Profiling caregivers: Hormonal variation underlying allomaternal care in wild red-bellied lemurs, *Eulemur rubriventer*

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ABSTRACT

Neuroendocrine evidence suggests that paternal care is mediated by hormonal mechanisms, where hormonal changes in expectant and new fathers facilitate infant care. In species with obligate and extensive paternal care such as humans, androgen levels decline once males are paired and have offspring, and in direct response to offspring care. Facultative infant care is widespread in the Order Primates, but the underlying hormonal mechanisms are largely unknown. We found that wild, red-bellied lemurs living in family groups (two adults and their presumed offspring) varied in the amount of care they provided infants. The more fathers invested in helping infants (measured as a composite of carrying, holding, huddling, grooming, and playing), and specifically the more they huddled and groomed with infants, the higher their fecal androgen (fA) levels, contrary to expectations. Carrying was negatively related to fA levels. Helping by subadults and juveniles was not related to their own fA levels. Elevated fA levels during infant dependence have been observed in other vertebrate species, and are thought to reflect reinvestment in mating rather than investment in dependent offspring. However, red-bellied lemurs do not mate until after infants are weaned, and they have long-term pair-bonds, suggesting that elevated fA levels play a role in offspring care. These results support a growing body of research suggesting that elevated androgen levels do not inhibit protective infant care.

1. Introduction

In most mammals, infants are completely dependent on others for their food, protection, and transportation. Usually the burden of raising offspring falls on the mother, and can be costly, especially when lactation and infant dependence are prolonged [1,2]. This cost can be mitigated somewhat if infant care is distributed across several individuals [3]. Allomaternal care (AMC), or care by anyone other than the biological mother, can include babysitting, nursing, and carrying, or other, less expensive behaviors like grooming [4–8]. AMC is significant because it can help reduce a mother's energetic burden of raising offspring (see [8,9]), while increasing energy invested in future [10] or current infants, supporting infant growth and survival (e.g., [11,12]).

AMC varies quite a bit in form and type, and is particularly notable and well-studied in cooperatively breeding species. In cooperative breeders, several caregivers (including babysitters, teachers, family members, and friends) may help a mother raise her offspring through habitually providing direct care and food to infants, and help is obligate [13–16]. Humans and callitrichines are the only primates that have cooperative breeding systems; AMC occurs nearly without fail [14]. AMC in these cooperatively breeding primate species is also adaptive, increasing reproductive output [17]. Phylogenetic comparisons indicate that mothers reproduce faster if they receive help, with shorter inter-birth intervals and faster growing offspring who also wean off breastmilk quicker for their body size [18–20]. And, evidence suggests that evolved neuroendocrine systems mediate paternal care in cooperatively breeding fathers: hormonal changes (e.g., in estradiol, prolactin, cortisol, and androgen levels) in expectant and new fathers occur [21–24], and are thought to facilitate infant care (see [25–27]).

Androgens, a class of hormones that includes both adrenal and gonadal steroids such as androstenedione and testosterone, respectively, have been associated with paternal care in mammals, birds, anurans, and fish. Lowered gonadal androgen levels may allow for bond formation with infants as part of a life-history trade-off between mating and parenting (the 'Challenge Hypothesis', [28]; see [29]). Within mammals, research testing this relationship has largely focused on

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cooperatively breeding primate and rodent species. First, direct interactions with offspring have been associated with reduced testosterone levels in hamster, human, and marmoset fathers [30–32]. Second, castrated Mongolian gerbil fathers perform more parental behaviors than intact males [33,34]. Third, adult male testosterone and unspecified androgen levels in marmosets and tamarins, respectively, are lower after infants are born than during the mating season [35,36] (though this also occurs in species without AMC [37]). Fourth, human fathers (whether or not they are in committed relationships) have lower testosterone levels than single, childless men [31,38–40]. Similarly, men and male African striped mice have lowered testosterone levels after they transition from competing for mates, into maintaining a bond with a pair-mate and offspring [41,42]. This change is expected because androgens like testosterone are costly to maintain at high levels (see [43]), are commonly associated with aggression and mate competition (e.g., [36,44]), and could therefore impede bond maintenance and suppress parental care. Maintaining lower androgen levels also may help individuals avoid the costs of immunosuppression associated with elevated androgen levels, in turn reducing the likelihood of disease transmission to infants [45].

In contrast, several other studies of cooperatively breeding mammals with biparental care have found the opposite relationship, demonstrating that AMC and elevated androgen levels are not incompatible. In the first days after an infant's birth, tamarin fathers' testosterone levels increase if they have previously experienced parenthood [46], and rodent fathers who are more responsive to infants have higher testosterone levels [47,48]. These different findings may reflect differences in mating competition after an infant's birth [25,40,46,49,50]. For example, males of species with fast life histories may compete for mates, or invest in mating with their partner if she has a postpartum estrus, when infants are still dependent. In this case, higher testosterone levels in paternal caregivers may reflect mating effort rather than parenting, and also demonstrate that aggressive competition and paternal behavior may co-occur [48,51].

