# Organizational and Activational Androgens, Lemur Social Play, and the Ontogeny of Female Dominance

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**Abstract**

The role of androgens in shaping "masculine" traits in males is a core focus in behavioral endocrinology, but relatively little is known about an androgenic role in female aggression and social dominance. In mammalian models of female dominance, including the ring-tailed lemur (*Lemur catta*), links to androgens in adulthood are variable. We studied the development of ring-tailed lemurs to address the behavioral basis and ontogenetic mechanisms of female dominance. We measured behavior and serum androgen concentrations in 24 lemurs (8 males, 16 females) from infancy to early adulthood, and assessed their 'prenatal' androgen milieu using serum samples obtained from their mothers during gestation. Because logistical constraints limited the frequency of infant blood sampling, we accounted for asynchrony between behavioral and postnatal hormone measurements via imputation procedures. Imputation was unnecessary for prenatal hormone measurements. The typical sex difference in androgen concentrations in young lemurs was consistent with adult conspecifics and most other mammals; however, we found no significant sex differences in rough-and-tumble play. Female (but not male) aggression increased beginning at approximately 15 months, coincident with female puberty. In our analyses relating sexually differentiated behavior to androgens, we found no relationship with activational hormones, but several significant relationships with organizational hormones. Notably, associations of prenatal androstenedione and testosterone with behavior were differentiated, both by offspring sex and by type of behavior within offspring sexes. We discuss the importance of considering (1) missing data in behavioral endocrinology research, and (2) organizational androgens other than testosterone in studies of female dominance.

*Key words*: aggression; androstenedione; female dominance; imputation models; masculinization; play; strepsirrhine primate; testosterone

**Introduction**

Historical frameworks of sexual selection are predominantly focused on the development of traditional sex roles. In these frameworks, males develop costly ornamentation, weaponry, and multimodal sensory cues to compete with other males and attract females, whereas females, owing to their greater reproductive investment, are thought to be the 'choosy' sex (e.g. Bateman, 1948; Trivers, 1972). With theoretical and empirical work highlighting the limitations of such traditional frameworks (Drea, 2005; Kokko & Jennions, 2008; Bro-Jørgensen, 2011; Rosvall, 2011; Clutton-Brock & Huchard, 2013), scholars have become increasingly interested in animal models in which the typical roles are reversed to varying degrees (reviewed in e.g. Eens & Pinxten, 2000; Stockley & Campbell, 2013). Here, we turn to one such species, the ring-tailed lemur (*Lemur catta*), to examine the endocrine correlates of behavioral development and the ontogeny of female dominance.

Under the theory of mammalian sexual differentiation (Jost, 1953), many elements in the constellation of "masculine" traits across species should be attributable to androgenic mechanisms (e.g. Young, Goy, & Phoenix, 1964). Endocrine studies of males have supported this link for several nonreproductive behavioral traits, including aggression, social dominance, rough-and-tumble play, and scent marking (Johnson, 1973; Goy & McEwen, 1980; Meaney & Stewart, 1981; Monaghan and Glickman, 1992; Hines & Kaufman, 1994; Archer, 2006; delBarco-Trillo et al., 2016), providing a framework for examining the extent to which 'exogenous' androgens in female mammals might also play an organizational and/or activational role in the emergence of these traits (e.g. nonhuman primates: Goy, 1978; 1981; humans: Money & Ehrhardt, 1973). In naturally 'masculinized' female mammals, several key species have emerged as models for the actions of endogenous androgens, but studies have produced variable results (Drea, 2009; French et al., 2013). In the most morphologically and behaviorally masculinized of female mammals, the spotted hyena (*Crocuta crocuta*), androgens, including androstenedione ($A_4$) and testosterone (T), are clearly implicated in the organization and

activation of these traits (Glickman et al., 1987, 2006; Michael G. Baker, MA thesis, University of California, Berkeley, 1990; Drea et al., 1998; Dloniak, French, & Holekamp, 2006). Nevertheless, T concentrations in adult female spotted hyenas do not rival those of males; outside of pregnancy, they show the typical mammalian sex difference (Glickman et al., 2006). In another, highly masculinized female, the fossa (*Cryptoprocta ferox*), no exceptional, postnatal endocrine pattern has been detected; females possess androgen concentrations significantly lower than those of males, even during periods of transient masculinization (Hawkins et al., 2002). In the female-dominant rock hyrax (*Procavia capensis*), T concentrations in females modestly exceed those of males, but do not predict social status (Koren, Mokady, & Geffen, 2006). Lastly, in the female-dominant meerkat (*Suricata suricatta*), sex differences in circulating androgen concentrations are absent or even reversed, and are related to breeding status in females (Clutton-Brock et al., 2006), with aggressively dominant females having exceptionally high concentrations of both $A_4$ and T, particularly during gestation (Davies et al., 2016).

The primary study system for the investigation of female dominance in primates is the suborder *Strepsirrhini* (e.g., Ramanankirahina, Jolly, & Zimmermann, 2011; Richard, 1987), in which females often dominate males indirectly (e.g. via feeding priority; Jolly, 1984) or directly (e.g. via overt aggression; Pereira et al., 1990) and in which female dominance is likely ancestral and hormonally mediated (Petty & Drea, 2015). The diurnal, group-living ring-tailed lemur has emerged as a prominent model of female dominance (e.g., Sauther, 1993), with females exhibiting a suite of traits consistent with masculinization, including intense aggression towards both sexes (Pereira et al., 1990; Drea, 2007; Charpentier & Drea, 2013), masculinized genitalia (Drea & Weil, 2008), and conspicuous scent-marking behavior that deploys informative olfactory cues that exceed those of males in their complexity (Scordato & Drea, 2007) and function as sexually selected ornaments (Boulet et al., 2010).

Early endocrine studies of *L. catta* adhered to traditional paradigms in that they were focused either on the role of estrogens in regulating female cycles (Bogart, Kumamoto, & Lasley, 1977; Evans & Goy, 1968; Van Horn & Resko, 1977) or on the role of T in regulating male cycles (Cavigelli & Pereira, 2000; Van Horn, Beamer, & Dixson, 1976). In one study that addressed the possibility of androgen correlates of female dominance, researchers examined fecal androgen metabolites in adults during the late non-breeding season to the onset of breeding, but as these metabolites were significantly lower in females than in males, the authors dismissed an androgenic or female-driven mechanism of masculinization in this species (Von Engelhardt, Kappeler, & Heistermann, 2000). Nevertheless, serum $A_4$ and T concentrations in adult females increase significantly when transitioning from non-breeding to breeding seasons and coincide with heightened female aggression, consistent with an activational role for androgens in adult, reproductively active females (Drea, 2007). Moreover, $A_4$ concentrations are greater in pregnant than non-pregnant ring-tailed lemurs, which may also suggest organizational effects of androgens on developing offspring (Drea, 2009).

Fundamental questions thus remain regarding the nature of female dominance in this species, including its behavioral basis and ontogenetic mechanisms. In the present longitudinal study, we performed the first behavioral endocrine examination of developing *L. catta*. Specifically, we collected repeat serum samples and behavioral observations from infant lemurs, following them through to adulthood. In addition, we collected serum samples from their mothers during gestation, which permitted an examination of potential organizational effects. Our aims were to more precisely characterize the emergence of female social dominance by examining differential trajectories of social play, aggression, and dominance interactions between the sexes, and to determine if androgen concentrations (in developing individuals or their mothers) predicted rates of observed behavior across time. Because logistical constraints limited the frequency of long-term blood sampling from

immature individuals, we adopted an analytical approach for the postnatal endocrine values based on missing data imputation. This approach was not necessary for the prenatal endocrine values. Following previous research (Drea, 2007), we anticipated that $A_4$, rather than T, would be the stronger predictor of aggressive/dominance behavior across ring-tailed lemur ontogeny.

