

Sex and seasonal differences in aggression and steroid secretion in *Lemur catta*: Are socially dominant females hormonally ‘masculinized’?

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Abstract

Female social dominance characterizes many strepsirrhine primates endemic to Madagascar, but currently there is no comprehensive explanation for how or why female lemurs routinely dominate males. Reconstructing the evolutionary pressures that may have shaped female dominance depends on better understanding the mechanism of inheritance, variation in trait expression, and correlating variables. Indeed, relative to males, many female lemurs also display delayed puberty, size monomorphism, and ‘masculinized’ external genitalia. As in the spotted hyena (*Crocuta crocuta*), a species characterized by extreme masculinization of the female, this array of traits focuses attention on the role of androgens in female development. Consequently, I examined endocrine profiles and social interaction in the ringtailed lemur (*Lemur catta*) to search for a potential source of circulating androgen in adult females and an endocrine correlate of female dominance or its proxy, aggression. I measured serum androstenedione (A_4), testosterone (T), and estradiol (E_2) in reproductively intact, adult lemurs (10 females; 12 males) over four annual cycles. Whereas T concentrations in males far exceeded those in females, A_4 concentrations were only slightly greater in males than in females. In both sexes, A_4 and T were positively correlated, implicating the Δ^4 -biosynthetic pathway. Moreover, seasonal changes in reproductive function in both sexes coincided with seasonal changes in behavior, with A_4 and T in males versus A_4 and E_2 in females increasing during periods marked by heightened aggression. Therefore, A_4 and/or E_2 may be potentially important steroidal sources in female lemurs that could modulate aggression and underlie a suite of masculinized features.

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Introduction

Unlike most mammals (Ralls, 1976), many strepsirrhine primates display a social organization that is characterized by female dominance over males (Jolly, 1966; Richard, 1987). This unusual trait has received considerable attention and its behavioral regulation has engendered significant debate: Some researchers suggest that males defer to females only in feeding contexts, via ‘male deference’ (Hrdy, 1981) or ‘female feeding priority’ (Jolly, 1984), whereas others suggest that females maintain elevated status through overt aggression against males, via ‘female dominance’ (Kappeler, 1990a; Pereira et al., 1990). Regardless of the gradations in intersexual social relationships, functional explanations for the evolution of this trait, that invoke

benefits to the female, are often linked to reproductive energetics, maternal investment, or nutritional intake (Jolly, 1984; Richard and Nicoll, 1987; Tilden and Oftedal, 1995; Young et al., 1990); nevertheless, the proximate mechanism remains a mystery.

Insight into a potential mechanism of female social dominance may derive from other unusual features of strepsirrhines, including sexual size monomorphism (Kappeler, 1990b), absence of bimaturation (Leigh and Terranova, 1998), and ambiguous (Hill, 1953; Ioannou, 1971; Petter-Rousseaux, 1964) or moderately ‘masculinized’ (Drea and Weil, submitted for publication) external genitalia. This array of male-like behavioral, physiological, and morphological traits calls attention to the possible role of androgens in female lemur development. Although female animals display aggression in a variety of social contexts, the neuroendocrine mechanisms that govern female competitive behavior remain

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poorly understood. Consequently, using the ringtailed lemur (*Lemur catta*) as a model – one with evolutionary ties to humans – I examine a potential route of hormonal mediation in female aggression and explore the hypothesis that female strepsirrhines may be masculinized by endogenous androgens.

An hormonal mechanism of social dominance ideally could explain an entire suite of male-like characteristics in the female, as well as patterns of individual or species variation. For instance, contemporary understanding of sexual differentiation requires that androgens circulate in the female fetus to produce male-like genitalia (Jost, 1953). Female exposure to prenatal androgens also delays puberty in the masculine direction (Goy and Robinson, 1982). Finally, androgens might enhance female aggressiveness, and hence dominance, by functioning as organizing hormones during fetal life or activating hormones in adulthood (Beatty, 1979; Goy and McEwen, 1980; MacLusky and Naftolin, 1981). Here, I explore the potential activating role of steroids in female aggression, recognizing that a contemporaneous hormone–behavior relationship is also affected by earlier organizational influences.

The suite of masculine traits in female lemurs recalls another exceptional mammal—the female spotted hyena (*Crocuta crocuta*). For instance, the external genitalia of female *L. catta* vaguely resemble those of the male, i.e. in terms of enlargement and elongation of the clitoris, as well as clitoral placement of the urinary meatus (Drea and Weil, submitted for publication). Likewise, but more strikingly, the external genitalia of female *Crocuta* closely mimic those of the male: The clitoris is elongated to form a fully erectile pseudopenis and the labia are permanently fused to form a pseudoscrotum (Frank et al., 1990; Matthews, 1939; Neaves et al., 1980). Thus, female *Crocuta* are unique among mammals in that they lack an external vagina and require mating and parturition to occur through a urogenital canal that traverses the hypertrophied clitoris. Importantly, the present discussion addresses only the shared morphological characteristics between the external genitalia of female lemurs and hyenas.

In addition, male and female *L. catta* mature at similar ages (Sussman, 1991) and achieve similar adult sizes (Drea and Weil, submitted for publication; Kappeler, 1990b). Somewhat more unusually, female *Crocuta* mature later than do males and achieve greater mass (Glickman et al., 1992; Kruuk, 1972; Matthews, 1939). In both species, which live in ‘multimale–multifemale’ societies (*L. catta*: Jolly, 1966; Sussman, 1991; *Crocuta*: Frank, 1986; Kruuk, 1972), females unconditionally dominate their male companions (*L. catta*: Jolly, 1966; Kappeler, 1990a; Pereira et al., 1990; Sauther, 1993; *Crocuta*: Drea and Frank, 2003; Frank, 1986; Kruuk, 1972; Smale et al., 1993). Thus, both species have promiscuous mating systems in which females exercise significant reproductive control (Drea and Wallen, 2003; Drea et al., 1999; East et al., 1993; Sauther, 1991) and gain other advantages, such as priority of access to resources.

Unlike in strepsirrhines, for which there is a dearth of hormonal data, the reproductive endocrinology of the spotted hyena has received considerable attention. Beyond the contribution of androgen-independent mechanisms to genital

development (Drea et al., 1998; Glickman et al., 1998, 2006), sexual differentiation in the female spotted hyena has both a prenatal and a postnatal hormone component, reflected by elevated androgen concentrations during the fetal period and throughout life (Dloniak et al., 2004; Drea et al., 1998; Glickman et al., 1987, 1992; Licht et al., 1992, 1998; Lindeque et al., 1986; Racey and Skinner, 1979). Prior investigators identified the prohormone Δ^4 androstenedione (Androst-4-ene-3,17,dione; A_4) as the primary circulating androgen in the female spotted hyena and established that females have higher circulating concentrations of A_4 than do males, more than 90% of which has an ovarian source (Glickman et al., 1987, 1992; Licht et al., 1992; Racey and Skinner, 1979). Although typically dismissed as a weak or inactive androgen because it fails to bind to androgen receptors, A_4 is readily converted to either testosterone (T) or estrone in the presence of appropriate enzymes. Free A_4 is therefore an ideal substrate for local conversion to active androgens and estrogens, either of which could produce powerful effects on behavior and morphology.

