

RESEARCH ARTICLE

Hormonal Changes During the Mating and Conception Seasons of Wild Northern Muriquis (*Brachyteles arachnoides hypoxanthus*)

KAREN B. STRIER^{1*}, JESSICA W. LYNCH¹, AND TONI E. ZIEGLER²

¹Department of Anthropology, University of Wisconsin–Madison, Madison, Wisconsin

²Wisconsin Primate Research Center, University of Wisconsin–Madison, Madison, Wisconsin

We investigated hormonal and behavioral changes in wild male and female northern muriquis (*Brachyteles arachnoides hypoxanthus*) at the Estação Biológica de Caratinga, Minas Gerais, Brazil, during a 6-mo period that encompassed the onset of the 1998–1999 mating and conception seasons. Individual females resumed mating with the resumption of ovarian cycling, which was not synchronized among them or related to their cortisol levels. Females experienced two to seven cycles prior to conceiving, and the first conception occurred 2 mo after the onset of the group's mating season. There were no differences in female cortisol levels across their premating, mating, and conception conditions. Cortisol levels were significantly higher in females than in males prior to the conception season, consistent with the prediction that energy reserves may be associated with breeding readiness in females, but not males, in this species. The sustained elevation in male cortisol occurred after the peak in their sexual activity, which resulted in the first conception of the year. Male cortisol levels were positively correlated between years that were similar in rainfall, but differed in the timing of sexual and reproductive events. The timing of cortisol elevations in males appears to be generally regulated by environmental cues, but is responsive to fine-tuning by social and behavioral cues related to the unpredictable timing of reproductive opportunities within their extended mating season. *Am. J. Primatol.* 61:85–99, 2003. © 2003 Wiley-Liss, Inc.

Contract grant sponsor: Liz Claiborne and Art Ortenberg Foundation; Contract grant sponsor: National Geographic Society; Contract grant sponsor: Margot Marsh Biodiversity Foundation; Contract grant sponsor: Graduate School, University of Wisconsin–Madison; Contract grant sponsor: National Center for Research Resources; Contract grant number: 00167, to Wisconsin Primate Research Center.

*Correspondence to: Karen B. Strier, Department of Anthropology, University of Wisconsin–Madison, 1180 Observatory Drive, Madison, WI 53706. E-mail: kbstrier@facstaff.wisc.edu

Received 10 October 2002; revision accepted 11 August 2003

DOI: 10.1002/ajp.10109

Published online in Wiley InterScience (www.interscience.wiley.com).

Key words: breeding readiness; reproductive seasonality; muriqui; *Brachyteles arachnoides hypoxanthus*; ovarian hormones; cortisol; energy reserves

INTRODUCTION

Seasonal reproductive activity is characteristic of many nonhuman primate species [Bronson, 1989; Lindburg, 1987]. In some seasonal breeders, the mating and conception seasons are effectively the same, whereas in others, conceptions are confined to a much shorter season than the period during which mating occurs. Breeding readiness, or the preconditioning needed to ensure full reproductive capability, is highly variable across species and between males and females of the same species. However, whether this variation reflects the degree to which mating and conception seasons coincide is not known.

In primates with a high temporal overlap in mating and conception seasons, male hormonal changes associated with breeding readiness often precede the resumption of ovarian cycling and behavioral estrus in females. For example, in captive male squirrel monkeys, cortisol elevations and increases in body mass typically occur about 1 mo prior to testosterone elevations and the resumption of ovarian cycling in females [Wiebe et al., 1988; Schiml et al., 1996, 1999]. Male rhesus macaques also exhibit prebreeding season elevations in body mass and cortisol [Bercovitch, 1992], and in gray mouse lemurs, male cortisol elevations and increases in testicular size occur 4–6 weeks earlier than the onset of female estrus [Genin & Perret, 2000; Perret & Aujard, 2001]. In these species, prebreeding-season gains in male body mass and cortisol levels have been attributed to physiological preparations in anticipation of reduced food intake during their temporally restricted mating and conception seasons [Bercovitch, 1992; Perret & Aujard, 2001].

Similar hormonal changes may also be associated with the onset of the conception season in species that mate over longer periods, as well as with rank-related differences in male behavior in species in which the mating and conception seasons coincide. In tufted capuchin monkeys, for example, mating occurs throughout the year, but male cortisol (and testosterone) levels only rise and remain elevated at the onset of the 1–2-mo conception season, when patterns of female behavioral estrus also change [Lynch et al., 2002]. In Japanese macaques, males do not exhibit prebreeding season elevations in cortisol, but during the mating and conception season, high-ranking males have significantly higher cortisol levels and engage in more energy-costly mate guarding than low-ranking males, whose cortisol levels are positively related to their mating frequencies [Barrett et al., 2001].

In contrast to males, elevations in female cortisol levels during the restricted mating and conception seasons of certain species, such as squirrel monkeys [Schiml et al., 1996, 1999] and ring-tailed lemurs [Cavigelli, 1999], have been attributed to the effects of ovarian cycling and pregnancy. Social and behavioral cues that mediate female cortisol levels in nonseasonal breeders (e.g., black tufted-ear marmosets [Smith & French, 1997] and common marmosets [Saltzman et al., 1998]) can also result in rank-related differences in female cortisol levels among seasonal breeders, such as ring-tailed lemurs [Cavigelli, 1999; Cavigelli et al., 2003].

Cavigelli et al. [2003] hypothesized that in addition to behavioral differences, the higher glucocorticoid levels of high-ranking ring-tailed lemurs might reflect dietary differences that affect their ability to maintain greater fat stores than

low-ranking females in the wild. Similar relationships between the effects of energy reserves on female reproduction have been suggested for other seasonal breeders (e.g., wild sifaka, in which heavier females are more likely to reproduce than lighter females [Richard et al., 2000]) and for species with longer, less predictable cycling-to-conception delays (e.g., Hanuman langurs, in which females in visibly good physical condition conceive faster than females in poor condition [Koenig et al., 1997]). Bercovitch [1987] found that, independent of rank, the body weights of cycling olive baboon females were negatively correlated with the number of months to conception. This indicates that energy reserves may be as important in baboons and other species that experience relatively long or unpredictable cycling-to-conception delays as they are in more strongly seasonal breeders that respond predictably to environmental cues and typically conceive within one to two cycles.