## Methods

*Subjects*

The study population of ring-tailed lemurs (N = 33) derived from three social groups, living at the Duke Lemur Center (DLC) in Durham, NC, and followed during a five-year period, from 2003-2008. The focal subjects comprised 24 infants (8 males, 16 females), studied from age 3 – 30 months (from infancy, through juvenility, and into adulthood), and nine of their mothers, studied during 13 of the pregnancies that produced these offspring. The animals occupied one of three separate forested enclosures (1.5, 3.3, or 5.8 ha), and all had access to indoor, heated enclosures. The animals were confined indoors when outdoor temperature highs fell to 4 °C, and they regained forest access after temperatures exceeded this limit for three consecutive days. The animals received daily rations of a commercially available primate chow, supplemented with fresh fruit and vegetables (Drea, 2007). When semi free-ranging, the animals supplemented their diet by foraging in the forest. The subjects were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals (protocol #A-245-03-07).

*Blood sampling*

We collected blood samples from the 24 focal offspring, targeting 10 samples per individual, collected approximately every three months (total *N* of offspring blood samples = 198). Moreover, as part of an earlier study of gestational hormones (Drea, 2011), we had collected blood samples at

least once per trimester, at approximately equal intervals, from nine dams during 13 pregnancies, while they were carrying the focal offspring (total *N* of mothers' blood samples = 48). We used these samples to match the mothers' late-term androgen concentrations to offspring behavior.

Blood samples were obtained with the assistance of DLC veterinary personnel. The animals were corralled and separated into indoor enclosures before being handled and sampled individually (mean delay between capture and blood draw = 3.97 min, SD = 2.99 min). Nearly all blood sampling occurred during the morning between 9:00 and 11:00 AM (mean time: 10:05, SD = 45.86 min), in accordance with previous research in this species (e.g. Drea, 2007). The samples were taken from the femoral veins of awake, gently restrained animals. Blood samples were transferred to serum separator tubes, left to clot at ambient temperature, and centrifuged prior to decanting the serum for storage at -80 °C. Any missing blood samples owed to animal transfers to other institutions, deaths or health issues (for reasons unrelated to the present study), or insufficient age at the conclusion of the study period.

*Hormone measurements*

We performed immunoassays for two androgens of interest: $A_4$ and T. Serum samples from offspring were analyzed with enzyme immunoassays (EIA; ALPCO diagnostics, Salem, NH, USA); serum samples from mothers were analyzed with radioimmunoassays (RIA; see Drea 2011). The $A_4$ assay has a sensitivity of 0.04 ng/ml for EIA, and 0.1 ng/ml for RIA. The T assay has a sensitivity of 0.02 ng/ml for EIA, and 0.05 ng/ml for RIA. The intra- and inter-assay coefficient of variation (CV) for the $A_4$ EIA was 7.62% and 11.58%, respectively; the intra- and inter-assay CV for the T EIA was 13.08% and 5.44%, respectively. Inter-assay CVs were calculated from high and low controls provided by the manufacturer. CVs for RIA measurements were previously published in Drea (2011). Because two of the blood samples from offspring had insufficient volume, our values relied

on 196 measurements of $A_4$ versus 198 measurements of T. Whenever circulating hormone concentrations in our samples were below the threshold of detection for the assay, we assigned a value equal to the lowest observed hormone concentration from our assays: 0.005 ng/ml for $A_4$; 0.001 ng/ml for T.

*Behavioral observations*

We obtained behavioral data, throughout the calendar years, on a subset ($N$ = 18 or 78%) of the offspring. Two researchers blind to the specific goals of the study recorded the offspring's behavioral interactions (with any group member) during 20-minute, focal-observation sessions (for a total of 315 hours of observation). The median observation time per animal was 19.56 hours, with an observation density ranging from 0-200 minutes per month. The reduced subject sample size and the variation in animal observation periods (with 88% of observations being performed in the warmer months of May-October; Electronic Supplementary Material Fig. S1) owed to the constraint that we performed behavioral observations only while the entire social group was semi free-ranging. Behavior was recorded and entered into handheld computers (Psion Workabout) in an actor-action-recipient format, using an established ethogram (Drea, 2007; Drea and Scordato, 2007; Electronic Supplementary Material, Table S1). We focused on counts of dyadic play, aggression, and dominance interactions, with separate counts of initiating and receiving behavior. Counts of behavior within each category were summed for analysis. A random sample of five videotaped focal-observation sessions were additionally scored by an independent coder; Cohen's kappa for all behavior of interest was 0.80, indicating strong agreement between raters (Hallgren, 2012).

*Analyses*

To best leverage the largely asynchronous data available for offspring (i.e., blood samples collected at approximately regular intervals vs. behavioral observations that were concentrated during the warmer months of the year; Fig. S1), we grouped our longitudinal analyses into three sets. In the first two sets of analyses, we respectively considered the offspring's endocrine and behavioral trajectories throughout development: the former provides information on patterns of circulating androgens; the latter provides information on patterns of play, aggression, and dominance interactions. The third set of analyses contains models that predict frequencies of offspring behavior from androgen concentrations, based on a) interpolation from the offspring's temporally proximate blood samples (i.e., to address activational effects of hormones on behavior), and b) the offspring's prenatal endocrine environment, based on its mother's androgen concentrations during late pregnancy (i.e., to address putative organizational effects of hormones on behavior; see e.g. Glickman et al., 2006). We performed all analyses using multilevel generalized linear models in the R packages lme4 (Bates, Maechler, Bolker, & Walker, 2015) and mgcv (Wood, 2017). Both packages permit the specification of models that account for the repeated sampling of focal animals, whereas the latter also permits the specification of non-linear associations between variables of interest. We calculated tests of statistical significance using Satterthwaite approximated degrees of freedom (reported in our results rounded to the nearest whole number). Dataset and R code for all results are available at https://osf.io/7q42t/.

*Offspring endocrine analyses.* To describe androgen trajectories during postnatal development, as well as sex and age differences in androgen concentrations, $A_4$ and T were each regressed on age (in months) and sex, with animal ID included as a random intercept.

*Offspring behavioral analyses.* Behavioral counts were binned by month and tallied for two outcomes of interest: dyadic play, and the sum of aggression and dominance-related behavior. Counts of initiation and reception were analyzed separately (following e.g. delBarco-Trillo et al.,

2016); in our supplementary materials, we present models examining initiation and reception aggregated (Tables S2-S5). Each of these behavioral outcomes was analyzed in a generalized additive model (GAM; Wood, 2017) to model non-linear trends in behavior; we used a negative binomial distribution in our GAMs to account for the zero-inflation and overdispersion of dependent variables. All models also included an offset term to account for varying observation time in a given month (see e.g. UCLA Statistical Consulting Group, 2019), group size at the time of observation to account for the number of potential social partners, as well as age and sex (the primary predictors of interest).