Several lines of evidence link A_4 to female dominance in spotted hyenas. For instance, the hyena placenta is characterized by high 17β -hydroxy-dehydrogenase activity, which converts A_4 to T. Increasing plasma T levels in pregnant females are transferred to developing fetuses of both sexes, providing a mechanism for female expression of male-typical traits (Licht et al., 1992, 1998; Yalcinkaya et al., 1993). Mothers with the highest androgen concentrations during pregnancy produce the most aggressive offspring (Dloniak et al., 2006), whereas anti-androgen treated mothers (Drea et al., 1998) produce infants that show both reduced A_4 concentrations and less severe sibling aggression during the early postnatal period (Drea et al., unpublished data). Lastly, ovariectomy during the juvenile period significantly reduces female aggression towards males in adult life, implicating an activational role of ovarian hormones, including either androgens and/or estrogens, in female dominance (Baker, 1990).

The behavioral and morphological similarities between female *Crocuta* and *L. catta* support a comparative endocrine approach to define factors relevant to feminine development, independent of phylogenetic constraints. In early studies of lemur reproductive endocrinology, researchers assessed hormone concentrations from serum or plasma samples (Bogart et al., 1977; Evans and Goy, 1968; Van Horn and Resko, 1977; Van Horn et al., 1976), whereas current investigators typically rely on hormone assays derived from fecal preparations (Cavigelli and Pereira, 2000; Von Engelhardt et al., 2000). In both cases, the investigators report on steroid production either in only one sex or for only a portion of the year, such that comparative data on the full reproductive cycle are unavailable. With one exception (Von Engelhardt et al., 2000), investigators also follow traditional paradigms, focused on assessing the role either of estrogens and progestogens in regulating female reproductive cycles (Bogart et al., 1977; Van Horn and Resko, 1977) or of T in regulating male reproductive cycles and aggression (Bogart et al., 1977; Cavigelli and Pereira, 2000; Evans and Goy, 1968; Van Horn et al., 1976). There are no reports on measurement of A_4 in either sex. In the present study,

I obtained serum samples from adult lemurs of both sexes throughout the entire annual cycle, repeated for four breeding seasons. During the last two years, I also obtained data on social behavior, focusing on aggressive interactions. I report on both sex-typical (e.g. 17β -estradiol, E_2 , in females) and sex-atypical or ‘heterologous’ (e.g. A_4 and T in females) hormones.

In the present study, I examine on the bioavailability of A_4 to female lemurs. In spotted hyenas, adult, nonpregnant females have circulating A_4 concentrations that are elevated by comparison to (1) their own T concentrations, (2) A_4 concentrations of male conspecifics, and (3) A_4 concentrations of many other female mammals (Glickman et al., 1992). According to the present hypothesis, if A_4 is implicated in female dominance, the same general patterns should be evident in adult, nonpregnant female lemurs. Because ‘masculinization’ is less extreme in lemurs than in spotted hyenas, however, an important caveat is that these patterns may be less pronounced in lemurs. Moreover, if seasonal A_4 elevations occur in lemurs, they should be most apparent during periods characterized by heightened aggression. An additional goal of this study is to track correlated seasonal changes in steroid concentrations as a possible indicator of the major metabolic pathway involved in T production. If A_4 provides the main source of T via the progesterone or Δ^4 -pathway, as opposed to the dehydroepiandrosterone or Δ^5 -pathway, then animals should show a positive correlation in the production of these two androgens (Wichmann et al., 1984).

Methods

Animals

The subjects of the endocrine study were 22 captive-born, gonadally intact ringtailed lemurs, including 10 females (age range: 2–20 years) and 12 males (age range: 2.5–20 years; Table 1). These animals were the adult members of three semi-free ranging social groups housed at the Duke Lemur Center (DLC), in Durham, NC (see housing details below). I studied the animals during four periods: an 8-month period from Dec 1999 to Jul 2000, a 6-month period from Oct 2001 to Mar 2002, and two contiguous 12-month periods from Aug 2003 to Jul 2005 (Table 1). I obtained concurrent behavioral data on the subset of 17 adult lemurs (8 females, 9 males) studied during the latter 24-month period. As data collection occurred over a six-year span, the animals that served as subjects in the endocrine study varied across years. Moreover, as the social groups comprised animals of all ages, some of the younger lemurs became focal subjects at maturity. Although, in the wild, males and females typically mate for the first time at about 2.5–3 years of age (Sussman, 1991), conceptions at younger ages (including 1.5 years) occur at the DLC (unpublished records). Thus, I consider these animals to be reproductively mature at about 2 years. Parenthetically, 15 of the subjects in this study (8 females and 7 males) were also subjects in a study of external genital morphology in which the researchers describe moderate ‘masculinization’ of females (Drea and Weil, submitted for publication).

Female *L. catta* exhibit strictly seasonal estrous cycles, with limited periods of receptivity to the male; however, females are polyestrous, cycling up to three times per season at about 40-day intervals (Evans and Goy, 1968; Jolly, 1966; Van Horn and Resko, 1977). Despite estrous synchrony at the species level, individual females show some asynchrony (Pereira, 1991), in that group members cycle within 1 to 3 weeks of each other (Jolly, 1966; Sauther, 1991). Thus, herein, the breeding ‘season’ refers to the span of time encompassing potentially three breeding peaks rather than representing one continuous season. In the Northern Hemisphere, seasons are shifted by 6 months from those in Madagascar (Van Horn, 1975) such that the breeding season at the DLC begins in late October, with a peak in conceptions occurring in early November (the first cycle). Most births occur in March, therefore, but some occur as late as June.

Table 1

Demographics of 22 ringtailed lemurs that served as subjects during four study periods

Study period	Females			Males		
	Subject and DLC #	Ages (years)	Breeding status	Subject and DLC #	Ages (years)	Breeding status
<i>Period 1: Dec 1999–Jul 2000 (8 months)</i>						
	1. 6280	10–11	Multiparous	11. 6435	8–9	Mature
	2. 6140	12–13	Multiparous	12. 6322	10–11	Mature
	3. 5847	15–16	Multiparous	13. 5811	16–17	Mature
<i>Period 2: Oct 2001–Mar 2002 (6 months)</i>						
	4. 6761	1–2 ^a	Nulliparous	14. 6688	5	Mature
	6140*	14	Multiparous	6322*	12	Mature
<i>Periods 3 and 4: Aug 2003–Jul 2005 (24 months)</i>						
	5. 6796	1–3 ^a	Nulliparous	15. 6795	1–3 ^a	Pubertal–mature
	6761*	3–5	Parous	16. 6528	11 ^b	Mature
	6. 6709	6–8	Nulli/ primiparous	17. 6485	11–13	Mature
	7. 6711	6–8	Nulli/ primiparous	18. 6440	12–14	Mature
	8. 6276	14–16	Multiparous	19. 6368	13 ^c	Mature
	6140*	16–17 ^c	Multiparous	6322*	14–15 ^c	Mature
	9. 6159	16–18	Multiparous	20. 6268	15–16 ^b	Mature
	10. 5984	18–20	Multiparous	21. 6191	16–17 ^c	Mature
	–	–	–	22. 5969	18–19 ^b	Mature

Ages are given for the period of time that each animal was sampled, reflecting the following: ^a animals that came of age during the study period (and whose prepubertal samples were excluded from analyses); ^b animals that were removed from or introduced into the social group during the course of the study, and; ^c animals that died during the course of the study.

* Animals served as subjects in earlier study periods.