While cooperative breeding is highly derived and unusual in primates, other, more facultative forms of AMC are common [17]. AMC is widespread in primates [8,19,52,53], and occurs at much greater frequency in the Order compared with other mammalian Orders (e.g., [17,54–56]), suggesting early or repeated and strong pressure for it to have evolved [9]. Comparative study of species with less derived versions of AMC can inform on the selective pressures and mechanisms that allowed cooperative breeding to evolve in our own species. Yet, the neuroendocrine correlates of facultative AMC are largely unexplored.

Here, we study AMC in a strepsirrhine primate, the red-bellied lemur (*Eulemur rubriventer*), to determine if the same mechanisms involved in paternal care in cooperative breeders may also underlie facultative AMC. We also extend this analysis (androgen changes associated with specific AMC behaviors) to siblings, which has not been investigated in any primate species to our knowledge. We investigated whether individual-level differences in AMC can be explained by differences in hormones thought to be important in AMC (in addition to infant age, and helper age-sex class). Our first goal was to document variation in AMC. From the infant's perspective, we determined how much care is received from different helpers; from the helper's perspective, we determined how much of their own effort is invested in infant care, and in what ways. Our second goal was to determine if variation in AMC is associated with variation in fecal androgen (fA) levels. We analyzed fA levels with *Total AMC*, which includes grooming, holding, huddling, carrying, and playing, because other studies have used these as indicators of paternal investment [57–61]. In a previous study of 5 adult and 3 subadult male red-bellied lemurs (n = 133 samples; Tecot, unpublished data), we found that at birth all individuals' fA levels plummeted sharply, as also observed in humans (see [62]). However, there was substantial variation in fA levels in the weeks after infants were born. Based on these data, as well as studies of cooperative breeders lacking a postpartum estrus, we predict that

helper fA levels will be inversely related to the amount of care they provide to infants.

2. Materials and methods

2.1. Subjects and site

We studied one population of red-bellied lemurs, *Eulemur rubriventer*, around the Vatoharanana trail system of Ranomafana National Park, Madagascar, a montane rain forest at approximately 1200 m above sea level (described in [63–66]). This population of lemurs has been the subject of study off and on since 1988, and is part of a long-term study by the Ranomafana Red-Bellied Lemur Project. Individuals are identified by sexual dichromatism (to determine sex), body size (to determine age), pelage color, and distinct scars and facial features (to determine individual identity) [67]. In this study, infants were < 1 year, juveniles were 1–2 years, and subadults were > 2 years and had not yet dispersed from their natal groups. One and two-year-olds are easily distinguished from each other and older group members, and subadults and older offspring are relatively easily distinguished from the adult breeding pair. Groups consist of strongly bonded monogamous family units comprising an adult female, adult male, and their offspring, though turnover in adult group membership does occur occasionally [68–71]. A relatively late birth season (November) in the first year of the study allowed us to observe all groups prior to infant birth in each year. No turnovers occurred preceding the births of infants in our study, increasing our confidence that presumed fathers were biological fathers. Red-bellied lemurs live in egalitarian groups with very little aggression [70], so dominance relationships were not expected to interfere with infant care-related hormone changes. Direct mate competition is rarely observed [70]. There is no evidence of postpartum estrus. The mating (April–July) and infant care (Sept–Feb) seasons are distinct with no overlap [72]. No infants were born to a female with dependent offspring, allowing us to focus on hormone changes only associated with infant care.

2.2. Allomaternal care behaviors

Behavioral data were collected from September 2013–March 2015, including two reproductive seasons that spanned conception through weaning, totaling 18 months and 2722 h of data (Season 1: 568 h; Season 2: 2153 h). Thirteen groups, each with one infant, were sampled, for a total of 56 individuals (Supplemental Table S1). Groups were sampled, on average, once per week, although difficulty locating some groups meant uneven sampling effort, both across Seasons and among subjects. Uneven sampling across Seasons also occurred because we increased our team of research assistants and technicians from Season 1 (n = 2–4) to Season 2 (n = 6–8). Focal data on behavioral states were collected from each infant (n = 13) every 5 min to determine the proportion of care each infant received, and from whom [73]. In addition to recording behavioral states, the nearest neighbor, partner(s) ID, and partner care behavior were recorded. Infant mortalities did occur during the data collection period. In Season 1, the infant (Lety) from Barimaso group died after ~1 month. In Season 2, infants in Barismaso, Cities, and Snack groups died after 1.5, 2.5, and 3 months, respectively (see Supplemental Table S1). For the purposes of this study, we only analyzed behavior and corresponding androgen data from the times when infants were present.

We were also interested in care as a proportion of each individual's activity so that we could relate their effort to individual fA levels. Though we did not associate care behavior with androgens in mothers, behavioral states of all non-infants, including mothers (n = 43; Supplemental Table S1), were recorded every 5 min using group scans [73], and if they were interacting with an infant, infant care behaviors (groom, play, carry, hold, huddle) were recorded as well. We defined groom and play after [74]. We defined 'carry' as the behavior that

occurs when an animal forages, moves, or travels with an infant clinging to their body, usually dorsally, ventrally, or on the side; 'hold' as the behavior that occurs when a stationary individual grasps an infant with its arms or hands; and 'huddle' as a behavior that occurs during rest in which an individual is stationary, holds an infant such that their bodies are touching, and wraps its tail around both bodies. We used these data to determine individual behavioral investment in allomaternal care behaviors. We repeatedly calculated inter-observer reliability among the field team (n = 2–8) until all observers were within 95% agreement [75].