*Analyses predicting offspring behavior from hormone concentrations.* Temporal asynchrony between postnatal hormonal and behavioral sampling presented a challenge for a subset of our analyses. In our long-term, developmental study, in which hormone concentrations can fluctuate substantially during the transition between life-history stages, correlational analyses that rely on large temporal 'bins' spanning multiple months would likely introduce substantial error in prediction. To address this issue for our focal offspring, we treated the unobserved hormone concentrations during the months between samples as missing data, and imputed those missing data based on prior and subsequent hormone concentrations.

Imputation of missing postnatal endocrine data was performed using the Amelia package (Honaker, King, & Blackwell, 2011) in R. Amelia uses a bootstrapped expectation-maximization algorithm (see Honaker & King, 2010) to generate *m* simulated datasets with missing values imputed for variables of interest (in this case, hormone concentrations). Analyses of interest predicting behavior from hormones are then performed on the *m* datasets separately, with tests of statistical significance calculated by accounting for uncertainty of imputations and disagreement in the estimated values across the models (Rubin, 1987).

Amelia has the option to create several varieties of diagnostic plots to inspect the plausibility and fit of imputed data. Here, we present two such plots based on our imputed data. Fig. 1 compares the distribution of imputed values for $A_4$ and T to the distribution of observed values. Fig. 2 displays overimputation plots for $A_4$ and T. These overimputation plots depict the results of a validation check in which the observed concentrations of these hormones are sequentially treated as missing—that is, for each observed value of $A_4$ and T, hundreds of imputations are performed as if that value were missing, and a confidence interval is constructed based on these imputations. As a guideline, the developers of Amelia suggest that at least 90% of the confidence intervals should cross the diagonal line on a plot of imputed versus observed values, because this would mean that the imputation model has captured the true, observed value at least 90% of the time. Despite a preponderance of missing data (i.e., only one out of three months observed per individual), these plots jointly and strongly indicate that our imputation model generated plausible values for the missing hormonal observations.

Using the imputed data, we performed a series of negative binomial GAMs predicting dyadic play, and the sum of aggression and dominance behavior (i.e., mirroring those described above in *Offspring behavioral analyses*). Once again, all models included group size at the time of observation, age, sex, and an offset term for observation time. Here, we added predictor terms for concurrent $A_4$ and T to assess potential activational effects of androgens on outcome behavior.

Finally, in a parallel set of models, we considered maternal $A_4$ and T during the last trimester of pregnancy as predictor terms to assess potential organizational effects of androgens. These models did not rely on any imputed data. In supplementary analyses, we substitute the mother's third trimester $A_4$ and T with her mean values (Table S6), but here we present results from the third trimester only, as this period represents the critical window for organizational effects on offspring behavior (Goy et al. 1988; Glickman et al., 2006).

Offspring and maternal $A_4$ and T measurements were log-transformed prior to analysis for two purposes: 1) to better capture effects that are thought to be a function of proportional, rather than absolute, changes in hormone concentrations (Jones, 1996) and 2) to improve linearity with other variables in statistical models (cf. Sherry, McGarvey, Sesapasara, and Ellison, 2014).

**Fig. 1.** Density plots of observed and imputed (a) androstenedione and (b) testosterone concentrations in male and female ring-tailed lemurs. Observed values are represented by the black line, imputed values are represented by the red line. All values are log-transformed.

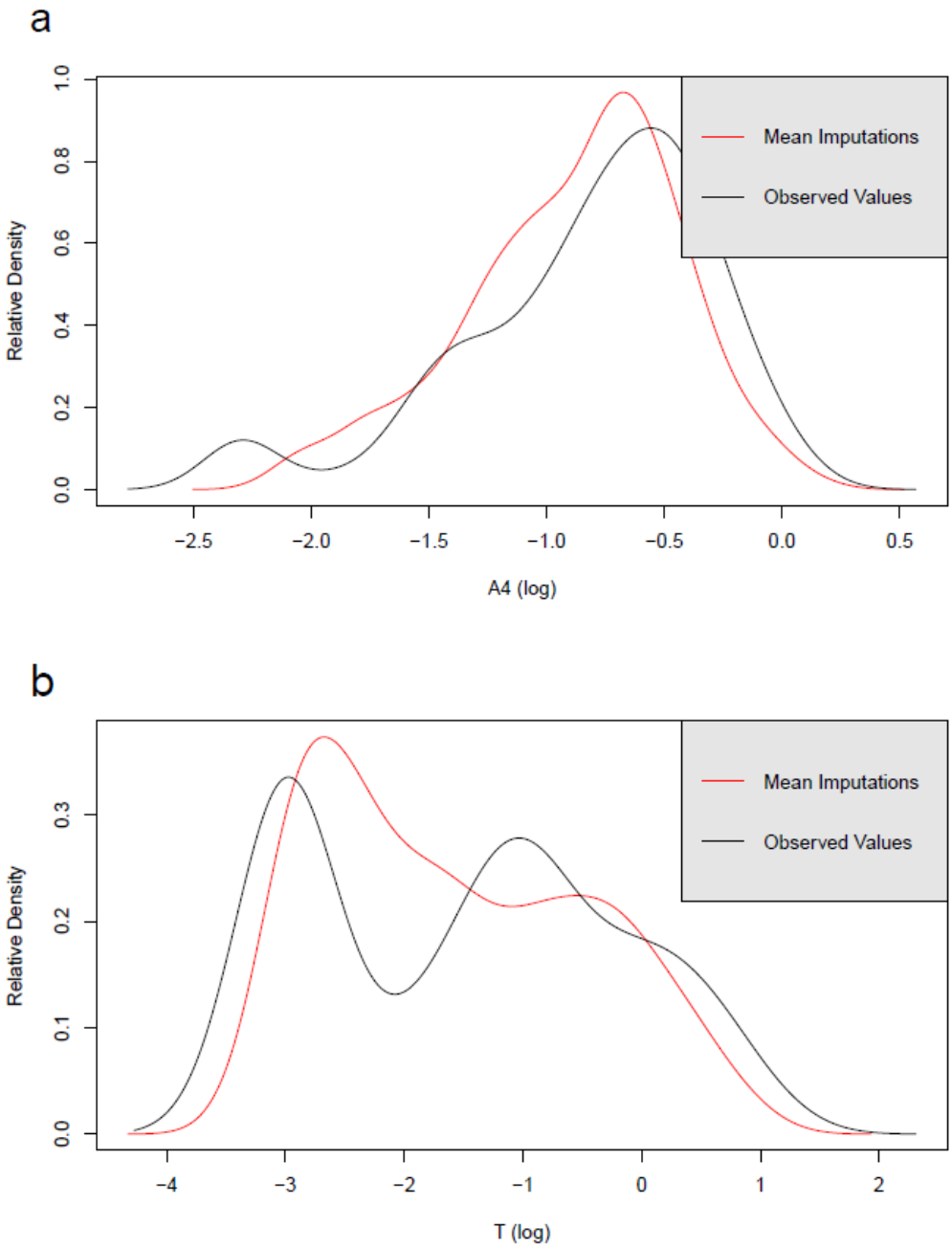
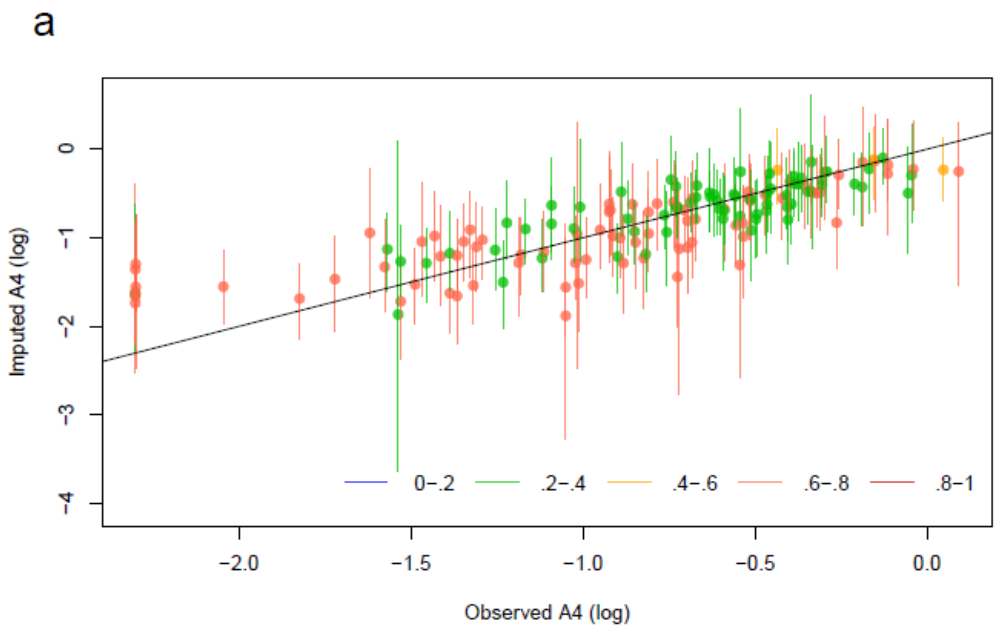
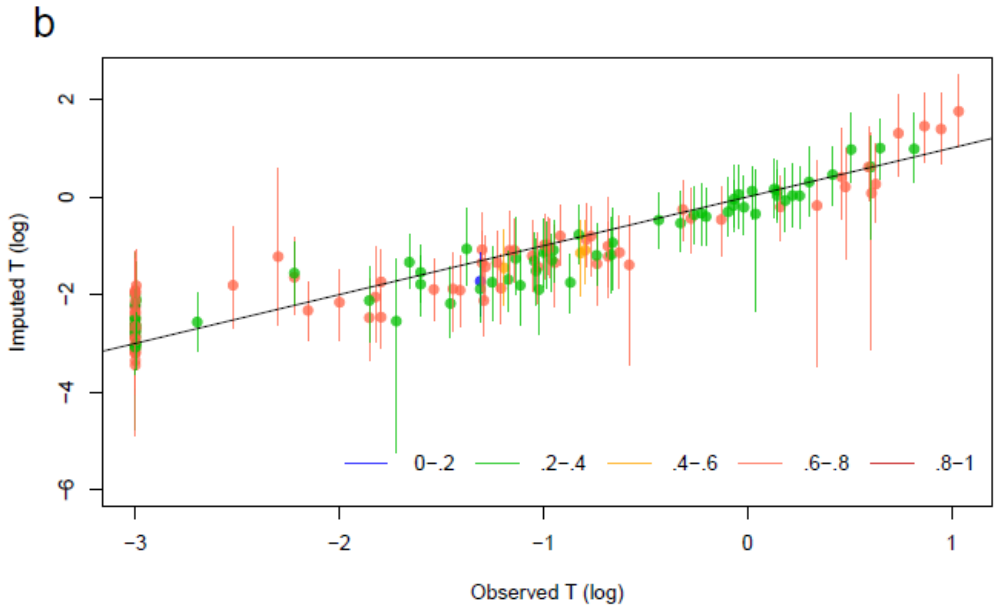