Although each study period (Table 1) encompassed most or all of a breeding season, the female data presented herein exclude values obtained during pregnancy. I achieved year-round endocrine sampling of females (see protocol below) by obtaining samples during the breeding season from females that were not pregnant at the time. Pregnancies, initially suspected by observed matings or copulatory plugs, were subsequently confirmed by monthly palpation, occasional obstetric ultrasound scanning, and, ultimately, by parturition. I estimated conception dates by subtracting 135 days (the gestation length of *L. catta*: Evans and Goy, 1968) from the date of parturition. I excluded from the analyses all blood samples or behavior obtained during these intervening periods. The remaining ‘nonconceptive’ values derived from females that may have lacked mating opportunities, mated but failed to conceive or became pregnant in a later cycle. No female subject had an unusual reproductive history and, to date, each has conceived at least once. Data on the hormonal correlates of prenatal development and the behavior of pregnant females will be presented elsewhere (Drea, unpublished data).

Housing

The subjects were housed socially in one of three semi free-ranging groups, each occupying a separate, forested enclosure (1.5, 3.3, or 5.8 ha), with access to indoor, heated rooms or thermostatically controlled nest boxes. During inclement weather, the social groups were kept in heated indoor areas (110, 193, or 246 ft²), two of which had an attached outdoor run (278 or 347 ft²) enclosed by chain-link fencing and containing branch supports. By convention, the top-ranking male of multimale groups remains with the adult females and infants during winter lock-ups, while the other adult male(s) of the group are housed in adjacent runs (about 100 ft²), often with shared fences that permit visual and olfactory access between animals. The separation serves to mitigate the intense male–male aggression that erupts during the breeding season and that might otherwise escalate in confined quarters.

The subjects were fed daily rations of a commercially available primate diet (Purina® Monkey Diet 5038, PMI Nutrition International, Inc., Brentwood, MO 63144, USA), supplemented with fresh fruit and vegetables. When the animals were free ranging, they additionally complemented their diet with food foraged from the forest. Water was always freely available. The animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Blood sampling

With the assistance of DLC veterinary personnel, we obtained blood samples about once (sometimes twice) monthly per subject (for a total of $n=141$ male and $n=112$ female samples). The regular monthly sample occurred within the first week of each month and, when possible, the second sample occurred 2 weeks later. When two samples were obtained from one individual in a given month, the values were averaged. Logistical constraints of a longitudinal study on an exotic species contributed to an unequal representation of samples at different times of the year. These constraints included unrelated animal illnesses or deaths and male transfers to mitigate aggression or inbreeding (see note of Table 1).

On blood-draw days, animals that previously had been corralled into their indoor enclosures were netted and processed individually, to minimize the time delay between capture and blood draw (mean \pm S.E.M. = 5.31 ± 0.26 min). Handling occurred primarily in the morning (between 8:15 and 12:30 h, mean \pm S.E.M. = $10:07 \pm 0:24$ h). Using a 23-gauge needle and syringe, we drew blood samples (3 cc) from the femoral vessels of awake, manually restrained animals, all of which were habituated to these procedures. We immediately transferred the blood samples to serum separator tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ 07417, USA), allowed them to clot at ambient temperature, centrifuged them at $1500 \times g$ for 20 min, and stored the decanted serum at -80°C . All research protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Duke University (protocols #A457-99-09, A245-03-07).

Hormone assay

The hormone assays were performed in the Endocrine Core Laboratory at the Yerkes National Primate Research Center using previously validated procedures. To control for interassay variation, (1) all samples were processed together, (2) all samples from a given subject's breeding cycle were run in the same assay, and (3) samples from males and females were equally distributed between assays. Serum A_4 and T were determined using commercial radioimmunoassay (RIA) kits (Diagnostic Systems Laboratories, Webster, TX 77598, USA). The A_4 assay has a sensitivity of 0.1 ng ml^{-1} using a $50 \mu\text{l}$ dose, with an intra- and inter-assay coefficient of variation (CV) of 5.09% and 12.4%, respectively. The T assay has a sensitivity of 0.05 ng ml^{-1} using a $50 \mu\text{l}$ dose, with an intra- and interassay CV of 6.3% and 5.0%, respectively. Serum E_2 was determined using a modification (Pazol et al., 2004) of a commercially available RIA kit (Diagnostic Products Corp., Los Angeles, CA 90045, USA). Prior to assay, samples ($250 \mu\text{l}$) were extracted twice with 5 ml of anesthesia grade ether. Following solvent evaporation, the samples were reconstituted with $250 \mu\text{l}$ of zero calibrator and $200 \mu\text{l}$ aliquots were assayed in duplicate. The E_2 assay has a sensitivity of 5 pg ml^{-1} using $200 \mu\text{l}$ of extracted serum, with an intra- and interassay CV of 7.9% and 6.9%, respectively. Sample values of E_2 were corrected for extraction efficiency, which exceeded 80%.

Behavioral observation

During the last 24-month period of study, endocrine sampling was coupled with behavioral observation. Accordingly, four observers, blind to the goals of this study, watched all group members year round (weather permitting), each during two 20-min focal periods per week. Using an ethogram that included all forms of social, reproductive, developmental, and communicative behavior (e.g. Drea and Scordato, in press), the observers recorded states and events in real time on handheld computers (Psion 'Workabout', Noldus Information Technology, Inc., 751 Miller Drive, Suite E-5, Leesburg, VA 20175-8993, USA). From that larger dataset, I extracted frequencies of unequivocal dominance/subordination or 'aggressive' interaction (including supplants, cuffs, lunges, chases, withdrawals, and bites) occurring between adult subjects. I did not include more subtle expressions of social dominance, such as those involving grooming or inter-individual proximity. These data derived from

nearly 390 h of adult focal observation, obtained while the lemurs were semi-free ranging (i.e. only when the social group was intact). Because of inclement weather and animal injuries or pregnancies, certain winter months are underrepresented in the behavioral dataset.

Statistical analyses

For the endocrine analyses, I assigned the minimum detectable limit of the assay to the few samples that had undetectable E_2 concentrations, and confirmed normality of distribution and homogeneity of variances. I first evaluated adult sex differences in serum hormone concentrations, averaged across the year, by Student's *t*-test. In subsequent analyses, I treated males and females separately. For analyses of age effects, I collapsed subjects into four age bins (2–5, 6–10, 11–15, and 16–20 years) and conducted one-factor analyses of variance for repeated measures (rANOVA). I evaluated the effects of social context on male androgen secretion by Student's *t*-test.

In initial analyses of seasonal effects, I compared monthly patterns of androgen production using two-factor (androgen \times month) rANOVAs, for which I resolved significant interactions by comparing androgen production within each sampling time point, using *F*-tests for simple effects and a Bonferroni correction for multiple comparisons (Sokal and Rohlf, 1981). I further evaluated the main effects of month for each steroid, independently, using one-factor rANOVAs. As production of the different androgens showed a linear relationship in both sexes, I evaluated the correlation between A_4 and T in both male and female subjects using the Pearson correlation. Lastly, I divided the year into three 'seasons,' each encompassing nonbreeding (Jul–Oct), breeding (Nov–Feb), and birthing (Mar–Jun) activity. As the majority of blood draws occurred early in the month, I assigned transitional months to the earlier season (e.g. Oct to nonbreeding and Feb to breeding). I evaluated main effects of season for each steroid, independently, using one-factor rANOVAs and the Tukey test for pairwise comparisons among means. Because variances for male androgen and female estrogen values were not homogeneous in these comparisons, I used log-transformed data in the analyses, but present the actual values in all figures.