We investigated hormonal and behavioral changes in wild male and female northern muriquis (*Brachyteles arachnoides hypoxanthus*) over the same 6-mo study period, from August 1998 through January 1999, to evaluate their responses to social and environmental cues during transitions between their seasonal nonmating, mating-only, and conception conditions. In northern muriquis, the annual conception season is concentrated within a longer mating season, and female cycling-to-conception delays are highly variable. Previous studies on muriquis have shown that the resumption of female ovarian cycling coincides with the resumption of mating [Strier & Ziegler, 1994, 1997; Ziegler et al., 1997], and that sustained elevations in male cortisol are delayed relative to the onset of the annual mating season and individual males' sexual activity [Strier et al., 1999]. Here, we examine whether female cortisol levels fluctuate with season or their reproductive condition, and whether the timing of male cortisol elevations is associated with environmental cues or behavioral changes related to the onset of the conception season.

We expected that female muriqui cortisol levels would rise with the resumption of ovarian cycling and conception, as in other seasonally-breeding primates, and that as a consequence, female cortisol profiles would differ from those of males under the same environmental conditions. In addition, if relatively long, unpredictable cycling-to-conception delays of female muriquis result in high energy demands during their extended mating season, their cortisol levels prior to the onset of cycling and mating may be higher than those in males.

To distinguish between the possible effects of environmental and behavioral stimuli on the timing of sustained elevations in male cortisol, we compared male cortisol profiles during the present study period with those of the same males over the corresponding weeks from another year. The 2 years had similar rainfall patterns, but differed in the timing of the mating and conception seasons, and in the frequency and distribution of male copulations. If sustained elevations in male cortisol levels are triggered by environmental cues, one would expect them to occur at the same time in both years. Alternatively, if male cortisol elevations are triggered by behavioral changes, one would expect them to occur at different times corresponding to annual variation in the temporal patterns of sexual activity and the onset of conceptions.

MATERIALS AND METHODS

Study Site and Subjects

Behavioral and hormonal data were collected between 1 August 1998 and 31 January 1999 from members of one group of muriquis inhabiting the protected

forest at the Estação Biológica de Caratinga (EBC)/RPPN Feliciano Miguel Abdalla, located in Minas Gerais, Brazil (19°50'S, 41°50'W). The 65+ members of this study group have been the subjects of behavioral and demographic studies since 1982, and of fecal hormone studies since 1990 [Strier, 1999]. All group members are fully habituated, and can be individually distinguished by their natural markings by trained observers who follow the murequis on a near-daily basis.

Rainfall at this site is strongly seasonal, and murequi births are concentrated during the dry season (June–September) [Strier et al., 2001]. Gestation has been calculated to be 216.4 ± 1.5 days (mean \pm SD; median=216 days, $n=5$), or 7.2 months [Strier & Ziegler, 1997], and most conceptions occur during the mid-rainy season (November–February).

Annual mating seasons can be distinguished by a 1–5-mo interval during which no copulations occur. For example, the last observed copulation of the 1997–1998 mating season, which preceded the present study period, occurred on 25 April 1998. No copulations were observed until August 1998, even though the group was monitored daily during this interval.

Data Collection

Copulations involving any of the 14 adult males or 19 adult females in the study group were recorded whenever they were observed during the course of systematic behavioral and fecal sampling. Copulations were distinguished by whether or not they terminated in ejaculations, whenever visibility permitted such an observation. Fecal samples were collected from six adult males and five adult females. To facilitate interannual comparisons, the male subjects were the same individuals that had been monitored during a previous study of sexual behavior and fecal hormones in 1996–1997 [Strier et al., 1999]. The five female subjects were chosen based on their prior reproductive histories. No consistent relationship has yet been found between the timing of their previous parturitions and when females resume cycling and conceive [Strier & Ziegler, 1994, 1997; Strier, 1996], but we could predict which females would resume cycling and conceive during the 1998–1999 study period based on the year (1996) in which their previous infants had been born.

Fecal samples were collected at 4–5-day intervals from each male subject, and at 1–2-day intervals from each female subject, except during the period of 23 December to 11 January (when no observers were present) or when the target individuals could not be located, in which case their feces were collected at the earliest opportunity. This sampling regime yielded a median of 55 fecal samples (range: 48–56) per male, and 75 fecal samples (range: 69–88) per female. We obtained ≥ 1 sample per week over the 24 weeks in which collections were made from three of the five females and five of the six males. We were unable to collect samples from one female (PL) during week 2, one female (BR) during weeks 4–5, and one male (NI) during week 21.

The individuals targeted for fecal collection were followed until they defecated in a discrete location and a fresh fecal sample could be collected. The feces were scooped into 50-ml polypropylene vials, which were labeled by individual, date, and time of collection. The vials were stored on ice in insulated pouches until late afternoon, when they were transferred to a freezer (-20°C) until they were processed at the field station.

Fecal samples were collected from males before 1100 hr, and from females throughout the day. Previous analyses indicated that collection times were not related to progesterone or estradiol levels in females [Strier & Ziegler, 1994], or to

testosterone or cortisol levels in males [Strier et al., 1999]. To evaluate whether collection time might have affected our interpretation of female cortisol levels, we compared all pairs of successive daily samples in which one sample was collected before 1100 hr and the other was collected after 1100 hr from the same female. A total of 26 pairs of days met these criteria, with two to eight pairs per female. A paired t-test on log-transformed values indicated that morning and afternoon samples obtained on successive days from the same females did not differ significantly ($t(25) = -1.6, P = 0.12$).