**Fig. 2.** Overimputation plots for (a) androstenedione and (b) testosterone concentrations in male and female ring-tailed lemurs. Points represent the mean imputation for each observed value treated as missing; whiskers represent 90% confidence intervals for these imputations. Symbol color (as coded in the legend) represents the fraction of missing observations in the pattern of 'missingness' (see Honaker, King, & Blackwell, 2011). All values are log-transformed.



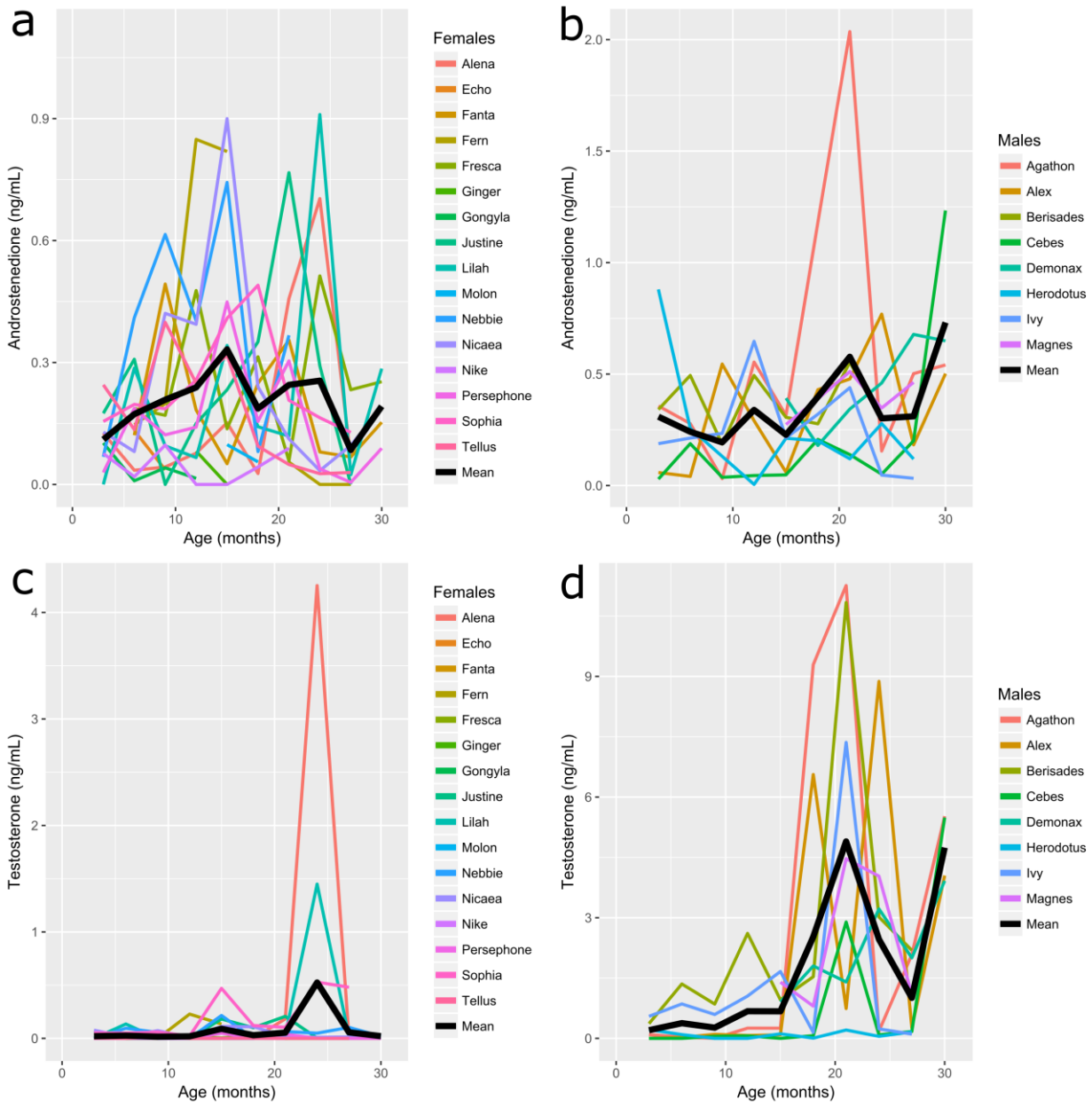

## Results

*Sex, age, and individual variation in androgen concentrations*

Immature and adolescent ring-tailed lemurs showed clear sex differences in their concentrations of both $A_4$ and T (Fig. 3). Controlling for age, we estimated that young males in our study had $A_4$ concentrations approximately 1.6 times greater than did young females ($t_{23} = 2.67$, $P =$