For the behavioral analyses, I tallied monthly occurrences of adult aggression for each focal individual, separated by the sex of the actor and/or recipient, and converted these frequencies to hourly rates. I then collapsed the actor and recipient categories for within-sex interactions to produce separate monthly tallies of male–male and female–female interaction, and separately calculated between-sex interaction. The latter were unidirectional (i.e. male-directed female aggression), as is characteristic of this unambiguously female-dominant species. As the number of adult animals varied by group and across portions of the year, I corrected these monthly rates for the number of potential adult partners of each sex and entered these values into three separate rANOVAs to test for monthly patterns in aggression. I also compared aggression across the

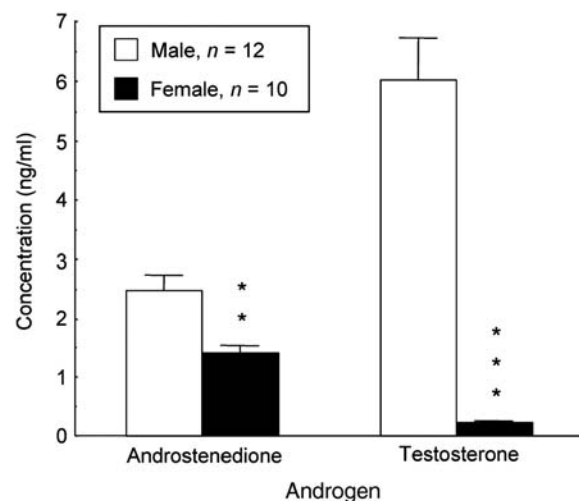


Fig. 1. Mean \pm S.E. annual serum androstenedione and testosterone concentrations of adult ringtailed lemurs (Student's *t*-test comparing between sexes: ** $P < 0.005$; *** $P < 0.001$).

same three seasons, as defined previously; however, it is important to note that, because behavioral data spanned the entire month, transitional periods were more ambiguous (e.g. end of Oct arguably could be considered breeding season). For all analyses, I considered differences at $P < 0.05$ to be significant.

Results

Sex differences in steroid production

Ringtailed lemurs showed clear sex differences in androgen production (Fig. 1). On average, serum concentrations of both A_4 and T were significantly elevated in males by comparison to females (A_4 : $t_{20} = 3.370$, $P < 0.005$; T: $t_{20} = 7.297$, $P < 0.001$). Male A_4 and T concentrations also appeared to be elevated by comparison to other non-lemurid species (Table 2). Whereas T concentrations were far (26x) greater in males than in females, A_4 concentrations in males were less than double (1.7x) those of females (Table 2; Fig. 1).

In the female ringtailed lemur (as in the female spotted hyena), mean T values fell within the ranges reported for other female mammals (Table 2). Mean A_4 concentrations in female lemurs fell within the low range of spotted hyena values (Table 2): They were unremarkable compared to some species (e.g. human, baboon), but elevated by comparison to other species

(e.g. dog, rabbit, rat). E_2 concentrations in female lemurs ranged from 5 to 69.18 pg ml^{-1} (mean = 22.70 ± 2.84). Within this adult sample, no age effects on hormone secretion were evident in either males or females (data not shown).

Social context and male endocrine profiles

Because of the different housing conditions of males during portions of the winter breeding season, I first investigated the relationship between social context and endocrine profiles. Winter housing conditions (which applied intermittently from Nov–Mar) had no significant effect on male endocrine profiles (Fig. 2): In each of these months, males ($n = 4$) housed alone or with a male companion showed similar androgen concentrations as did males ($n = 7$) housed with females (A_4 : $t_9 < 0.927$, *n.s.*; Fig. 2a; T: $t_9 < 0.999$, *n.s.*; Fig. 2b). Therefore, in subsequent monthly and seasonal analyses of hormone concentrations, I combined all males, regardless of social context.

Seasonal patterns in male endocrine profiles

Mean monthly androgen concentrations are depicted for all males sampled (Figs. 3 and 4a); however, because of missing samples, I used for the analyses a subset of males ($n = 5$) that

Table 2
Plasma or serum concentrations of testosterone and androstenedione in adult male and nonpregnant female mammals

Species	Testosterone (ng ml^{-1})		Androstenedione (ng ml^{-1})		Reference(s)
	Male mean (range)	Female mean (range)	Male mean (range)	Female mean (range)	
Ringtailed lemur	6.0 (0.3–48.8) 2.8/13.5 ^a (0.2–56.9 ^b) 1.2–7.1 (0.2–20.7) (0.2–2.9)	0.2 (0.1–0.7)	2.4 (0.1–9.5)	1.4 (0.1–5.2)	Present study Van Horn et al., 1976 Bogart et al., 1977 Evans and Goy, 1968 Van Horn et al., 1976
Galago	2.6 ^a –3.9 (<0.1–13.4)				Van Horn et al., 1976
Mouse lemur	5.4–65.8 ^b 9.0–60.0 25.5 ^b –50 ^b				Schilling and Perret, 1993 Perret, 1992 Aujard and Perret, 1998
Rhesus monkey	(5.0–17.7)	(0.2–0.8) 0.3 ≈ 0.3 –0.5	(0.7–1.4)	1.6 ≈ 0.9 –1.2	Feder, 1985 Billiar et al., 1985 Lovejoy and Wallen, 1990
Baboon	(0.2–1.8) ^c	0.1–0.2		1.2–2.0	Feder, 1985
Human	4.6–6.4	(0.3–0.6) 0.5 (0.3–1.1)	1.1	(0.9–3.0) 2.2 (1.0–3.8)	Feder, 1985 Cashdan, 2003
Spotted hyena	0.8–10.5	0.3–1.2	0.7–3.4	1.7–5.6	Glickman et al., 1992; Goymann et al., 2001
Brown hyena	15 (5.4–23)	0.52	10.8 (9.9–11.7)	(8.5)	Racey and Skinner, 1979
Striped hyena	8.5 (0.4–18.7)	0.64 (0.4–1)			Racey and Skinner, 1979
Dog	2.5 0.8–4.2		1.1		Wichmann et al., 1984 Feder, 1985
Cattle	4.5 (1–20)	($\ll 0.1$ –0.3) <0.1	0.1 (0.1–7.1)	0.3 (0.1–0.8)	Concannon and Castracane, 1985 Wichmann et al., 1984 Feder, 1985
Rabbit	2.6 5 (0.5–10)	<0.1	0.7 2.5	<0.1	Wichmann et al., 1984 Feder, 1985
Hamster	(0.1–10)	(0.1–0.2)		(1.0–1.9)	Feder, 1985
Rat	1.8 (1.7–7.7)	(0.1–0.2)	1.1	(0.1–0.2)	Wichmann et al., 1984 Feder, 1985
Guinea pig	2.5 2.0 (1.2–6.7)		2.1 (0.3–1.7)		Wichmann et al., 1984 Feder, 1985

Values are provided as means (and/or ranges). \approx refers to approximate values derived from figures; ^a samples collected during darkness; ^b samples collected after inductive photoperiod; ^c species not specified.