Hormonal Assays

The frozen fecal samples were thawed at the field site, and then 0.1 g wet feces were extracted into an ethanol-water (50:50) solution, as described by Strier and Ziegler [1997]. The steroid extracts were stored at -20°C and then transported to the Wisconsin Primate Research Center for hormone analysis [Strier & Ziegler, 2000]. From the 5-ml extraction, 500 μl were subjected to solvolysis and extracted with ethyl acetate to free steroids from single and multiple conjugates [Strier et al., 1999]. Cortisol values were determined by enzyme immunoassay (EIA) [Ziegler et al., 1995]. The cortisol assay was validated for muriqui feces [Strier et al., 1999]. The intra-assay coefficients of variation (CVs) for low and high muriqui pools were 6.65 and 2.8, respectively, and the interassay CVs were 19.25 and 14.55, respectively ($n = 21$). Testosterone was analyzed by radioimmunoassay (RIA) [Strier et al., 1999]. The intra- and interassay CVs for a muriqui fecal pool were 3.12 and 7.24, respectively ($n = 6$). Estradiol and progesterone concentrations were measured by RIA [Strier & Ziegler, 1997]. The intra- and interassay CVs for a muriqui fecal pool were respectively 3.58 and 15.68 for progesterone ($n = 7$), and 4.19 and 15.44 for estradiol ($n = 7$).

Data Analyses

Daily tallies of all observed copulations permitted us to distinguish the dates and individuals involved. During the present study period, 282 copulations were observed, of which 186 involved the five female subjects and 112 involved the six male subjects whose hormones were also analyzed. At least 98 of the 112 copulations (87.5%) involving the male subjects terminated with ejaculation. Only one of the 14 remaining copulations was scored as terminating without ejaculation; the other 13 copulations could not be scored. We examined the distribution of male sexual activity by calculating the proportion of each male's copulations and confirmed ejaculations, if different, that were observed in each week of the study period.

We analyzed male testosterone, and male and female cortisol levels based on their individual conditions. We distinguished individual conditions based on the periods before and after they resumed mating, their first observed copulation (FOC), or (if different in the case of males) their first confirmed ejaculation (FCE) this year. We also divided each individual's post-FOC (or FCE) samples into their mating-only and conception periods. Female conception periods were distinguished from their mating-only periods based on when they conceived, which was evident from estradiol levels that rose and remained elevated without returning to baseline levels [Strier & Ziegler, 1997]. All six male subjects ejaculated when they copulated with the first female that conceived during her conception cycle, so

we used the period from each male's FCE to the first conception in the group to define his respective mating-only period.

Following previously established criteria [Strier & Ziegler, 1997; Ziegler et al., 1997], we used each female's progesterone and estradiol profiles to identify the resumption of her ovarian cycles. Cycle lengths were calculated as the number of days separating successive ovulations, and periovulatory periods were estimated as the day of ovulation ± 3 days. We mapped each female's copulations onto her ovarian hormone profiles to determine the coincidence between her FOC and the resumption of her ovarian cycles. We compared the cortisol levels of females prior to the resumption of their ovarian cycles, and during their periovulatory periods and the intervals between them.

To control for individual and sex differences in sample sizes, we calculated the average of individual weekly means (\pm SEM) in our analyses of cortisol and testosterone levels, and compared them across individual conditions. To examine interannual variation in male cortisol profiles, we plotted the average weekly cortisol levels in 1998–1999 against the corresponding levels from the same weeks in 1996–1997. We analyzed the 1996–1997 cortisol data in males based on their individual mating activities this year, as described above for the 1998–1999 study period. To assess the possible relationship between the timing of sustained cortisol elevations and the onset of conceptions across years, we identified the week in which cortisol levels rose and remained elevated, using previously established criteria [Strier et al., 1999].

We present the actual hormone levels in our descriptive statistics, but all parametric statistical analyses were performed on log-transformations of each individual's weekly averages [Sokal & Rohlf, 1969]. We used paired t-tests to compare individual steroid levels under different conditions, and independent t-tests to compare differences in male and female cortisol levels. We used general linear model repeated-measures analyses of variance (ANOVAS) to evaluate hormonal differences across individual premating, mating-only, and conception periods. Post-hoc analyses were done with the Tukey test. The relationship between male cortisol levels across years was assessed with a Spearman one-way correlation test. In all analyses, we considered $P \leq 0.05$ to be statistically significant, but we looked closely for consistent patterns in individual hormone levels between conditions whenever $P > 0.05$, because the small number of female and male subjects in our study made our power to detect statistical differences quite low.

RESULTS

Females

The first copulation signaling the onset of the 1998–1999 mating season occurred on 23 August, with the FCE observed 2 days later (25 August). The FOCs of each female occurred at different times (Table I), and in each case coincided with the resumption of their ovarian cycles. The average length of the ovarian cycles was 21.3 days (SD=5.2 days, median=20.0 days, $N=14$), which is nearly identical to cycle lengths calculated in other years.

Female cortisol levels ($N=5$) were similar before (mean= 47.7 ± 2.4 ng/g; median=45.4 ng/g) and after (mean= 44.4 ± 2.2 ng/g; median=46.4 ng/g; $t(4)=1.1$, $P > 0.40$) their FOCs. Cortisol levels during their periovulatory periods and the intervals between them were also similar ($t(4)=0.03$, $P > 1.0$), averaging 40.1 ± 5.6 ng/g (median=36.1 ng/g) and 40.3 ± 3.6 ng/g (median=38.5 ng/g), respectively.

TABLE I. Mating and Reproductive Patterns of Study Subjects

Individual	Previous infant's birthdate ^a	FOC (FCE) ^b	Estimated conception date ^c
Females			
NY	24 July 96	25 Aug 98	24–27 Dec 98
BR	4–5 July 96	12 Sep 98	23–26 Oct 98
PL	17–18 June 96	20 Sep 98	28–31 Dec 98
DD ^d	30–31 Oct 96	27 Oct 98	
RO	1–7 Oct 96	14 Nov 98	13–16 Dec 98
Males			
DI		23 Aug 98 (15 Sep 98)	
DA		30 Aug 98 (21 Sep 98)	
CL		2 Oct 98	
IV		2 Oct 98	
NE		2 Oct 98	
NI		20 Oct 98	

^aAll infants survived and were present when their mothers resumed cycling and copulating.

^bDate of the first observed copulation (FOC) by each individual during this study period. Dates of the first confirmed ejaculation (FCEs) of males are shown if different from the FOCs.

^cConception dates for two females (NY and PL) occurred during the period when no observers were present or fecal samples collected, and were estimated conservatively here by subtracting the mean \pm SD gestation (215–218 days) from the dates on which the infants born in 1999 were first sighted.

^dThis female did not exhibit any hormonal indications of conception during the present study period, nor did she give birth in 1999.