0.014), and T concentrations approximately 17 times greater ($t_{22}$ = 6.41, $P$<0.001). In females, the greatest mean $A_4$ concentration was observed at 15 months, with a smaller peak observed at 24 months. T production in females tended to be quiescent until a surge at approximately 24 months, followed by a return to minimal concentrations at 27 and 30 months. For males, beginning at approximately 15 months of age, $A_4$ and T exhibited a cyclic pattern of surges and drops roughly every six months.

**Fig. 3.** Androgen concentrations ($ng/ml^{-1}$) for female and male ring-tailed lemurs, from 3 – 30 months of age. Shown are individual (colored lines) and mean (bolded black line) values for both (a, b) androstenedione and (c, d) testosterone. Different y-axis scales are used for each panel to highlight variation within sex and androgen type.

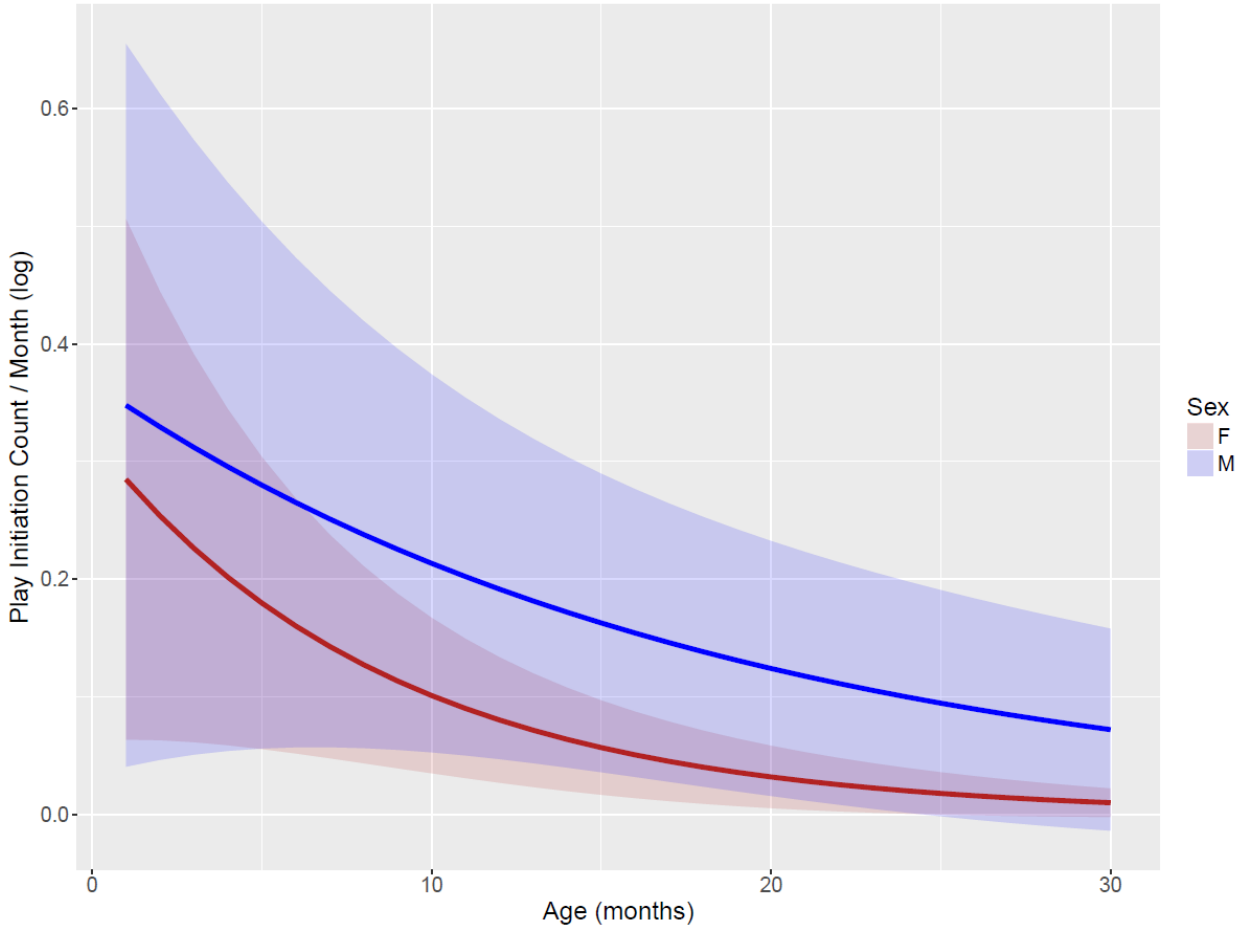



*Developmental patterns of play, aggression, and dominance*

*Play.* Uncharacteristically for most mammals, we found no overall sex difference in rates of play initiation ($z = 0.30$, $P = 0.765$). Members of both sexes initiated play at the highest rates early in the juvenile period, with rates decreasing overall as individuals matured ($z = -4.64$, $P<0.001$); however, this age effect was marginally qualified by an interaction with sex, such that the negative

relationship between age and play initiation was stronger for females than males ($z$ = 1.75, $P$ = 0.081; Fig. 4). As shown in Fig. 4, estimated rates of play initiation for both sexes became indistinguishable from zero at approximately 25 months of age. Rates of play received also decreased with age ($z$ = -4.36, $P$<0.001), although this relationship did not differ between the sexes ($P$ = 0.418).