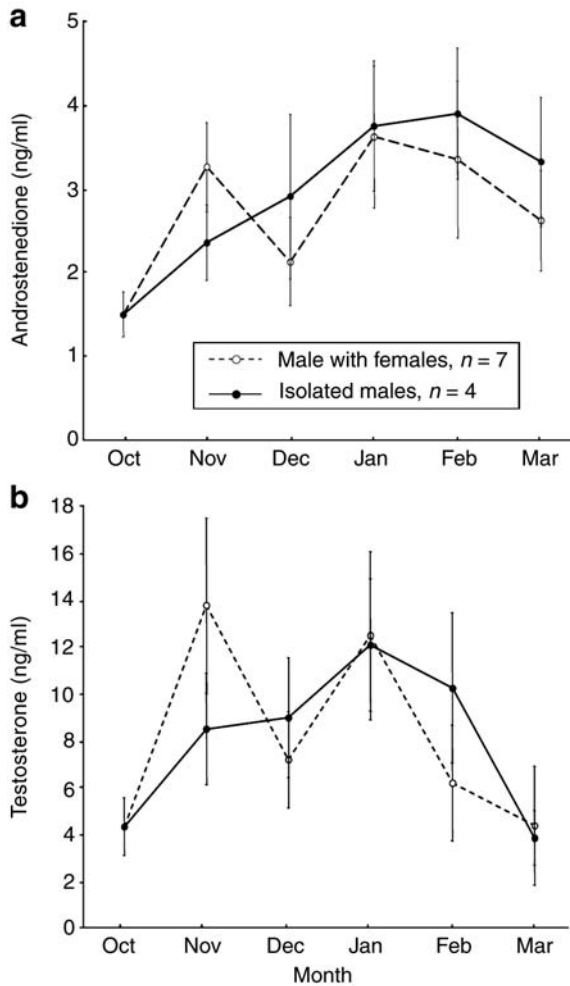


Fig. 2. Mean \pm S.E. monthly serum (a) androstenedione and (b) testosterone concentrations of male ringtailed lemurs housed in different social conditions during the breeding season (Nov–Feb) and transition months. All males were housed with females in Oct.

were represented in 8/12 months (excluding Aug, Apr, May, and Jun). Overall, male ringtailed lemurs had significantly higher circulating concentrations of T than A_4 (main effect of androgen: $F_{1,4}=25.718$, $P<0.01$). They also displayed significant monthly differences in total androgen secretion (main effect of month: $F_{7,28}=4.292$, $P<0.005$) and in the relative monthly concentrations of the two steroids (interaction effect: $F_{7,28}=4.638$, $P<0.005$; Fig. 3): Whereas T concentrations exceeded A_4 concentrations during peak breeding season (Nov–Jan; $P<0.001$, where $\alpha=0.006$), T and A_4 concentrations were comparable during the birthing (Mar) and nonbreeding (Jul and Sep) seasons. The difference in androgen production during transitional months did not reach statistical significance (Oct and Feb; $P<0.10$).

Despite significant differences between the absolute concentrations of A_4 and T across months of the year (Fig. 3), significant fluctuations in secretion patterns were evident for each androgen, independently (A_4 : $F_{7,28}=3.165$, $P<0.02$; T: $F_{7,28}=4.469$, $P<0.005$; Fig. 4a). On relative scales, both androgen profiles showed similar patterns of peaks and troughs: Mean concentrations of male A_4 and T were significantly

positively correlated across months of the year ($r_{10}=0.74$, $P<0.01$). Likewise, correlations between A_4 and T in individual males were strong in all but one animal (range R^2 : 0.45–0.94).

Lastly, androgen production varied significantly by reproductive season (A_4 : $F_{2,16}=6.450$, $P<0.01$; T: $F_{2,16}=21.755$, $P<0.001$; Fig. 4d). Fig. 4d depicts mean values derived using all animals available, but for the analyses, I used nine males for whom blood samples had been obtained at least once per season. Accordingly, mean A_4 concentrations increased two-fold during the breeding season compared to the nonbreeding season (Tukey: $P<0.05$). Mean T concentrations during the breeding season increased three- to four-fold over nonbreeding and birthing season values (Tukey: $P<0.01$ and $P<0.05$, respectively).

Seasonal patterns in female endocrine profiles

Mean monthly androgen (Figs. 3 and 4b) and estrogen (Fig. 4c) concentrations are depicted for all females sampled, but because of missing samples, I used for the analyses a subset of three females that were represented in 9/12 months (excluding Apr, May, and Jun). Unlike males, females showed greater overall production of A_4 than of T (main effect of androgen: $F_{1,2}=83.609$, $P<0.02$; Fig. 3). Like males, however, females showed significant monthly differences in total androgen secretion (main effect of month: $F_{8,16}=2.843$, $P<0.05$) and in the relative monthly concentrations of these two androgens (interaction effect: $F_{8,16}=4.043$, $P<0.01$): Whereas, in females, A_4 concentrations exceeded T concentrations in every month ($P<0.001$, where $\alpha=0.005$), the magnitude of the difference was greater during the breeding and birthing seasons (Nov–Jun) than during the nonbreeding season (Jul–Oct).

Monthly effects were evident independently for A_4 ($F_{8,16}=3.503$, $P<0.02$; Fig. 4b) and E_2 ($F_{8,16}=2.938$, $P<0.05$; Fig. 4c); however, the males' peak in androgen secretion (Nov) preceded by about 1 month the females' peak in E_2 secretion (a two- to three-fold increase in early Dec). The annual pattern

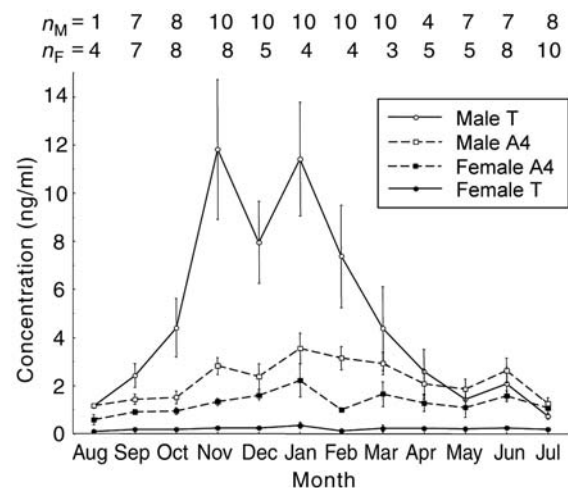


Fig. 3. Mean \pm S.E. monthly serum androstenedione (A_4) and testosterone (T) concentrations of adult ringtailed lemurs. Data points are plotted on the same scale to illustrate absolute differences. Male and female sample sizes for each month are provided at the top of the graph.