The first conception during the 1998–1999 breeding season occurred on 23–26 October. The four female subjects that conceived during this study experienced two to seven ovarian cycles (Table I). None were observed to copulate after they conceived. Female cortisol levels were lower during their respective mating-only periods (mean = 39.8 ± 3.6 ng/g, median = 37.4 ng/g, $n=5$) than during both their pre-mating (see above) and conception periods (mean = 50.6 ± 2.4 ng/g, median = 48.8 ng/g, $N=4$; $F=3.9$, $df=2$, 11 [due to one female that did not conceive], $P < 0.06$; Fig. 1). Although $P > 0.05$, the consistency of the pattern across females suggests that the lower cortisol levels during their mating-only periods may be a real effect.

Males

Similarly to the females, the male subjects resumed copulating at different times (Table I). There was no difference in male testosterone levels ($N=6$) before their respective FCEs (mean = 57.6 ± 4.4 ng/g, median = 58.5 ng/g) and afterward (mean = 55.7 ± 4.3 ng/g, median = 54.7 ng/g; $t(5) = -0.6$, $P=0.6$), nor did their testosterone levels differ across their pre-FCE, mating-only (mean = 54.2 ± 8.6 ng/g, median = 50.5 ng/g), and conception (mean = 55.5 ± 3.1 ng/g, median = 56.4 ng/g; $F=0.03$, $df=2$, 15, $P=0.77$) conditions.

In contrast to the females, male cortisol levels ($N=6$) were significantly higher after their respective FCEs (mean = 46.9 ± 3.4 ng/g, median = 45.8 ng/g) than before (mean = 26.7 ± 1.9 ng/g, median = 28.1 ng/g; $t(5) = -5.5$, $P=0.003$). Male cortisol levels prior to their FCEs were also similar to those during their mating-only periods (mean = 24.8 ± 4.3 ng/g, median = 25.4 ng/g; $t(5) = 1.0$, $P=0.40$), but were significantly higher after the first conception in the group (mean = 53.4 ± 4.4 ng/g, median = 51.5 ng/g) than during both their pre-FCEs ($t(5) = -6.2$, $P=0.002$).

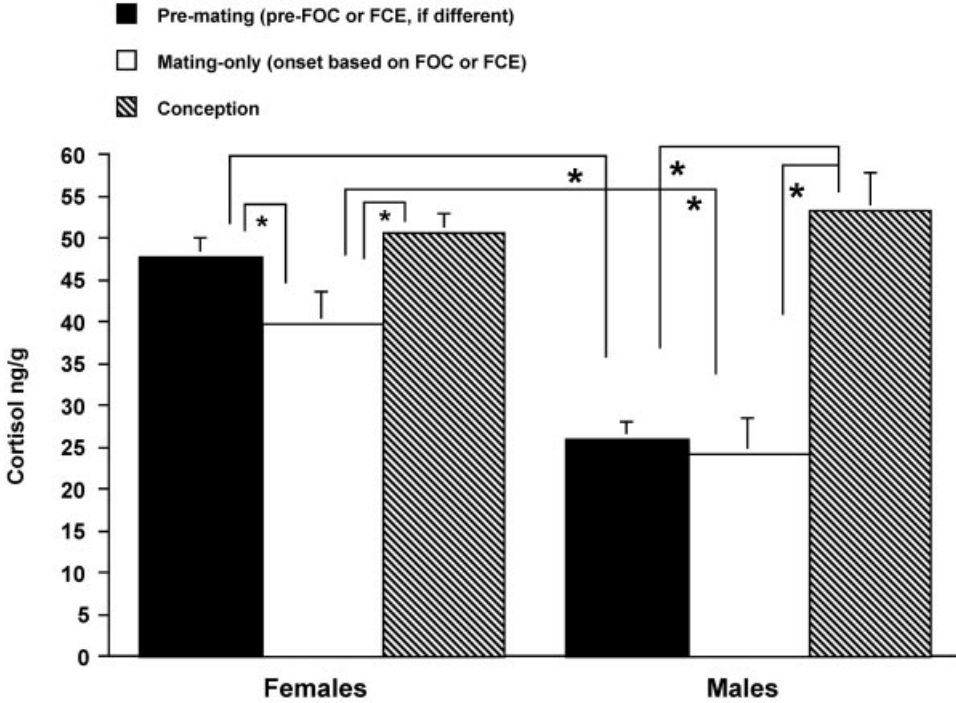


Fig. 1. Cortisol levels by individual conditions. Mean \pm SE cortisol levels of females and males during their respective pre-mating (pre-FOC or FCE), mating-only, and conception periods. Asterisks indicate differences, as described in the text, for between condition comparisons among females ($P < 0.06$) and males ($P \leq 0.01$). Cortisol levels were significantly higher in females than in males during the pre-mating ($t(9) = 6.1$, $P < 0.001$) and mating-only ($t(9) = 2.2$, $P = 0.05$) periods, but there was no sex difference in cortisol levels during the conception period ($t(8) = 0.7$, $P = 0.50$).

and mating-only periods ($t(5) = -3.9$, $P = 0.01$). The conception season was the only period in which male cortisol levels were not significantly lower than those of females (Fig. 1).

Independently of when the males resumed copulating, and of their FCEs, male sexual activity peaked during the week that preceded the first conception of the season. For all males, cortisol levels rose and remained elevated the week of October 31, or 2 weeks after their peak sexual activity and 1 week after the first conception of the season occurred (Fig. 2).

Interannual Variation in the Onset of Mating and Conceptions

The onset of the 1996–1997 mating season occurred on 27 September, with the FCE observed on 10 October, and the first conception on 26–29 November. Compared to 1998–1999, therefore, in 1996–1997 mating resumed 35 days later, the FCE was observed 46 days later, and the first conception occurred 32–38 days later. Rainfall patterns in the years preceding each of the study periods, and in the corresponding months were nearly identical and did not deviate until December, after the onset of both conception seasons (Fig. 3A).