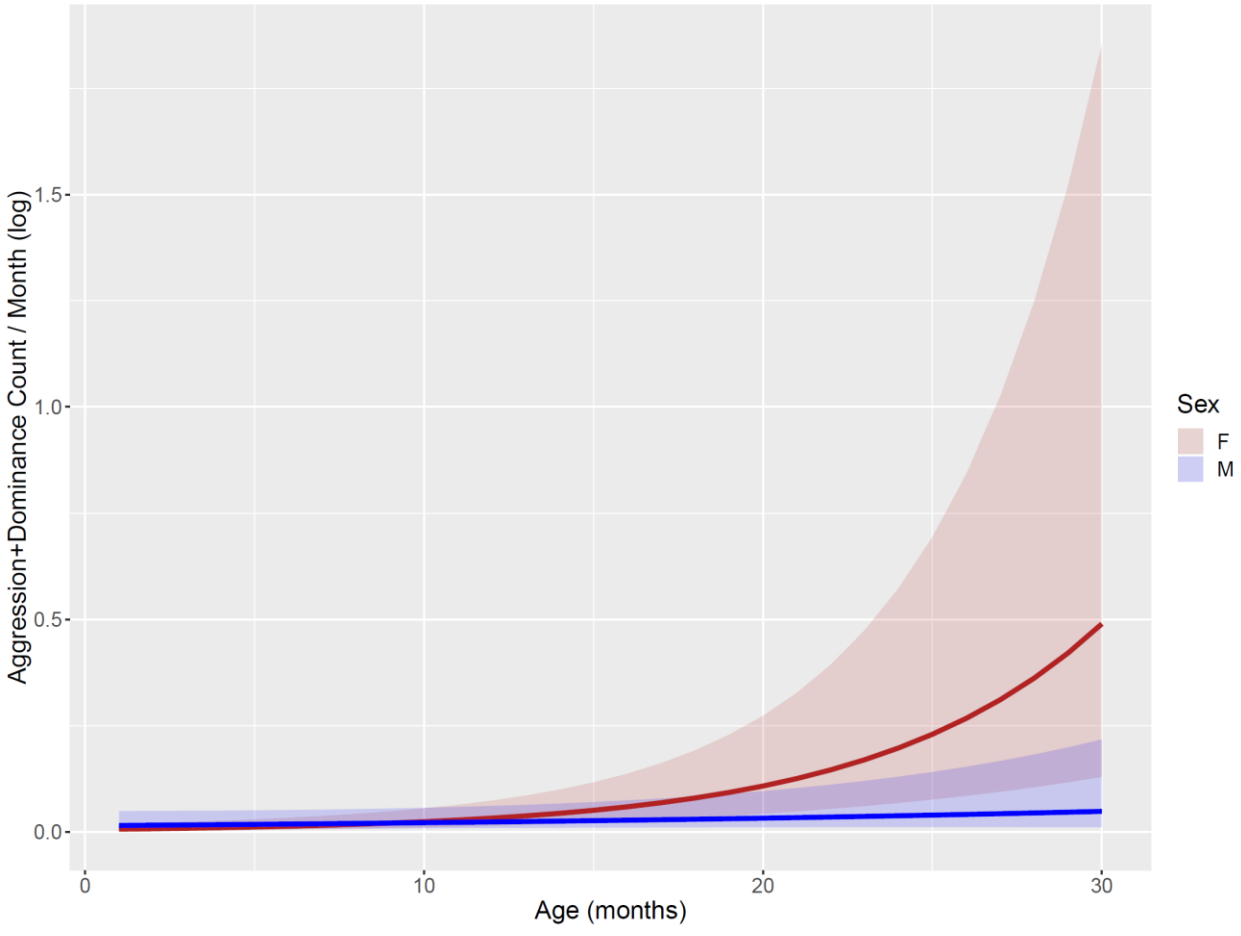
**Fig. 4.** Predicted rates at which young ring-tailed lemurs of both sexes initiate dyadic play. Shaded bands represent 95% confidence intervals.




*Aggressive/dominance behavior.* We also detected no overall sex difference in rates of aggressive/dominance behavior initiated ($z$ = 1.44, $P$ = 0.149). Rates increased with age ($z$ = 4.83,

$P$<0.001), but this increase was significantly steeper in females ($z$ = -2.46, $P$ = 0.014; Fig. 5). Until approximately 12 months, aggression rates of males and females were indistinguishable; however, while male rates continued to remain low, female rates of aggression/dominance surged. Rates of aggressive/dominance behavior received also increased with age ($z$ = 2.89, $P$ = 0.004), although this rate of increase did not differ between the sexes ($z$ = 0.003, $P$ = 0.997).

**Fig. 5.** Predicted rates at which young ring-tailed lemurs of both sexes initiate aggressive/dominance behavior. Shaded bands represent 95% confidence intervals.




*Concurrent/activational androgenic predictors of behavior*

Using our imputed datasets, we found no evidence that an individual's concurrent $A_4$ or T concentrations predicted their rates of initiating or receiving play (for $A_4$, *P*s = 0.988 and 0.968, respectively; for T, *P*s = 0.813 and 0.889). The same was true of analyses excluding imputed values (see Table S7). Our models also provided no evidence for activational androgens predicting either initiation or reception of aggression/dominance (all *P*s>0.482).

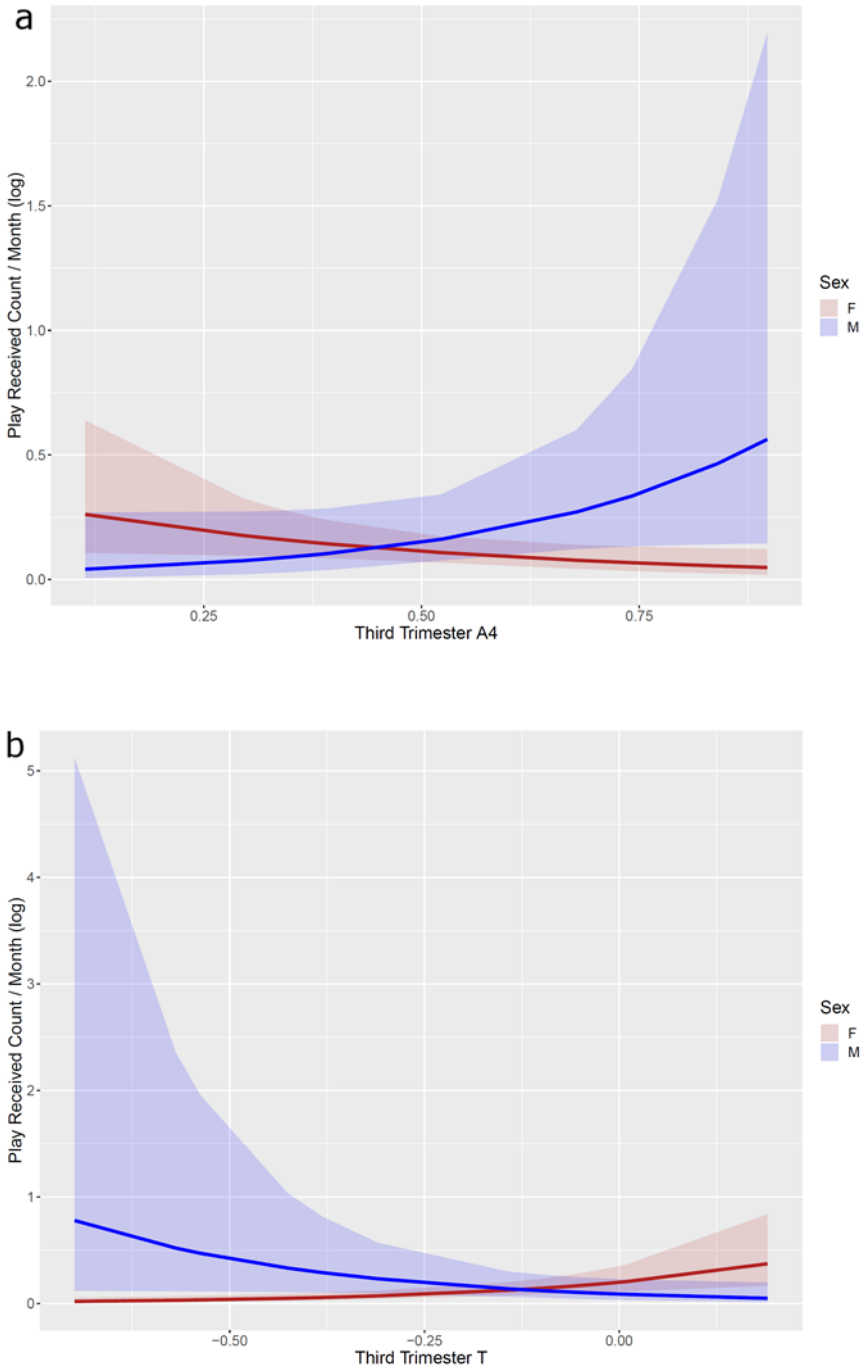
After controlling for an individual's androgen concentrations, age maintained its strong negative relationship to rates of play initiation ($P<0.001$). The age × sex interaction reported above for the behavioral analyses weakened slightly with the inclusion of the subjects' androgen concentrations ($P = 0.129$), although the effect size was nearly the same across both analyses ($\gamma = 0.06$).