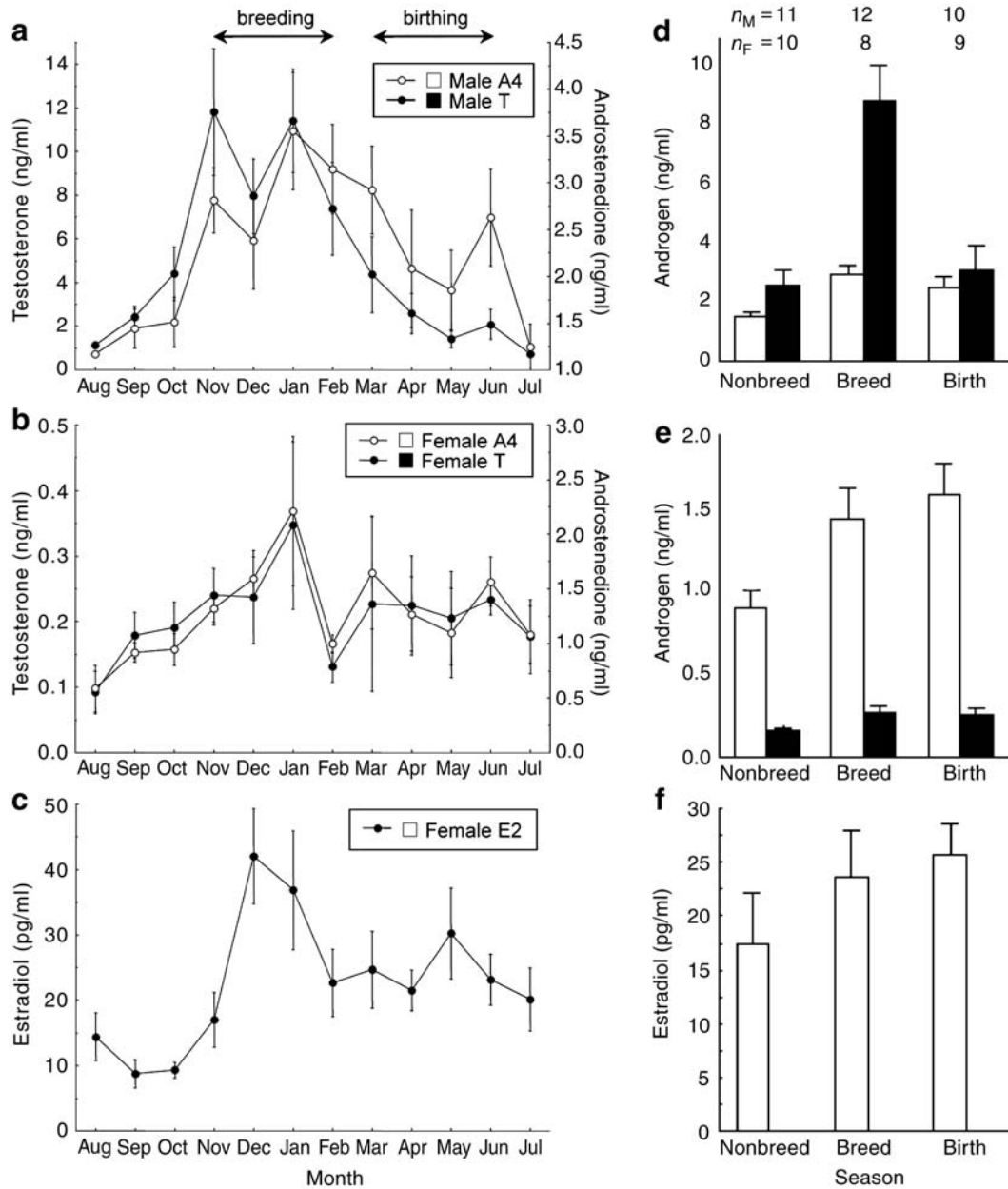


Fig. 4. Mean \pm S.E. monthly (a–c) and seasonal (d–f) serum androstenedione (A₄), testosterone (T), and estradiol (E₂) concentrations of adult (a, d) male and (b–c, e–f) female ringtailed lemurs. Monthly data points in a–b are the same as in Fig. 3, but are plotted on relative scales to illustrate correlated seasonal patterns. Arrows in a–c delineate the breeding and birthing seasons. Seasonal means represent 4-month periods that respectively encompass the nonbreeding (Jul–Oct), breeding (Nov–Feb), and birthing (Mar–Jun) seasons. Sample sizes in a–c are as in Fig. 3; sample sizes in d–f are provided for each season at the top of d.

of female T secretion, although virtually identical to that of A₄, was not statistically significant ($F_{8,16}=0.800$, *n.s.*; Fig. 4b). Nevertheless, the linear relationship between mean A₄ and T was positively and more strongly correlated in females ($r_{10}=.93$, $P<0.001$) than it had been in males. Individually, strong correlations between A₄ and T were evident in all but one female (range R^2 : 0.76–0.93).

Lastly, female A₄ ($F_{2,12}=3.946$, $P<0.05$; Fig. 4e) and E₂ ($F_{2,12}=7.430$, $P<0.01$; Fig. 4f) production changed significantly across the reproductive seasons. Despite showing a similar seasonal pattern, T production did not change significantly ($F_{2,12}=2.140$, $P=0.160$, *n.s.*; Fig. 4e). Figs. 4e, f depict mean values derived using all animals available, but for

the analyses, I used seven females represented at least once per season. For this subset, mean A₄ concentrations in the birthing season exceeded those of the nonbreeding season (Tukey: $P<0.05$) and E₂ concentrations in the birthing and breeding seasons exceeded those of the nonbreeding season (Tukey: $P<0.01$ and $P<0.05$, respectively).

Seasonal patterns in aggressive interactions

Mean monthly aggressive interactions between males (Fig. 5a), females (Fig. 5b), and the two sexes (female-on-male aggression: Fig. 5c), are depicted for all males and nonpregnant females that were subjects of focal observation. Because of