As in 1998–1999, male cortisol levels ($N = 6$) in 1996–1997 varied with individual pre-FCE, mating-only, and conception conditions ($F = 6.9$, $df = 2$, 13 [due to two males for which no samples were obtained during their mating-only periods], $P = 0.009$). Post-hoc analyses indicated that cortisol levels were

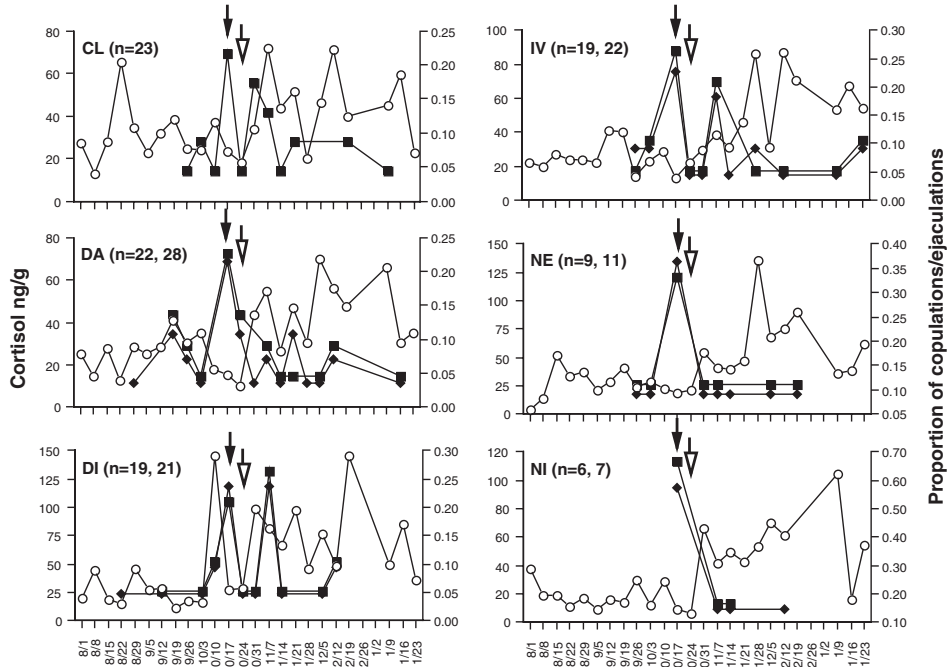


Fig. 2. Individual male cortisol profiles and sexual activities. Shown are each male's weekly cortisol levels (open circles) and the proportion of his total confirmed ejaculations (closed squares) and copulations (closed diamonds), if different. Black arrows indicate peaks in weekly ejaculation frequencies; white arrows indicate the first conception week.

significantly higher after the onset of the first conception (mean=86.8±9.2 ng/g, median=93.9 ng/g) than during the pre-FCE (mean=42.3±6.8 ng/g, median=34.7 ng/g; $P=0.012$) or mating-only periods (mean=45.0±9.3 ng/g, median=39.6 ng/g; $P=0.04$). On average, male cortisol levels were nearly twice as high (97.3%) after the first conception in 1996 than before, similar to the corresponding increase (100.1%) in 1998.

Weekly cortisol levels over the 2 years were significantly related ($r_s=0.595$, $N=23$, $P<0.01$). In 1996, the sustained elevation in male cortisol occurred between the weeks of 14 November and 21 November, or 2–3 weeks later than in 1998. This delay was less than the corresponding temporal difference in the onset of the two conception seasons, and resulted in a different pattern of sustained cortisol elevations relative to the first conceptions in the 2 years (Fig. 3B).

Weekly copulation and ejaculation rates were lower and less synchronized among the six male subjects in 1996–1997 than in 1998–1999. Although all six of the males mated (with ejaculation) within the same 8-day period, the maximum weekly total ejaculations was much lower ($N=5$) in 1996–1997 than in 1998–1999 ($N=26$). Nonetheless, as in 1998, male cortisol levels in 1996 rose and remained elevated following the peak in the males' sexual activity.

DISCUSSION

Our findings confirm and extend previous investigations into the hormonal changes associated with the annual resumption of mating and conception in wild muriquis. As in previous years, the resumption of female ovarian cycling