From these data, we calculated two measures of AMC: *Total AMC*, measured as the total proportion of scans a helper was observed performing any AMC behavior; and *AMC type*, measured as the proportion of scans in which a helper was observed performing each of the five AMC behaviors (described above).

2.3. Fecal sample collection, processing, and androgen assays

To analyze fA levels, fresh fecal samples were collected opportunistically from known individuals during behavioral data collection on focal groups, as described in Tecot [66]. Samples were only collected in the morning, before noon, to control for any circadian effect on steroid excretion (see [76]), and only if they were uncontaminated by urine, other individuals' feces, or stream water. Samples were placed in aluminum foil, flattened, labeled, and desiccated by a fire within 6 h of collection (following [77], with modifications in [66,78]). Once dry, samples were combined into a Ziplock bag with desiccant. Samples were transported to the Laboratory for the Evolutionary Endocrinology of Primates in the US for analysis at the end of each field season.

To extract steroids, each sample was ground to a dust using a mortar and pestle, and seeds were removed using a sifter. 2.5 ml of purified water and 2.5 ml of 100% ethanol were added to 0.1 g of dried feces. When < 0.1 g was available, multiple samples from the same individual in the same day were combined to reach 0.1 g, or a smaller amount of sample was used and its weight accounted for during concentration calculations. Samples were centrifuged for 10 min at 3000 rpm and homogenized on a multi-vortexer for 10 min, the supernatant was poured off, and the fecal pellet was disposed of. To release conjugated steroids, 4 ml of ethyl acetate was added to 1 ml of the supernatant, then homogenized for 8 min and centrifuged for 3 min. The top layer was aspirated off, dried down, and resuspended in 1 ml 100% ethanol.

Fecal androgen levels were determined through enzyme immunoassay (EIA), based on procedures employed by Ginther and colleagues [79] for testosterone. 100 μl of each sample was dried and reconstituted in 300 μl of assay buffer and vortexed, and 100 μl was aliquoted onto a 96-well assay plate in duplicate. Testosterone antibody was acquired from C. Munro (R156/7, University of California, Davis). R156/7 has a high cross-reactivity with testosterone (100%) and 5alpha-dihydrotestosterone (57.37%), both of which are gonadal androgens, and low cross-reactivity with androstenedione (0.27%) and other tested analytes (< 0.05%) of adrenal origin. Absorbance was read at 415 nm with a background absorbance of 570 nm, on a Biotek Epoch microplate spectrophotometer. All samples were assayed in duplicate and are expressed in ng/g feces. The assay was biochemically validated for red-bellied lemur feces: 1) serially diluted fecal sample extract was parallel to the androgen standard, F = 2.91(13,14), p = 0.112; and 2) accuracy of added fecal pool extracts to the androgen standard curve points was 83.68 ± 11.57% standard error of the mean, n = 8. The inter-assay coefficients of variation (CV) were 11.6 ± 7.8% for the high pool and 12.6 ± 3.1 for the low pool, and the intra-assay CVs were 4.4 ± 7.7% for the high pool and 2.5 ± 2.9% for the low pool.

2.4. Data analysis

2.4.1. Allomaternal care behaviors

We conducted all statistical analyses in R version 3.3.2 [80]. We used descriptive statistics followed by non-parametric Kruskal-Wallis and Bonferroni-corrected Wilcoxon signed ranks comparisons to explore individual differences in AMC behaviors (*Total AMC*, hold, huddle, carry, groom, play) across age-sex classes. We analyzed focal infant scans to investigate AMC from the infant perspective, including relative amounts of care received from each member of its social group by age-sex class. We also used focal group scans to investigate AMC from the perspective of the helper, including the relative proportions of scans helpers were observed in each of the five *AMC types*, as well as in the proportion of scans engaged in AMC overall (*Total AMC*).

2.4.2. Androgens × AMC type

We explored the relationship between fA and AMC behaviors (both *Total AMC* and *AMC type*) using generalized linear mixed-effect models (R version 3.3.2, lme4 package; [81]). Models included fA as the dependent variable, and individual nested within family group nested within Season (2013–2014 or 2014–2015) as random effects to control for repeated sampling of individuals and groups across different sampling seasons (see Supplemental Table S1).

Individual androgen levels were measured in 246 fecal samples (mean = 9.84 ± 5.76 samples per individual per study season; Season 1: n = 78 samples, mean = 7.80 ± 4.49 samples/individual; Season 2: n = 168 samples, mean = 11.20 ± 6.25 samples). Weekly samples were collected from a subsample of 18 adults, subadults, and juveniles (presumed fathers and siblings, respectively) from nine social groups (see Supplemental Table S1) during 42 consecutive weeks, from each group's adult female's late gestation (weeks − 16 to 1 week before birth) through birth (week 0) and postpartum (weeks 1 to 26) (n = 401 observations). In cases where births were not observed, birth date was estimated based on the time (in days) since the group was last observed, and the size of the infant. Because androgen data were non-normally distributed (high kurtosis), they were natural log-transformed prior to analysis.