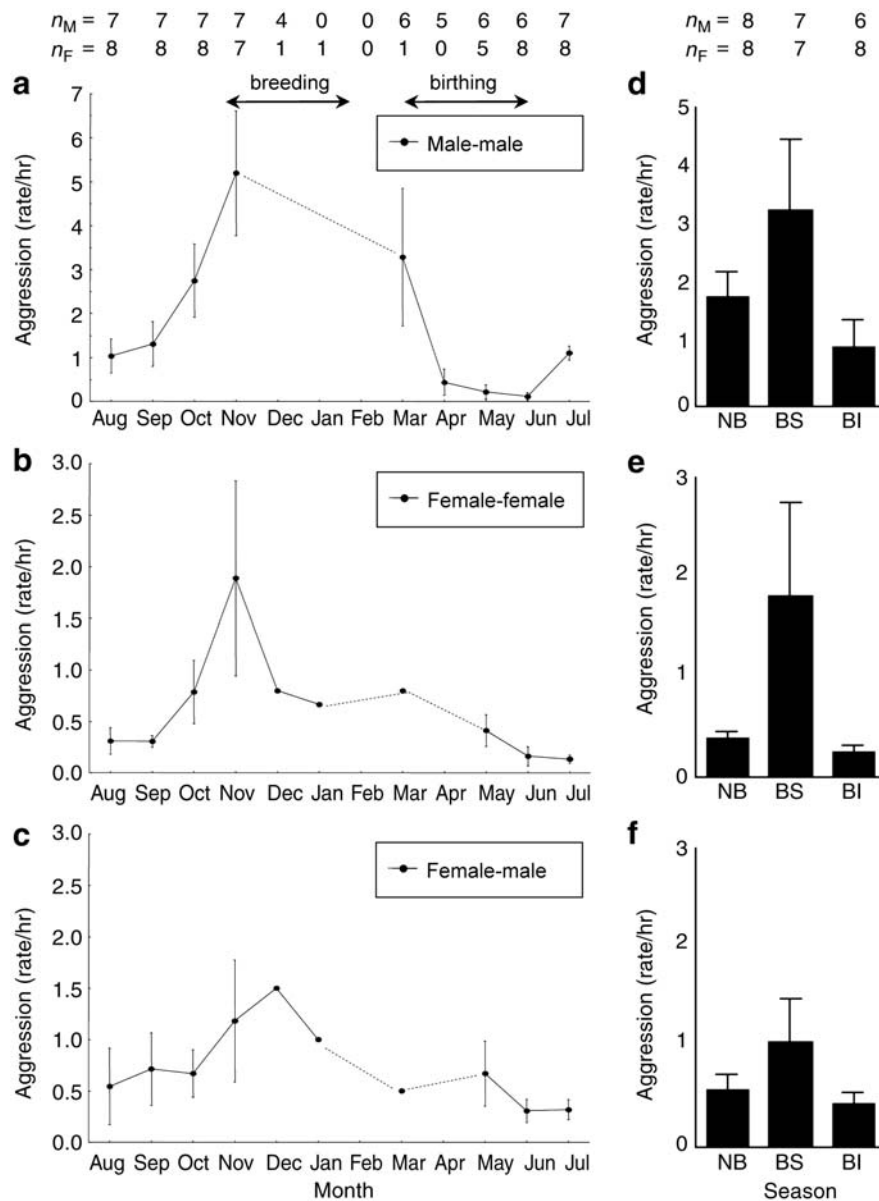


Fig. 5. Mean \pm S.E. monthly (a–c) and seasonal (d–f) rates of (a, d) intrasexual male, (b, e) intrasexual female, and (c, f) intersexual female-on-male aggression in ringtailed lemurs, corrected for the number of potential partners. Dashed lines reflect missing data points for intervening months. Arrows in a–c delineate the breeding and birthing seasons. Focal samples sizes for males and females are provided in a–c and d–f above the top graph, respectively.

missing samples (e.g. male winter lock up) and/or pregnancies, I used for the analyses a subset of five males that were represented in 9/12 months (excluding Dec, Jan, and Feb) and seven females that were represented in 6/12 months (excluding Dec–May). Despite excluding the most aggressive periods of the year, I found significant monthly differences in rates of male–male aggression ($F_{8,32}=2.955$, $P<0.02$; Fig. 5a) and female aggression against males ($F_{5,30}=2.827$, $P<0.05$; Fig. 5c). A similar pattern of activity obtained in rates of female–female aggression, but showed no significant monthly change ($F_{5,30}=1.160$, $n.s.$; Fig. 5b).

Given the same constraints as in the prior analyses, mean seasonal patterns of aggression are depicted for all animals available in Figs. 5d–f. For the analyses, I relied on six males and seven females represented at least once per season. Despite

seemingly enhanced aggression during the breeding season, relative to the other seasons, none of the individual categories of aggression showed reliable differences (male–male: $F_{2,10}=1.137$, $n.s.$, Fig. 5d; female–female: $F_{2,12}=1.964$, $n.s.$, Fig. 5e; female–male: $F_{2,12}=2.619$, $n.s.$, Fig. 5f); however, when the two categories of female-initiated aggression were combined, seasonal differences became statistically reliable (female–female + female–male: $F_{2,12}=4.807$, $P<0.05$), with female aggression in the breeding season exceeding that in the birthing season (Tukey: $P<0.05$).

Discussion

To enhance our understanding of the processes regulating feminine development and to gain insight into the evolution of

female social dominance in Malagasy lemurs, I explored the possibility that both natural sex–role reversal (i.e. female display of masculine behavioral traits) and genital mimicry (i.e. female display of masculine morphological traits) characteristic of this lineage might be associated with ‘heterologous’ hormones (i.e. female production of androgens) in adult animals. More specifically, to shed light on the neuroendocrine mediation of aggression in both sexes of ringtailed lemurs, I searched for similar patterns of seasonal variation in sex steroids and aggression that might suggest gross activational effects of hormones. Whereas both T and A_4 were elevated in male ringtailed lemurs during portions of the year marked by heightened aggression (i.e. the breeding season), both A_4 and E_2 were elevated in females. Thus, although androgens may play a functionally significant role in normal female development (Staub and De Beer, 1997), the present findings in females cannot be used to distinguish between a potential androgenic or estrogenic mechanism of female dominance. Consequently, the hypothesis that female ringtailed lemurs may be behaviorally, physiologically, and morphologically masculinized by exposure to endogenous androgens must await further testing, particularly through studies at the organizational stage of development.

Endocrine profiles of ringtailed lemurs revealed the traditional mammalian pattern in that adult males displayed significantly higher T concentrations than did adult females. This sex difference is consistent with the only other study addressing the possibility that androgens might regulate female dominance in ringtailed lemurs (Von Engelhardt et al., 2000); however, the present interpretation is quite different. In the prior study, the authors reported low concentrations of fecal T in nonpregnant, female subjects that were of uncertain age and were examined during a brief portion of the year (from late nonbreeding season to the onset of breeding). Because this finding was contrary to their hypothesis that female dominance should be associated with higher T concentrations in the female than in the male, the authors dismissed an androgenic or even female-driven (e.g. estrogenic) mechanism of social dominance in this species (Von Engelhardt et al., 2000).

That conclusion may have been premature, however, as their T finding is entirely consistent with the hyena model on which they had reportedly based their prediction. Specifically, in longitudinal endocrine studies of spotted hyenas, sampled at all postnatal ages, both in the field and in captivity, researchers report significantly lower T concentrations in females than in males (Dloniak et al., 2004; Frank et al., 1985; Glickman et al., 1987, 1992; Goymann et al., 2001). Moreover, because mean T concentrations in female spotted hyenas, as in female ringtailed lemurs, are similar to those of other female mammals that lack masculine traits, prior hyena research does not support a prediction of elevated T during adulthood in female-dominant species.

Instead, the main prediction generated by the spotted hyena model, relevant to adult female hormone profiles, relates to the availability of an androgenic precursor to T or E_2 . In female spotted hyenas, that androgenic source was revealed as elevated A_4 concentrations relative to those of conspecific males and females of most (but not all) other species, as well as to

conspecific female T concentrations (Glickman et al., 1987, 1992). In this study, female lemurs had appreciable A_4 concentrations, with peak values within the lower range of values expressed by female spotted hyenas, but these were not greater in the female lemur than in her male conspecifics. A_4 concentrations in female lemurs also were greater than those of some, but not all, other female mammals. Finally, mean A_4 concentrations in the female lemur were seven times her mean T concentrations. Importantly, none of these factors (alone or in combination) are diagnostic of masculinization—they merely point to the bioavailability of a potentially critical precursor. Thus, the endocrine profiles of female ringtailed lemurs, while not equivalent to those of female spotted hyenas, may be consistent with the lemur’s more moderate expression of masculine features.

Such comparative analyses of hormone profiles should be interpreted with caution, moreover, as circulating hormones in adulthood may reveal little about their activational consequences and even less about any organizational contribution. Endocrine patterns similar to those described herein, and similar even to those described for adult female spotted hyenas, are not necessarily associated with ‘masculine’ females. In the only published study of brown hyena (*Parahyaena brunnea*) reproductive endocrinology, Racey and Skinner (1979) reported an A_4 concentration of 8.48 ng ml^{-1} in one adult female, a value that is elevated by any standard; yet, brown hyenas have typically dimorphic external reproductive organs (Racey and Skinner, 1979). Unlike many carnivores, the sexes are roughly size monomorphic (Mills, 1990). Unfortunately, the female brown hyena’s social status in relation to males remains unclear, with reports of linear co-dominance between the sexes (Owens and Owens, 1978; 1996), nonlinear dominance (Goss, 1986), and absence of dominance (Mills, 1990, Yost, 1980). Although it is possible that *Parahyaena* shares some features of *Crocuta* reproductive endocrinology, the extent to which hormonal influences and developmental processes differ or converge is yet unknown and the implication of elevated A_4 concentrations remains poorly understood. Nevertheless, it is neither the absolute nor relative quantity of A_4 in adulthood that matters in the masculinization of spotted hyenas so much as the conversion of A_4 to T during pregnancy. Therefore, the next step in this investigation will be to examine endocrine correlates of lemur pregnancy.