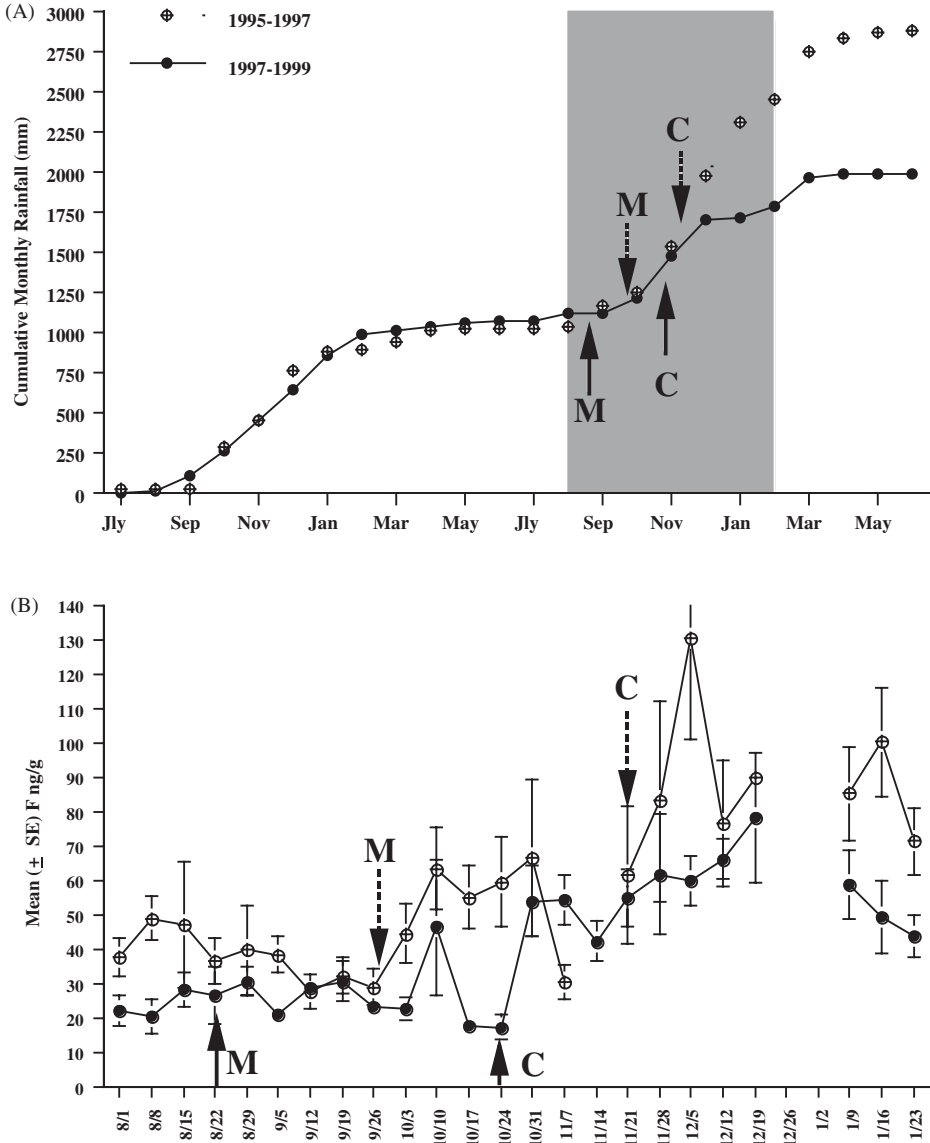


Fig. 3. Interannual variations in rainfall patterns, male cortisol profiles, and reproductive events. The onset of the mating seasons (M) and conception seasons (C) of the 1998–1999 (solid arrows) and 1996–1997 (dotted arrows) study periods are shown. **A:** Cumulative rainfall, with shaded area corresponding to the weeks for which male cortisol levels between years are compared. **B:** Weekly mean \pm SE male cortisol levels. The 1996–1997 cortisol levels are from Strier et al. [1999], reanalyzed by weeks corresponding to those in 1998–1999.

triggered the onset of the 1998–1999 mating season, and there were no hormonal changes associated with pre-mating season readiness in males. However, in contrast to our predictions, we found no evidence that cortisol levels in female muriquis are affected by their individual reproductive conditions. Although the power of our statistical comparisons across female conditions and seasons was limited by the small number of subjects in our sample, all of the females had lower cortisol levels during their mating-only periods, after they resumed cycling, than

before. This difference is in the opposite direction to that expected if their cortisol levels rise with the onset of ovarian cycling.

The significantly higher levels of cortisol in females compared to males before and during their individual mating periods are consistent with the notion that female muriquis undergo physiological changes associated with prebreeding fat storage similar to those seen in males of other species with more pronounced reproductive seasonality [Bercovitch, 2000; Whitten, 2000]. Although we lack data on changes in body mass in female muriquis, positive associations between energy reserves and reproduction have been found in females with strong breeding seasonality (e.g., gray mouse lemurs [Perret & Aujard, 2001] and sifaka [Richard et al., 2000]), as well as in females with longer, more variable cycling-to-conception delays (e.g., Hanuman langurs [Koenig et al., 1997] and olive baboons [Bercovitch, 1987]). Preliminary data indicate that female muriquis devote less time to feeding when they are cycling and mating compared to other times [Nogueira, 2001], and it is possible that maintaining energy reserves sustains them over what can be an extended mating period with unpredictable cycling-to-conception delays. However, more cortisol data collected from a larger number of females (particularly during the postlactation and prebreeding periods), as well as data on the relationship between female body mass and cortisol levels, are required before we can more fully evaluate how female cortisol levels, energy stores, and reproductive conditions are related.

The lack of changes in male testosterone levels, and the delayed elevation in male cortisol levels relative to the onset of the FCEs were consistent with previous findings based on the FOCs [Strier et al., 1999]. Male muriquis respond to cycling females by mating with them, but there is no evidence that hormonal changes precede their sexual activity. Rather, the timing of sustained elevations in male cortisol appears to be regulated in general by environmental cues, but is responsive to fine-tuning from social and behavioral cues.

Compared to the 1998–1999 period, in 1996–1997 the onset of mating and conceptions occurred later, rates of sexual activity were lower, and male mating patterns were less consistent. Yet, in both years, male cortisol levels rose and remained elevated within a 2–3-week period, subsequent to the corresponding peaks in male sexual activity. Although peak sexual activity preceded the first conceptions in both years, the sustained elevations in male cortisol occurred the week *after* the first conception in 1998, but 0–1 weeks *before* the first conception in 1996. Thus, while elevations in male sexual activity preceded both the onset of the conception seasons and sustained elevations in male cortisol, there was no consistent relationship between male cortisol elevations and the onset of the two conception seasons.

The effects of sexual activity on male cortisol elevations may be similar to those described in rhesus macaques, in which rapid elevations in cortisol have been positively related to ejaculation frequencies [Phoenix et al., 1977], and in low-ranking Japanese macaques, in which cortisol levels have been positively correlated with mating frequencies [Barrett et al., 2001]. However, in male muriquis, once cortisol is stimulated by heightened sexual activity, it remains elevated. Barrett et al. [2001] suggested that the higher cortisol levels of high-ranking male Japanese macaques may sustain them during the longer consortships they maintain with females, compared to low-ranking males. Although male muriquis do not mate-guard or maintain exclusive consortships, cortisol is known to mobilize energy for a rapid physical response [Sapolsky, 1993]. Sustaining high cortisol levels could enhance the abilities of males to respond to subsequent mating and reproductive opportunities during the ensuing conception season.

In muriquis, the number of sexually active males and females increases as the mating season progresses. The effort spent monitoring both other males and the cycling schedules of multiple females may result in reduced feeding time or efficiency. Although data on variations in male muriqui feeding patterns relative to sexual activity are not yet available (Cázar et al., unpublished results), there is indirect evidence that monitoring other males affects male mating success through scramble competition instead of contest competition. Males spend more than 50% of their time in close proximity to one another, and their associations with each other appear to be a better predictor of their mating success than the amount of time they spend associating with females [Strier et al., 2002]. The sustained cortisol elevations in male muriquis may therefore be a response that prepares them for reduced food intake during the subsequent conception season. This response is similar to that associated with prebreeding season elevations in body mass and cortisol levels in male squirrel monkeys, rhesus macaques, and gray mouse lemurs [Bercovitch, 1992; Perret and Aujard, 2001], and the elevated cortisol levels associated with consortships in high-ranking male Japanese macaques and tufted capuchin monkeys [Barrett et al., 2001; Lynch et al., 2002].