Because we were interested in the fA × AMC relationship specifically, we reduced the androgen dataset to include only the postpartum period (n = 346 observations, Week 0–Week 26). We then aligned weekly androgen values to a corresponding behavioral dataset that included weekly measures of AMC, which we calculated as the proportion of group scans during which an individual was observed engaging in each type of AMC behavior, as well as a composite *Total AMC* score. For weeks with missing androgen data, we imputed mean individual fA values calculated from postpartum samples.

To predict fA levels, we created models that included the fixed factors of infant age (in weeks since birth), helper age-sex class (adult male, subadult male, subadult female, juvenile male, juvenile female), helper affiliation (measured weekly as the proportion of scans in which an individual groomed or huddled with another group member, other than the infant), and measures of AMC. We included helper affiliation to ensure that any relationship between AMC and fA levels was not due to general affiliative behavior. The first set of models included *Total AMC* as a fixed effect. The second model set included weekly measures of each of the five *AMC types* (groom, hold, huddle, play, carry). Because *Total AMC* is a composite of the five *AMC types*, we never included it as a predictor variable in models where *AMC types* were also used.

Prior to analyses, we assessed predictor variables in each model set for collinearity using variance inflation factors (VIFs) (R version 3.3.2, usdm package [82]). VIFs were low across predictor variables (1.00–1.57); however, *AMC types* carry and hold were moderately correlated ($r^2 = 0.599$). To be conservative, we calculated the residuals of carry by regressing it against hold prior to including it in our final model set.

We assessed model performance using an adjusted measure of Akaike's Information Criterion (AICc) with the "dredge" function in the MuMIn package [83]. We evaluated models using the change in AICc scores (ΔAICc) and Akaike weight value (w). The 'best model' was the model with the lowest AICc score. As is the convention, we considered models within 2 ΔAICc scores to be equally good (reviewed in [84]).

We used a standard model averaging technique to estimate the effect sizes and significance values for each relevant parameter. To estimate the relative effect sizes of each term that appeared in any of the top models, we averaged the models in each of the 95% confidence sets (i.e. $\Delta AIC_C < 10$). Model averaging with this threshold of confidence provides an additional and conservative method of estimating the effects of a given predictor [85].

We used likelihood ratio tests to compare final models to a null model with no fixed effects, thus verifying the statistical significance of the final model; we expected significant differences.

2.5. Ethics statement

This research was approved by the University of Arizona Institutional Animal Care and Use Committee (protocol 13-470) and Madagascar National Parks (055/15/MEEMEF/SG/DGF/DCB.SAP/SCBSE), and adhered to the guidelines set forth by the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates.

3. Results

3.1. Allomaternal care behavior

Infants received a majority of care from their mothers (90.82% ± 10.14 SD, n = 13), followed by adult males (mean = 6.55% ± 9.66 SD, range = 0–36.95%, n = 16), subadults (females: mean = 2.38% ± 1.30 SD, range = 0.65–5.14%, n = 3; males: mean = 1.45% ± 1.30 SD, range = 0.73–2.55%, n = 3), and juveniles (females: mean = 1.35% ± 0.79 SD, 0.00–2.67%, n = 4; males: mean = 2.36% ± 1.57 SD, 0.67–5.47%, n = 9) (Table 1). The predominant form of AMC received by infants was huddling (86.06%), followed by holding (4.66%), playing (4.43%), carrying (2.97%), and grooming (1.88%). Care type received was, however, dependent on helper age. The majority of care received from adult males was huddling (86.65%), followed by holding (5.69%), carrying (4.22%),

grooming (1.97%), and playing (1.48%), whereas AMC involving juveniles and subadults was primarily huddling (92.45%), followed by playing (5.60%), grooming (0.83%), holding (1.04%), and carrying (0.07%). Infants typically received AMC from a single individual at a time (71.4% - 98.8% of observations for carry, groom, hold, and play involved only one group member). However, huddling – the most common form of AMC – most often occurred with several group members (93.72% of huddling observations included at least two group members in addition to the infant), and usually involved at least one adult (e.g., an adult was present in 94.07% of observations involving huddling in non-adults; Fig. 1).

From the helper point of view, all individuals included in this study were observed performing some form of infant care behavior in at least one of the two seasons, with the exception of one subadult female and one juvenile female in Season 2 (i.e., 93.3% of group members helped; Fig. 2). Three adult males were studied in both sampling seasons; in these cases, *Total AMC* and *AMC types* were averaged across seasons prior to statistical comparison. In one case, a subject moved up an age class (e.g., a juvenile in Season 1 became a subadult in Season 2), and was included as separate data points in the analysis (Supplemental Table S1).

