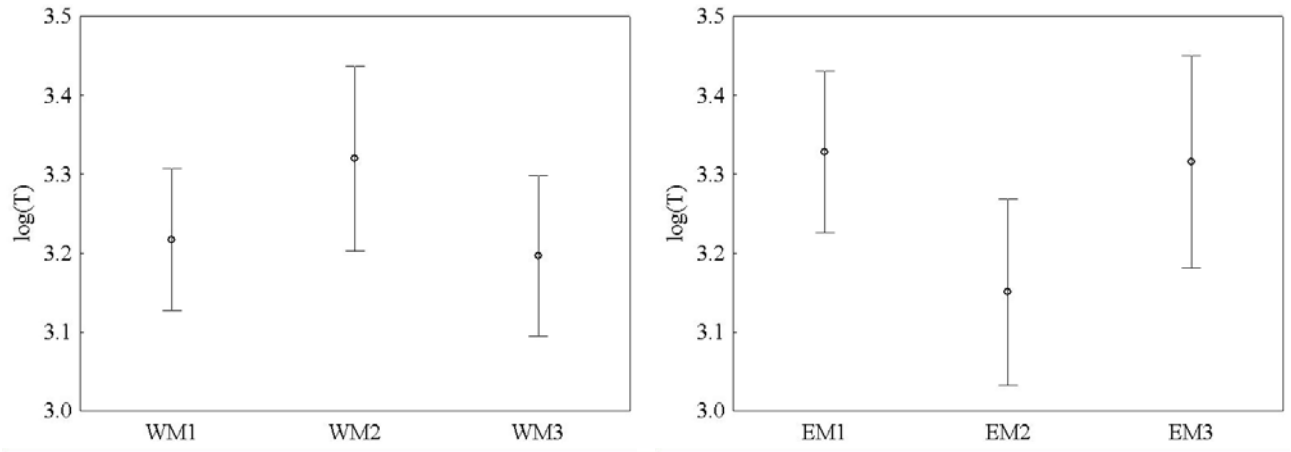

Fig 3 a & b

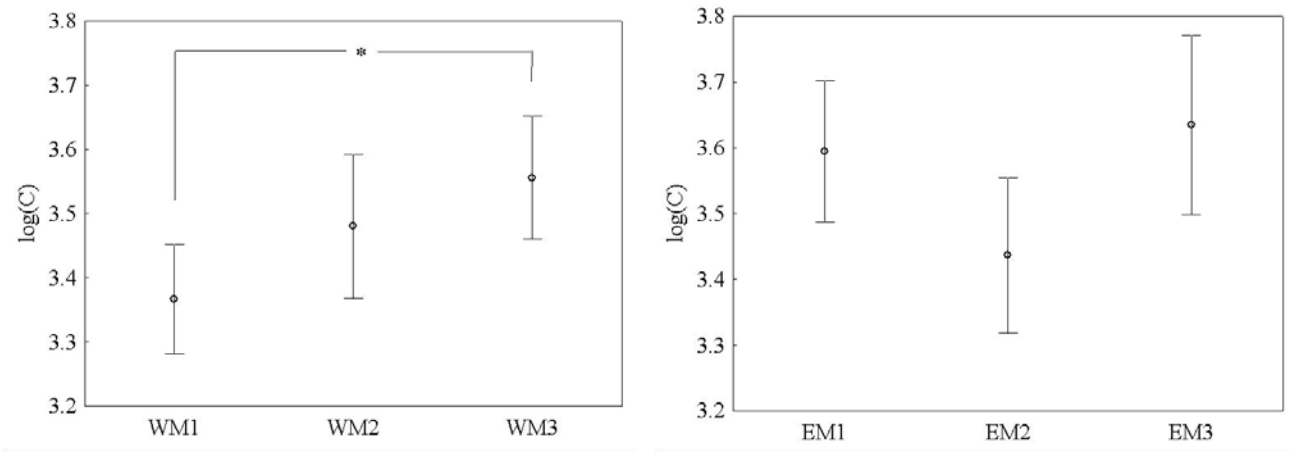

fig 4 a & b

TABLE I: Composition of Study Groups

Individual	Sex	Age class[1]	Mother/Father[2]	Demographic notes
Group W				
WF1*	f	adult		died 03.12.01
WM1	m	adult		emigrated 14.12.01
WM2	m	adult		
WM3	m	adult		
WM4	m	juvenile	WF2/WM1	born 22.2.00; emigrated 5.12.01
WM5	m	juvenile	WF2/WM1	born 22.2.00
WM6	m	infant	WF1/WM1	born 24.2.01
Group E				
EF1*	f	adult		
EF2	f	adult	EF1/EM3	emigrated 9/01
EF3	f	adult	EF1/EM3	emigrated 9/01
EM1	m	adult	EF1/EM3	
EM2	m	adult		
EM3	m	adult		
EM4	m	juvenile	EF1/EM3	born 5/00
EM6	m	infant	EF1/EM3	born 21.1.01
EF4	f	infant	EF1/EM3	born 21.1.01

[1] Age classes are defined as by Soini and Soini [1990] and refer to the age at the beginning of the study

[2] The determination of parenthood is described in Huck et al. [submitted].

* Reproductively active female

TABLE II: Mean untransformed Testosterone and Cortisol Values and its Upper 95% Boundary of Adult Males of Group W during tenure of WF1 and Mean of Samples Collected After Immigration of a New Female (WF3). Values are given in ng/g dry weight. T-tests were done with log-10-transformed values.

	Mean (with WF1)	95 % boundary	Mean (with WF3)	T-test
Testosterone				
WM1	2333.6	5683.8	670.2	not possible (N = 1)
WM2	3633.2	11112.8	3910.3	$t_{51,3} = -0.76$, p = 0.45
WM3	2367.2	9117.7	7396.8	$t_{66,5} = -2.46$, **p = 0.02**
Cortisol				
WM1	3590.9	11502.8	3460.6	not possible (N = 1)
WM2	3815.3	8627.9	3360.3	$t_{49,3} = 0.18$, p = 0.86
WM3	6470.4	20417.4	8423.7	$t_{66,5} = -0.50$, p = 0.61

TABLE III: Mean Daily Inter-group Encounter Rates per Month

Month	Group W	Group E
January	0.6	0.621
February	0.864	0.519
March	0.690	0.414
April	0.762	0.556
May	1.000	0.800
June	0.690	0.667
July	0.733	0.467
August	0.828	0.630
September	1.000	0.519
October	0.871	0.571
November	1.040	0.522
December	0.913	0.571

TABLE IV: Product Moment Correlation Coefficients and their Significance of Mean Daily Inter-group Encounters Rates per Month and Mean Monthly (log-10-transformed) Hormone Levels

Individual	r	p
Testosterone		
WM1	-0.79	**0.002**
WM2	-0.09	0.78
WM3	0.45	0.14
EM1	-0.17	0.59
EM2	-0.45	0.15
EM3	0.09	0.77
Cortisol		
WM1	-0.27	0.39
WM2	0.00	0.99
WM3	-0.06	0.86
EM1	0.01	0.99
EM2	-0.26	0.42
EM3	-0.39	0.21