*Prenatal/organizational androgenic predictors of behavior*

By contrast with results based on concurrent androgen concentrations, prenatal androgen concentrations significantly predicted offspring play behavior. Specifically, maternal concentrations of $A_4$ during the last trimester of pregnancy negatively predicted rates of offspring play reception ($z = -2.20$, $P = 0.028$), but this was true for females only ($A_4$ × sex interaction: $z = 2.72$, $P<0.001$). See Fig. 6a. The main effect of $A_4$ predicting play initiation was non-significant, but ran in the same direction ($z = -1.63$, $P = 0.156$).

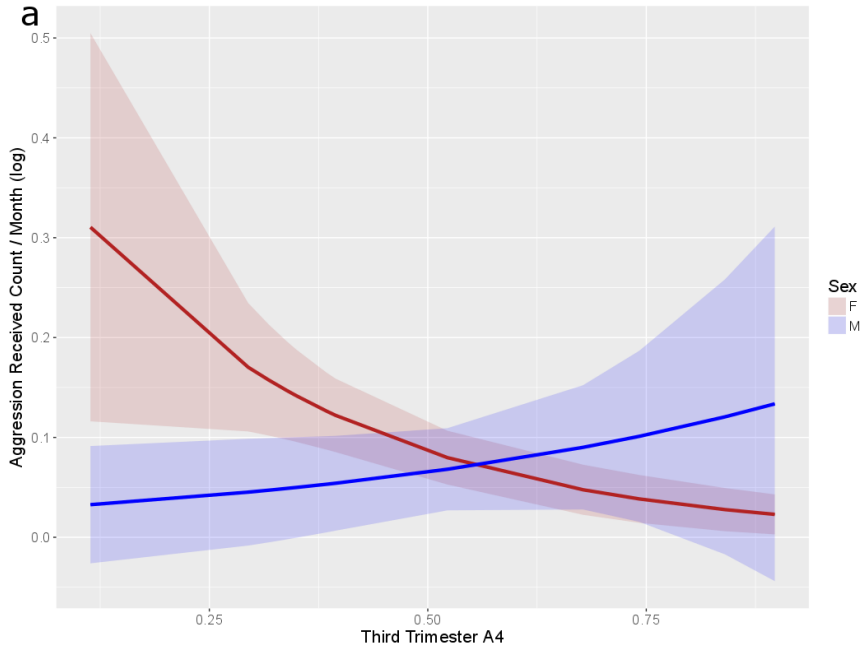
Maternal T concentrations during the last trimester of pregnancy positively predicted both the offspring's rates of play initiation and reception (both $P<0.005$), but as with $A_4$, the main effect for play received differed between males and females ($z = -3.62$, $P<0.001$), with a positive relationship for females and a negative relationship for males. See Fig. 6b.

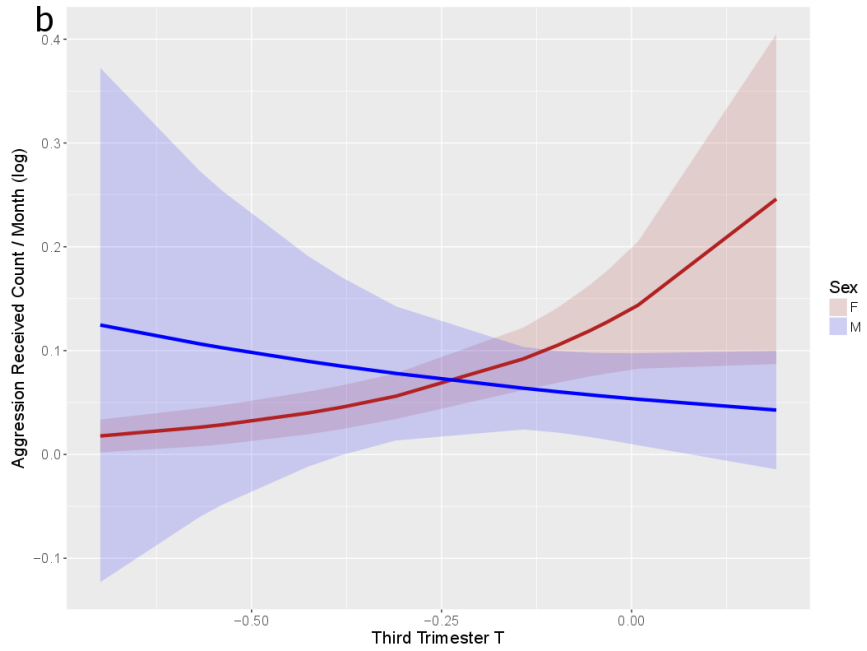
**Fig. 6.** Rates of play received by young ring-tailed lemurs of both sexes, as predicted by late-term maternal (a) androstenedione and (b) testosterone concentrations. Shaded bands represent 95% confidence intervals.



Although our models provided no evidence of organizational androgens predicting the initiation of aggression and dominance interactions (all *P*s>0.482), we found strong and opposing relationships between maternal $A_4$ or T concentrations and the rates at which offspring received aggression. Maternal $A_4$ negatively predicted aggression received by offspring overall ($\chi$ = -3.72, *P*<0.001), but this was qualified by an interaction with offspring sex, such that we observed a negative relationship to $A_4$ in females, but not males (expressed as a positive $A_4$ × sex interaction ($\chi$ =2.48, *P* = 0.014; Fig. 7a). Conversely, maternal T positively predicted aggression received by offspring overall ($\chi$ = 3.73, *P*<0.001), but once again this differed between males and females, with a positive relationship in females only (expressed as a negative T × sex interaction; $\chi$ =-2.15, *P* = 0.031; Fig. 7b).

**Fig. 7.** Rates of aggression received by young ring-tailed lemurs of both sexes, as predicted by late-term maternal (a) androstenedione and (b) testosterone concentrations. Shaded bands represent 95% confidence intervals.






## Discussion

At a proximate level, rough play, aggression, and social dominance in male mammals are often linked to their greater concentrations of circulating androgens (Monaghan and Glickman, 1992). Thus, when present in females, these behavioral characteristics suggest a possible role for organizational and/or activational androgens in female development (Davies et al. 2016; Dloniak et al.; Glickman et al. 1992; Ulibarri and Yahr, 1996). Consistent with findings in adult conspecifics (Drea, 2007) and indeed most mammals (MacLusky & Naftolin, 1981), young ring-tailed lemurs showed the typical sex differences in their androgen concentrations, particularly of T. Nevertheless, serum samples obtained across prenatal and postnatal development allowed us to differentiate both between different types of androgens and between potential organizational vs. activational effects. Accordingly, our results provide new evidence suggestive of an organizational androgenic role,

particularly for $A_4$, in explaining female social dominance and its related attributes in ring-tailed lemurs.