We observed striking individual variation, both in *Total AMC* and in individual behaviors by *AMC type* (Fig. 2). We found only a low-level correlation between individual *Total AMC* and the percent of time they affiliated with other non-infant group members (% AMC × % AFF; $r^2 = -0.307$), and no relationship between % AFF and any of the *AMC types* (% AFF × % any AMC type; average $r^2 = -0.10$, range = 0.001 to -0.29), suggesting that it is unlikely that an individual's proclivity to help was related to its tendency to affiliate overall. Individual differences in AMC behaviors were unrelated to helper sex (*Total AMC*: Wilcoxon rank sum, W = 5437, p-value = 0.45), but could be explained by helper age, both in terms of total investment (i.e., *Total AMC*: Kruskal-Wallis, χ^2 (2) = 21.694, df = 2, p < 0.001), as well as in the proportion of observations invested in each of the five main AMC types (Carry: χ^2 (2) = 24.86, p < 0.001; Groom: χ^2 (2) = 18.689, p < 0.001; Hold: χ^2 (2) = 16.12, p < 0.001; Huddle: χ^2 (2) = 18.00, p < 0.001; Play: χ^2 (2) = 22.82, p < 0.001; Fig. 2). For this reason, sexes were pooled into each of the three age classes for all further analyses. Subadults huddled with infants less than did either juveniles or adults (W = 5293.5, p = 0.001, W = 14,436, p < 0.001, respectively; Fig. 3). In fact, subadults invested significantly less (*Total AMC*) than did either of the other two age classes (Sub × Juv: W = 5381.5,

Table 1
Relative proportion of care received by infants by age-sex class in each season.

Season	Group	Infant	AdF	AdM	SubF	SubM	JuvF	JuvM
2013–2014	Authors	Poe	73.63	18.82	–	–	–	7.55
	Barimaso	Lety	83.41	11.12	–	–	–	5.47
	Cities	Tana	63.05	36.95	–	–	–	–
	LOTR	Precious	99.74	0.26	–	–	–	–
	Star Wars	Chewbacca	86.75	4.58	5.14	–	–	3.52
	Triangle	Hypotenuse	98.28	0.00	–	–	1.72	–
2014–2015	Africa	Monkey	96.98	3.02	–	–	–	–
	Barimaso	Nany	100.00	0.00	–	–	0.00	0.00
	Cities	Madrid	95.33	1.48	–	–	–	3.19
	Freax	Asterix	91.47	4.96	0.00	2.55	1.02	–
	GOT	Robb	88.05	11.95	–	–	–	–
	Lovelies	Candy	93.75	4.24	1.34	–	–	0.67
	Malagasy	Ovy	95.32	1.07	–	1.07	–	2.54
	Snack	Salto	97.86	0.80	–	–	–	1.34
	Speed	Bolt	92.49	4.84	–	–	2.67	–
	Star Wars	Obi	96.95	0.73	0.65	0.73	–	0.94
		Average	90.82	6.55	1.78	1.45	1.35	2.80
		SD	10.14	9.66	1.30	0.68	0.79	2.30
		Min	63.05	0.00	0.00	0.73	0.00	0.00
		Max	100.00	36.95	5.14	2.55	2.67	7.55

AdF = adult females; AdM = Adult males; SubF = Subadult females; SubM = Subadult males; JuvF = Juvenile females; JuvM = Juvenile males.
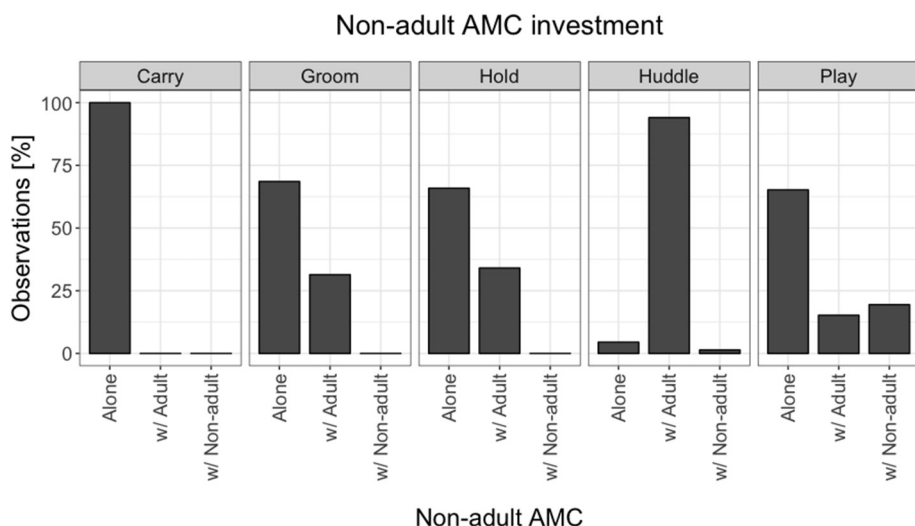
Fig. 1. Non-adult AMC behaviors with and without presumed parents (adults) and siblings (non-adults). AMC behaviors were unrelated to the presence of adults, except in the case of huddling. Subadults and juveniles huddled with adults in 94.01% of instances in which they also huddled with infants.
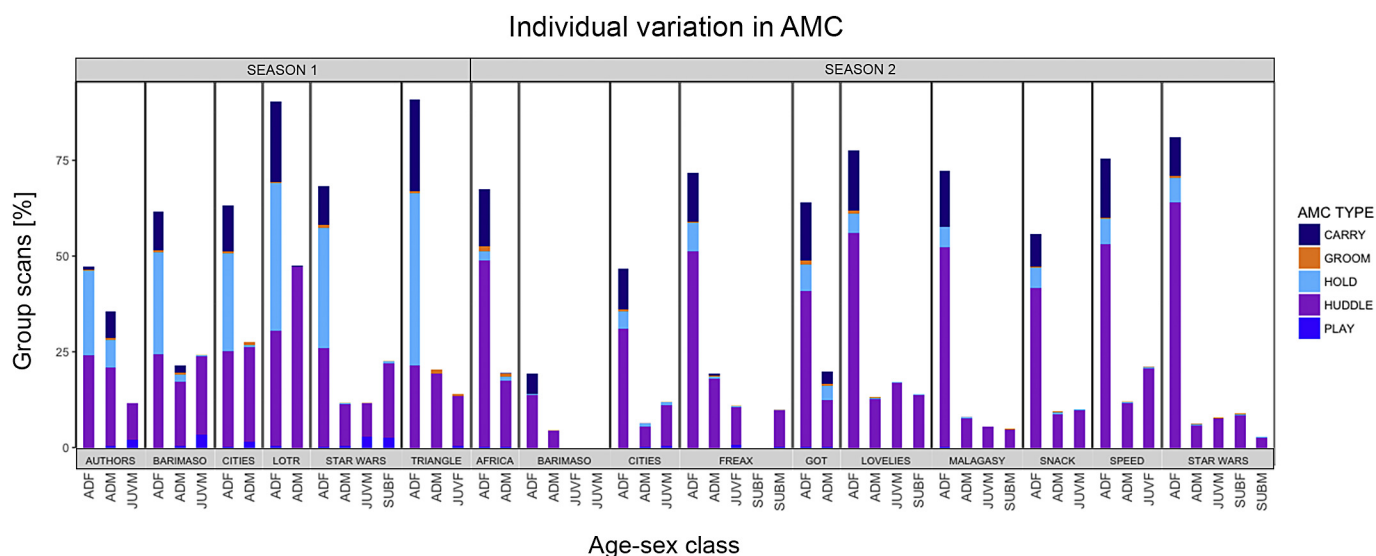