Heightened sexual activity, triggered by the unpredictable timing of female cycling, may contribute to the year-to-year fine-tuning of the sustained cortisol elevations in male muriquis within the temporal limits set by more regular environmental cues. However, it is not clear whether male behavior or hormones play a role in regulating female cycling-to-conception delays. The two to seven cycles that female muriquis exhibit prior to conception fall within the range of cycling-to-conception delays reported for other primates (e.g., mantled howler monkeys: seven to 10 cycles [Glander, 1980]; yellow baboons: median=4 cycles [Altmann et al., 1978]; mountain gorillas: median=5 cycles [Watts, 1991]; and chimpanzees: mean=4.3 cycles [Wallis, 1997]). A number of factors, including female age, reproductive experience, date of previous parturition, nutritional condition, and social rank, have been related to individual variations in cycling-to-conception delays in other species [Fedigan & Rose, 1995; Pusey et al., 1997; Bercovitch, 2000; Knott, 2001], but no consistent patterns in the attributes of female muriquis and variation in their cycling-to-conception delays have yet emerged [Strier & Ziegler, 1994, 1997; Strier, 1996].

Heightened sexual activities preceded the first conception in both years examined in this study, but not the subsequent conceptions for which behavioral data are available in these or other years. Thus, although intensive mating activity may stimulate both the onset of conceptions and male cortisol elevations, it does not appear to be necessary for subsequent conceptions that occur once male cortisol is elevated. Similarly, although the FCEs were delayed relative to the FOCs of the two male subjects that copulated earliest, the FCEs of other males in the group that also copulated at the onset of the mating season coincided with their FOCs, without resulting in female conceptions. Cortisol may affect male fertility by stimulating Leydig cell steroidogenesis, and leading to an increase in testosterone [Li, 1991], but at present there is no evidence that testosterone levels in male muriquis rise in response to cortisol elevation.

Recent analyses of primate social evolution have focused on the interacting effects of female numbers, estrus synchrony, duration of receptivity, and conception probabilities on male reproductive strategies [e.g., Mitani et al., 1996; Nunn, 1999a, b; van Schaik et al., 1999; Domb & Pagel, 2001]. Although cycling-to-conception delays have usually been considered in regard to females, the possible relationships between these delays and the behavioral endocrinology of male primates have largely been ignored. Understanding the ways in which

male hormones and behavior interact with female cycling-to-conception delays may be particularly important in studies of species (such as muriquis) that exhibit high individual and interannual variation in the timing of conceptions within the mating season.

ACKNOWLEDGMENTS

K.B.S. thanks the Brazilian government and CNPq for permission to conduct research in Brazil, G.A.B. de Fonseca and S.L. Mendes for their sponsorship during this study period, and E. Veado for logistical field support. E. Veado, J. Gomes, F. Mendes, J. Rímoli, A.O. Rímoli, F. Neri, P. Coutinho, A. Carvalho, L. Oliveira, C. Nogueira, S. Neto, W. Teixeira, R. Printes, M. Maciel, C. Costa, A. Oliva, L. Dib, D. Carvalho, N. Bejar, C.D. Cäsar, L.G. Dias, W.P. Martins, V.O. Guimaraes, J.C. da Silva, C. de Borba Possamai, R.C.R. de Oliveira, F.P. Paim, and M.F. Iurck contributed to the long-term collection of field data. We are grateful to D. Wittwer for consultation regarding the assay. We also thank M. Andrews, M. Novak, and anonymous reviewers for their comments on an earlier version of this manuscript, and J. Mitani and C. Snowden for their input and suggestions. This is publication #43-010 for the WPRC.

REFERENCES

- Altmann J, Altmann SA, Hausfater G. 1978. Primate infant's effects on mother's future reproduction. *Science* 201:1028–1030.
- Barrett GM, Shimizu K, Bardi M, Asaba S, Mori A. 2002. Endocrine correlates of rank, reproduction, and female-directed aggression in male Japanese macaques (*Macaca fuscata*). *Horm Behav* 42:85–96.
- Bercovitch FB. 1987. Female weight and reproductive condition in a population of olive baboons (*Papio anubis*). *Am J Primatol* 12:189–195.
- Bercovitch FB. 1992. Estradiol concentrations, fat deposits, and reproductive strategies in male rhesus macaques. *Horm Behav* 26:277–282.
- Bercovitch FB. 2000. Behavioral ecology and socioendocrinology of reproductive maturation in cercopithecine monkeys. In: Whitehead PF, Jolly CJ, editors. *Old World monkeys*. Cambridge: Cambridge University Press. p 298–320.
- Bronson FH. 1989. *Mammalian reproductive biology*. Chicago: University of Chicago Press. 325 p.
- Cavigelli SA. 1999. Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. *Anim Behav* 57:935–944.
- Cavigelli SA, Dubovick T, Levash W, Jolly A, Pitts A. 2003. Female dominance status and fecal corticoids in a cooperative breeder with low reproductive skew: ring-tailed lemurs (*Lemur catta*). *Horm Behav* 43:166–179.
- Domb LG, Pagel M. 2001. Sexual swellings advertise female quality in wild baboons. *Nature* 410:204–206.
- Fedigan LM, Rose LM. 1995. Interbirth interval variation in three sympatric species of neotropical monkey. *Am J Primatol* 37:9–24.
- Genin F, Perret M. 2000. Photoperiod-induced changes in energy balance in gray mouse lemurs. *Physiol Behav* 71:315–321.
- Glander KE. 1980. Reproduction and population growth in free-ranging mantled howling monkeys. *Am J Phys Anthropol* 53:25–36.
- Knott C. 2001. Female reproductive ecology of the apes. In: Ellison PT, editor. *Reproductive ecology and human evolution*. New York: Aldine de Gruyter. p 429–463.
- Koenig A, Borries C, Chalise MK, Winkler P. 1997. Ecology, nutrition, and timing of reproductive events in an Asian primate, the Hanuman langur (*Presbytis entellus*). *J Zool Soc Lond* 243:215–235.
- Li PS. 1991. Effect of cortisol on testosterone production by immature pig Leydig cells. *J Steroid Biochem Mol Biol* 38:205–212.
- Lindburg DG. 1987. Seasonality of reproduction in primates. In: Mitchell G, Erwin J, editors. *Comparative primate biology*. Vol. 2B. Behavior, cognition, and motivation. New York: Alan R. Liss. p 167–218.
- Lynch JW, Ziegler TE, Strier KB. 2002. Individual and seasonal variation in fecal