Rough-and-tumble play is the primary form of non-filial social interaction in primates, including lemurs (Gould, 1990; Pereira, 1993). Play behavior may provide an opportunity to practice skills used in adult life (particularly those relating to social bonding or aggression; see Gould et al., 1990; Palagi, 2018). Indeed, the dynamics of rough play in juvenile ring-tailed lemurs reflect those of aggression in adults (Pellis & Pellis, 1997). Nevertheless, play can also serve more immediate socialization functions (Drea et al. 1996), including the establishment of dominance relations (Panksepp, 1981; Pelligrini, 1995). We found that female infant and juvenile *L. catta* engaged in rough play at rates not significantly different from those of males, consistent with prior findings both in captivity (Pereira, 1993) and in nature (Gould, 1990).

The absence in lemurs of a strong sex difference in play could be seen as a null effect that potentially constrains data interpretation; however, we view it as particularly noteworthy, because mammals are generally strongly sex differentiated in their play behavior (e.g., Goy, 1970; Lovejoy & Wallen, 1988; Meaney, 1988; Geary, 1999; Auger & Olesen, 2009). In some species, male exposure to antiandrogens prenatally (e.g. Casto et al., 2003; but see Wallen, 2005) or postnatally (e.g. delBarco-Trillo et al., 2016) decreases play relative to untreated males. On the flip side, female exposure to exogenous androgens prenatally increases rough-and-tumble play relative to control females (Goy and Resko, 1972; Pellis, 2002; Young, Goy, and Phoenix, 1964). In naturally masculinized females, the absence (or reversal: Pedersen, Glickman, Frank, and Beach, 1990) of a sex difference in play could suggest naturally occurring organizational or activational effects of androgens in females. As in anthropoid primates, however, in which rough-and-tumble play requires organization without activation (Goy & Resko, 1972), our findings in strepsirrhine primates are

consistent with organizational (rather than activational) relationships between late-gestation maternal androgens and female offspring play behavior. The infant female lemur's prominent engagement in rough play could thus represent the earliest expression of her nascent social dominance over males.

Aggression too appeared not to exhibit a strong sex difference in immature ring-tailed lemurs, again consistent with prior findings in this species (Meredith, 2018). Despite a lack of behavioral sex differences in juveniles, scholars have noted clear female dominance by the time individuals reach puberty (ranging from ~16 months in captivity to ~30 months in the wild; Pereira, 1993; Meredith, 2018). This contrast once again leaves open the possibility that activational surges in androgens or unfolding organizational androgenic mechanisms that occur around the time of sexual maturation may contribute to adult patterns of female social dominance. It thus may be relevant that, although we found no significant evidence of overall activational effects of androgens on the behavior of either young male or female ring-tailed lemurs, a peak in female $A_4$ at 15 months of age coincided with a female advantage in the predicted rates at which young ring-tailed lemurs initiated aggressive behavior during ontogeny (perhaps marking the emergence of more overtly aggressive female dominance). It is unknown the extent to which this surge in androgens represents gonadal output (owing to puberty) versus adrenal output, although we think it to be predominantly gonadal based on previous work in anthropoid primates (Lovejoy & Wallen, 1990) and female-dominant spotted hyenas (Yalcinkaya et al., 1993). Nevertheless, the role of androgen surges (and their derivation) during this period of development warrants further study.

In contrast to our results examining activational androgens, we found strong associations of organizational androgens in relation to aggressive behavior, particularly in young females. Notably, females that were presumably exposed to greater concentrations of maternal $A_4$ late in fetal development were less likely to be aggressed against postnatally, whereas females that were

presumably exposed to greater concentrations of maternal T during this same period were more likely to receive aggression postnatally.

Our results are consistent with the notion that the organizational actions of prenatal $A_4$ and T are differentiated by fetal sex, raising a question about the potential specificity and mechanism of action for $A_4$. Although typically dismissed as a weak androgen (or prohormone), there is evidence that $A_4$ may exert androgenic effects directly, via binding to androgen receptors (Chen et al., 2004; Sonneveld et al., 2006). Interestingly, $A_4$, but not T, covaries with female competitive aggression in young women (Cashdan, 2003; Inoff-Germain et al., 1988). A specific role for $A_4$ in mediating female dominance could help explain male subordination despite the male's much greater T concentrations throughout life (see Yalcinkaya et al., 1993). Alternately (or additionally), $A_4$ could exert its effect indirectly, via enzymatic conversion to estrone (itself a precursor to other estrogens). Estrogens have been implicated in the regulation of female aggression in a wide range of species (e.g., Van de Poll et al., 1986, Finkelstein et al., 1997; but see Couse & Korach, 1999). Thus, the role of $A_4$ and T could differ between the sexes, including in relation to differential conversion of $A_4$ (to either T or estrone) based on locally available enzymes. Lastly, as previously suggested (Sherwin, 1988; Eens & Pinxton, 2000; Rosvall et al., 2012), it may be the case that the sexes differ in their densities or distribution of steroid receptors, or in their neural sensitivity to gonadal steroids. Thus, there are various endocrine-based explanations (i.e., related to the biosynthetic pathway or binding of steroids) to account for female dominance in the absence of a female bias in T, all of which merit further investigation.

These open questions highlight certain difficulties and constraints in behavioral endocrinology. Notably, it remains a challenge to obtain information about organizational effects of hormones on animal development without invasive or manipulative research. While most field work

is limited to studies of activational effects, based largely on correlative data, ours has extended this approach to the prenatal period (following e.g. Dloniak et al., 2006). Nevertheless, without evidence that maternal hormones actually reach the developing fetus (following e.g. Yalcinkaya et al., 1993), these patterns also remain correlative. Accordingly, we advocate caution in interpreting such findings.

Likewise, temporal asynchrony between hormonal and behavioral sampling presents a frequent challenge in behavioral endocrine studies, particularly in field or other non-laboratory contexts. Often, there is a desire to retain as many data points as possible, even if the constituent measurements are temporally distal. In such cases, researchers typically partition data into temporal 'bins' for correlational analyses. When bins span several months, however, researchers must assume that circulating hormone concentrations at one time point closely reflect concentrations at a time period up to several months in the past or future. The significant error potentially introduced by this assumption may be compounded in long-term, developmental studies such as ours, in which concentrations can fluctuate substantially during the transition between life-history stages. While the issue of asynchrony is often viewed as an unavoidable limitation, we propose an analytical pathway forward by fundamentally treating this issue as a missing data problem. Statisticians have developed a rich body of work to guide scientists in the handling of missing data (Little & Rubin, 2014), and we encourage behavioral endocrinologists to avail themselves of these methods. The benefits are clear: our use of a popular missing-data approach (i.e., multiple imputation) allowed us to perform correlational analyses on our postnatal hormonal and behavioral data points without making theoretically implausible assumptions regarding the stability of hormonal concentrations over multiple months. Using this approach gave us the ability to examine potential roles of both organizational and activational androgens in behavioral masculinization, with our results supporting

the fetal masculinization hypothesis of female dominance in lemurs (Drea, 2011; Petty and Drea, 2015).

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