Fig. 2. Infant care provided by each individual as a proportion of their total activity, by group and age-sex class. Season 1 = 2013–2014 field season; Season 2 = 2014–2015 field season. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

p < 0.001; Sub × Ad: W = 14,838, p < 0.001; Fig. 3). Adults groomed, held, and carried infants proportionately more than did either subadults or juveniles (Groom: Ad × Sub: W = 13,211, p < 0.001, Ad × Juv: W = 9886, p = 0.003; Hold: Ad × Sub: W = 13,306, p < 0.001, Ad × Juv: W = 9738.5, p = 0.014; Carry: Ad × Sub: W = 12,996, p < 0.001, Ad × Juv: W = 9787.5, p < 0.001; Fig. 2). Juveniles played more than either adults or subadults (Ad × Juv: W = 6640.5, p < 0.001; Sub × Juv: W = 5098.5, p < 0.001; Fig. 3).

3.2. Androgens

To investigate the relationship between fecal androgens and AMC behavior, we focused on the nine social groups with the greatest intensity of fecal and behavioral sampling (average = 36.36 observation days ± 22.25 SD, range = 14–98 observation days; Supplemental Table S1).

Fecal androgen levels (fA) changed significantly through time, and varied by age-sex class. The best model included both infant age and helper age-sex class as significant predictors of individual mean weekly androgen levels, χ^2 (5) = 15.257, p = 0.009. For every week postpartum, fA levels increased by 1.3%, β = 0.013, SE(β) = 0.007, t (318.700) = 1.967, p < 0.05. The variable age-sex class was also

significantly associated with fA values. Juvenile male and subadult female helpers had significantly lower fA values compared to adult male helpers; fA values were 45.1% (β = − 0.451, SE(β) = 0.204, t(20.300) = − 2.210, p = 0.039) and 127.4% (β = − 1.274, SE(β) = 0.394, t (18.000) = − 3.233, p = 0.005) lower than adult males, respectively.