- testosterone and cortisol levels of wild tufted capuchin monkeys, *Cebus apella nigritus*. *Horm Behav* 41:275–287.
- Mitani JC, Gros-Louis J, Manson JH. 1996. Number of males in primate groups: comparative tests of competing hypotheses. *Am J Primatol* 38:315–332.
- Nogueira CP. 2001. Ecologia e comportamento das fêmeas de muriqui (*Brachyteles arachnoides hypoxanthus*, Primates, Cebidae), em diferentes estados reprodutivos, na Estação Biológica de Caratinga-MG. Doctoral thesis, Universidade Federal de Minas Gerais, Belo Horizonte. 225 p.
- Nunn CL. 1999a. The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis. *Anim Behav* 58:229–246.
- Nunn CL. 1999b. The number of males in primate social groups: a comparative test of the socioecological model. *Behav Ecol Sociobiol* 46:1–13.
- Perret M, Aujard F. 2001. Regulation by photoperiod of seasonal changes in body mass and reproductive function in gray mouse lemurs (*Microcebus murinus*): differential responses by sex. *Int J Primatol* 22: 5–24.
- Phoenix CH, Dixson AH, Resko JA. 1977. Effects of ejaculation on levels of testosterone, cortisol and luteinizing hormone in peripheral plasma of rhesus monkeys. *J Comp Physiol Psychol* 91:120–127.
- Pusey A, Williams J, Goodall J. 1997. The influence of dominance rank on the reproductive success of female chimpanzees. *Science* 277:828–831.
- Richard AF, Dewar RE, Schwartz M, Ratsirarson J. 2000. Mass change, environmental variability and female fertility in wild *Propithecus verreauxi*. *J Hum Evol* 39: 381–391.
- Saltzman W, Schultz-Darken NJ, Wegner FH, Wittwer DJ, Abbott DH. 1998. Suppression of cortisol levels in subordinate female marmosets: reproductive and social contributions. *Horm Behav* 33:58–74.
- Sapolsky RM. 1993. The physiology of dominance in stable versus unstable social hierarchies. In: Mason WA, Mendoza SP, editors. *Primate social conflict*. Albany, NY: SUNY Press. p 171–204.
- Schimpl PA, Mendoza SP, Saltzman W, Lyons DM, Mason WA. 1996. Seasonality in squirrel monkeys (*Saimiri sciureus*). *Physiol Behav* 60:1105–1113.
- Schimpl PA, Mendoza SP, Saltzman W, Lyons DM, Mason WA. 1999. Annual physiological changes in individually housed squirrel monkeys (*Saimiri sciureus*). *Am J Primatol* 47:93–103.
- Smith TE, French JA. 1997. Social and reproductive conditions modulate urinary cortisol excretion in black tufted-ear marmosets (*Callithrix kuhli*). *Am J Primatol* 42:253–257.
- Sokal RR, Rohlf FJ. 1969. *Biometry*. New York: W.H. Freeman and Company. 776 p.
- Strier KB, Ziegler TE. 1994. Insights into ovarian function in wild muriqui monkeys (*Brachyteles arachnoides*). *Am J Primatol* 32:31–40.
- Strier KB. 1996. Reproductive ecology female muriquis (*Brachyteles arachnoides*). In: Norconk MA, Rosenberger AL, Garber PA, editors. *Adaptive radiations of neotropical primates*. New York: Plenum Press. p 511–532.
- Strier KB, Ziegler TE. 1997. Behavioral and endocrine characteristics for the reproductive cycle in wild muriqui monkeys, *Brachyteles arachnoides*. *Am J Primatol* 42: 299–310.
- Strier KB. 1999. *Faces in the forest: the endangered muriqui monkeys of Brazil*. Cambridge, MA: Harvard University Press. 138 p.
- Strier KB, Ziegler TE, Wittwer D. 1999. Seasonal and social correlates of fecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoides*). *Horm Behav* 35:125–134.
- Strier KB, Ziegler TE. 2000. Lack of pubertal influences on female dispersal in muriqui monkeys, *Brachyteles arachnoides*. *Anim Behav* 59:849–860.
- Strier KB, Mendes SL, Santos RR. 2001. Timing in births in sympatric brown howler monkeys (*Alouatta fusca clamitans*) and northern muriquis (*Brachyteles arachnoides hypoxanthus*). *Am J Primatol* 55:87–100.
- Strier KB, Dib LT, Figueira JEC. 2002. Social dynamics of male muriquis (*Brachyteles arachnoides hypoxanthus*). *Behaviour* 139:315–342.
- van Schaik CP, van Noordwijk MA, Nunn CL. 1999. Sex and social evolution in primates. In: Lee PC, editor. *Primate socioecology*. Cambridge: Cambridge University Press. p 204–240.
- Wallis J. 1997. A survey of reproductive parameters in the free-ranging chimpanzees of Gombe National Park. *J Reprod Fertil* 1009:297–307.
- Watts DP. 1991. Mountain gorilla reproduction and sexual behavior. *Am J Primatol* 24:211–225.
- Whitten PL. 2000. Evolutionary endocrinology of the cercopithecoids. In: Whitehead PF, Jolly CJ, editors. *Old World monkeys*. Cambridge: Cambridge University Press. p 269–297.
- Wiebe RH, Williams LE, Abee CR, Yeoman RR, Diamond EJ. 1988. Seasonal changes in serum dehydroepiandrosterone, androste-

- nedione, and testosterone levels in the squirrel monkey (*Saimiri boliviensis boliviensis*). *Am J Primatol* 14:285–291.
- Ziegler TE, Scheffler G, Snowdon CT. 1995. The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, *Saguinus oedipus*. *Horm Behav* 29:407–424.
- Ziegler TE, Santos CV, Pissinatti A, Strier KB. 1997. Steroid excretion during the ovarian cycle in captive and wild muriquis, *Brachyteles arachnoides*. *Am J Primatol* 42:311–321.