Peak endocrine activity in male and female ringtailed lemurs occurred over a 4–6-month period encompassing the mating season. Peak androgen concentrations in males coincided with peak conception rates in females, both occurring 1–2 months prior to peak steroid secretion in nonpregnant females. An additional peak in female endocrine activity accompanied the birthing season. These seasonal patterns replicate and extend previous reports for male (Bogart et al., 1977; Cavigelli and Pereira, 2000; Evans and Goy, 1968) and female (Van Horn and Resko, 1977; Von Engelhardt et al., 2000) ringtailed lemurs, and are consistent with patterns described for other male strepsirrhines (Brockman et al., 1998, 2001; Ostner et al., 2002; Perret, 1992; Schilling and Perret, 1993). An age-related reduction in T secretion has been

reported for male mouse lemurs (*Microcebus murinus*: Aujard and Perret, 1998), but no such pattern was evident in this study for ringtailed lemurs of either sex, despite the inclusion of aged animals.

Given strong diurnal patterns in male *L. catta* steroid production, with peak values occurring during the dark period (Van Horn et al., 1976), the present day-light estimates likely reflect minimum values, potentially for both sexes. Nonetheless, male T concentrations during the breeding season appear to be unusually high by comparison to other male mammals. In their ‘challenge hypothesis,’ Wingfield et al. (1990) posited that peak androgen concentrations (above the minimum required to support reproductive recrudescence) reflect male–male aggression, including territorial defense and mate guarding. Some researchers have invoked this hypothesis to explain elevated T levels in male lemurs during periods of intense competition over access to females (Cavigelli and Pereira, 2000; Ostner et al., 2002). Nevertheless, Wingfield et al. (1990) primarily cited evidence from monogamous species that show paternal care, stipulating that the relationship between T and aggression is expected to be less pronounced in males of polygynous species with little paternal investment (such as the ringtailed lemur), in which there has been selection for T levels to be elevated throughout the breeding season. Thus, for some species, “it is possible that photoperiodic cues or endogenous rhythms of reproductive activity regulate T secretion to a maximum effective level and that social cues have no further effect” (Wingfield et al., 1990, p. 842).

Although social factors do influence endocrine function in various strepsirrhine primates (reviewed in Izard, 1990), including, for instance, the social entrainment of female cycles (Jolly, 1966; Pereira, 1991), the depression of testicular hormones in subordinates (Perret, 1992; Schilling et al., 1984), and the occasional bursts of aseasonal testicular function (Brockman et al., 2001), socially mediated effects on the indices of reproductive competence (e.g. Cavigelli and Pereira, 2000) appear to be subtler than environmentally mediated effects (e.g. Perret, 1992). Contrary to predictions generated by the challenge hypothesis, periods of social instability among lemurs are not always associated with increases in male T (Ostner et al., 2002) and female presence does not necessarily mediate male T concentrations (Aujard, 1997; Schilling et al., 1984). It appears, therefore, that environmental cues may drive the reproductive cycles of male ringtailed lemurs, as they do those of females (Van Horn, 1975; Van Horn and Resko, 1977), and set maximal T ranges for males. Within this range, individual indices are then likely to be ‘fine tuned’ by social interaction or secondarily modulated by olfactory signals (e.g. Perret, 1992).

Another aspect of this study involved the seasonal link between endocrine measures and patterns of aggression. While male and female steroids predictably peaked during the aggressive breeding season, A_4 and E_2 concentrations in females were also elevated during portions of the year that, in this study, were less marked by heightened female aggression (i.e. the birthing season). Nevertheless, in other studies, both the breeding and birthing seasons have been characterized as

intensely aggressive (Evans and Goy, 1968; Pereira and Weiss, 1991; Sauther, 1991; Vick and Pereira, 1989; Von Engelhardt et al., 2000), further suggesting a link between these steroids and female dominance. Several key factors probably contributed to the reduced aggression observed in this study. First, resources are not limiting in captivity. Second, in the wild, social instability may arise with male transfer and defense of infants by residents (Brockman et al., 2001; Ostner et al., 2002), but male transfers were not a factor here. Third, captive management policies specifically aim to prevent or reduce aggression by staging introductions during the nonbreeding season, by housing animals in small, stable groups of known individuals, and by removing either the aggressors or the targets of aggression. Future studies will focus on female endocrine profiles and behavior during pregnancy and lactation to better understand this period in lemur reproductive biology.

The absence of a significant temporal pattern in female T may reflect a floor effect; otherwise, these negative data further implicate the availability of A_4 and/or E_2 , rather than the absolute concentration of T, in female aggression and social dominance. Indeed, A_4 , but not T, has been shown to covary with female competitive aggression in young women (Cashdan, 2003; Inoff-Germain et al., 1988) and researchers studying various taxa have reported a significant role for E_2 , either acting alone or synergistically with dihydrotestosterone, in the regulation of female aggression (reptiles: Rubenstein and Wikelski, 2005; Woodley and Moore, 1999; rodents: Albert et al., 1992; Lonstein and Gammie, 2002; Van de Poll et al., 1986; humans: Inoff-Germain et al., 1988). As previously suggested, it also may be that females are more sensitive than males to the behavioral effects of androgens (Sherwin, 1988) and/or that androgens and estrogens have distinct roles in the masculinization of female behavior (Simon and Whalen, 1987).

In summary, the male-like behavioral and morphological traits described for female ringtailed lemurs approximate those described for female spotted hyenas, but share only some of the endocrine correlates. Both species live in socially complex societies, characterized by female aggression toward and social dominance over males. In both species, females show enhanced body size and mass, and delayed puberty, relative to males, such that typical sexually differentiated traits are reversed or absent. Masculinization of genital morphology in female lemurs, however, is only superficially similar to that found in female hyenas—‘superficial’ in that no other female mammal shows permanent vaginal closure, clitoral mating, or clitoral delivery. These latter, uniquely hyena traits, however, are the very features of genital development and female reproduction that appear to be independent of androgenic control (Drea et al., 1998). Thus, on the one hand, a non-androgenic and yet unknown mechanism accounts for the basic phallic structure (Drea et al., 1998) and may drive genital growth (Glickman et al., 1998) in spotted hyenas (Glickman et al., 2006). On the other hand, the shared features of female hyena and lemur genitalia (i.e. clitoral elongation, clitoral thickening, and meatus length: Drea and Weil, submitted for publication) are precisely those that are tuned in hyenas by prenatal exposure to endogenous

androgens (Drea et al., 1998, 2002). Recent data are also consistent with a possible role for estrogens in masculinizing the genitalia of female spotted hyenas (Place and Glickman, 2004; Glickman et al., 1998, 2006).

At a proximate level, aggression and dominance in male mammals are often linked to higher concentrations of circulating androgens (Monaghan and Glickman, 1992). Although, when present in females, social dominance and aggressiveness do not necessarily imply hormonal masculinization, these characteristics, especially when coupled with other masculine features, suggest a possible role for androgens in feminine development. Strong positive correlations between A_4 and T also implicate the Δ^4 -pathway in the production of T, especially in the female; however, as A_4 in females may be of ovarian as well as adrenal origin (Davison and Bell, 2006), further studies will be needed to identify the major glandular source. While the influence of E_2 cannot be discounted and may prove to be crucial, A_4 may be a potentially important source of androgen and useful predictor of aggression in the female ringtailed lemur. Resolution of these issues will require further study, particularly during the prenatal period.

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