3.3. Androgens × allomaternal care

3.3.1. Androgens × Total AMC

Using the subsampled dataset, the best model predicting fA included infant age (weeks since birth), helper age-sex class, *Total AMC*, and the interaction between age-sex class and *Total AMC* (Table 2). This model performed significantly better than the null model, χ^2(6) = 19.285, p = 0.004. Only one other model was within 2 AICc scores; it included only helper age-sex class and *Total AMC* (Table 3). *Total AMC* predicted an increase in fA values, β = 0.006, SE(β) = 0.003, z = 2.019, p = 0.044. For every 1% increase in *Total AMC*, there is an associated 0.6% increase in fA. Juvenile male and subadult female helpers had significantly lower fA values compared to adult males, β = − 0.444, SE (β) = 0.210, z = 2.103, p = 0.035 and β = − 1.265, SE(β) = 0.397, z = 3.172, p = 0.002, respectively. Juvenile female and subadult male helpers did not differ significantly from adult males in their fA response
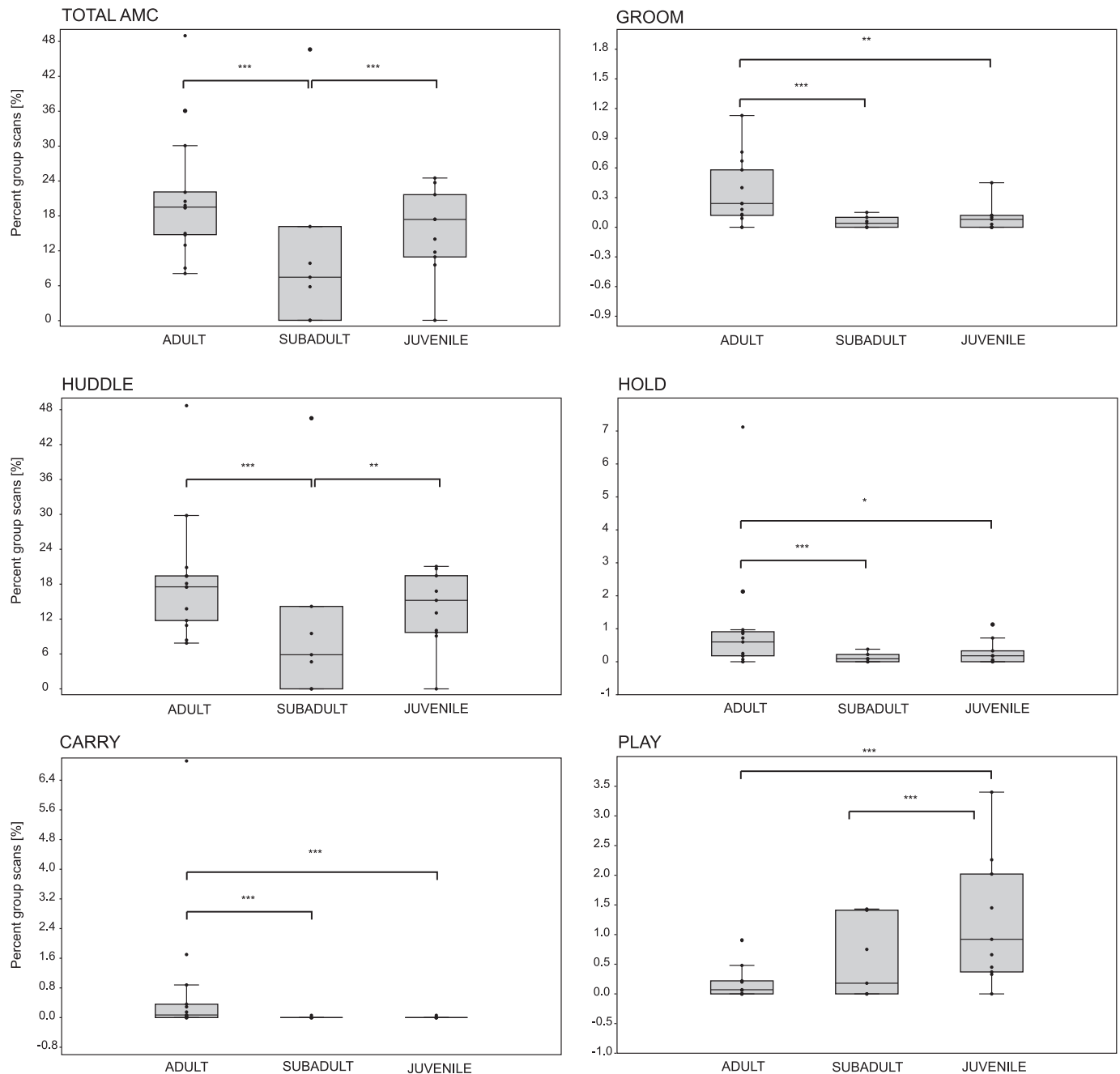
Fig. 3. Comparison of Total AMC and behavioral investment across AMC types by age-sex classes. Based on % group scans. Asterisk indicates significance level. ***p < 0.001, **p < 0.01, *p < 0.05.

to *Total AMC*. However, *Total AMC* in juvenile females was associated with a lower than expected fA response when compared to adult males (Table 2; Fig. 4).

3.3.2. Androgens × AMC Type

To then investigate which type of AMC behavior best predicted fA levels, we ran a second set of models replacing *Total AMC* with each of five *AMC types* (groom, carry, hold, huddle, play) as fixed factors. In this case, the best model included helper age-sex class, and grooming, carrying, and huddling behaviors, $\chi^2(7) = 27.648$, p < 0.001 (Table 2). There were four other models within 2 AICc scores, indicating that these models were equally as good (Table 3). Subadult female helpers had more than a magnitude lower fA values than adult males, $\beta = -1.251$, SE(β) = 0.400, z = 3.117, p = 0.002; juvenile male helpers also had lower fA values than males, though this result

only approached significance, $\beta = -0.406$, SE(β) = 0.211, z = 1.915, p = 0.055. Juvenile female and subadult male helpers did not differ significantly from adult males in their fA levels. The proportion of scans spent grooming and huddling predicted an increase in fA levels, $\beta = 0.176$, SE(β) = 0.066, z = 2.634, p = 0.008 and $\beta = 0.005$, SE(β) = 0.003, z = 2.038, p = 0.042, respectively, while carrying was associated with lower fA levels, $\beta = -0.039$, SE(β) = 0.019, z = 2.033, p = 0.042. We observed an ~18% increase in fA levels for every 1% increase in grooming behavior; a 0.5% increase in fA levels for every 1% increase in huddling behavior; and a ~4% decrease in fA levels for every 1% increase in carrying.

Finally, because our second set of models could not address whether fA responses were specific to a particular age-sex class, we built *AMC type* models for each age-sex class independently (i.e., three separate model sets for adult males, subadult males and females, and juvenile

Table 2

Generalized linear mixed models predicting fecal androgen levels (ng/g) as determined by infant age, helper age/sex class, weekly Total AMC or weekly AMC type, affiliation (% AFF), and the interactions of helper age-sex class with Total AMC and % AFF, respectively.

Model	Fixed factor	Factor level	Estimate	s.e.	t	p-value
Total Weekly AMC	**Age-sex class**	Juv. Female	0.203	0.505	0.401	0.688
	–	**Juv. Male**	**− 0.443**	**0.210**	**2.103**	**0.035**
	–	**Sub. Female**	**− 1.265**	**0.397**	**3.172**	**0.002**
	–	Sub. Male	0.260	0.255	1.015	0.310
	Infant age	–	0.012	0.007	1.686	0.092
	% Total AMC	**–**	**0.006**	**0.003**	**2.019**	**0.044**
	% Affiliation	–	0.000	0.003	0.136	0.892
	Age-sex class * % Total AMC	**Juv. Female**	**− 0.035**	**0.016**	**2.251**	**0.024**
		Juv. Male	− 0.003	0.006	0.430	0.667
		Sub. Female	− 0.007	0.009	0.863	0.388
		Sub. Male	− 0.010	0.009	1.170	0.242
	Age-sex class * % Affiliation	Juv. Female	− 0.009	0.016	0.518	0.604
		Juv. Male	− 0.003	0.006	0.540	0.589
		Sub. Female	− 0.005	0.009	0.507	0.612
		Sub. Male	− 0.001	0.006	0.185	0.853
Weekly AMC Type	**Age-sex class**	Juv. Female	0.070	0.461	0.151	0.880
	–	Juv. Male	− 0.406	0.211	1.915	0.055
	–	**Sub. Female**	**− 1.251**	**0.400**	**3.117**	**0.002**
	–	Sub. Male	0.283	0.254	1.107	0.269
	Infant age	–	0.011	0.007	1.538	0.124
	% Affiliation	–	0.000	0.003	0.016	0.987
	Carry	**–**	**− 0.039**	**0.019**	**2.035**	**0.042**
	Groom	**–**	**0.176**	**0.066**	**2.634**	**0.008**
	Hold	–	0.019	0.017	1.118	0.263
	Huddle	**–**	**0.005**	**0.003**	**2.038**	**0.042**
	Play	–	0.006	0.029	0.212	0.832

Interaction terms are indicated with an asterisk; bold text indicates statistical significance at the level of < 0.05.

males and females, respectively). For adult males, the best model included carry, groom, hold, and huddle. This result matched earlier results from the full *AMC type* models (i.e., those that include all age-sex classes). As before, this model performed significantly better than the null ($x^2(5) = 19.331$, p = 0.002); groom (p = 0.014), huddle (p = 0.014), and carry (p = 0.051) were significant predictors of fA levels in this model (Table 4). Grooming was associated with a 19.2% fA increase, whereas huddling was only associated with a 1% fA increase per 1% increase in this *AMC type*. Carrying was associated with a 4.2% decrease in fA per 1% increase in this behavior. Earlier results did not hold for subadults and juveniles. For subadults, the best model included infant age and helper sex, with only sex as a significant predictor of subadult fA levels. For juveniles, no models performed better than the null model.

4. Discussion

The results of this study demonstrate that primate species with facultative AMC are neuroendocrinologically attuned to the presence of infants. Contrary to expectations, we found that fA levels were positively associated with *Total AMC* in adult males (presumed fathers, hereafter referred to as fathers), demonstrating that elevated fA levels occur during infant care and are unrelated to reinvestment in mating and mate competition in this species. Positive associations between facultative AMC behavior and fA concentrations were also found in ursine colobus monkeys (*Colobus vellerosus*) and savanna baboons (*Papio cynocephalus*) [44,86]. In another study, siamang fA levels declined as proximity and contact with infants increased, suggesting that androgen levels decrease with AMC behavior, though the relationship between the two has not yet been investigated in this species [87]. AMC behavior was most common, and only associated with fA levels, in adult males, suggesting that developmental and experiential factors moderate the relationship between AMC and androgen levels. While carrying was negatively associated with fA levels, grooming and huddling were positively associated with fA levels, supporting the hypothesis that androgens play a role in protective infant care behavior [88,89]. We suspect that facultative AMC in other species will also be associated with changes in androgen concentrations, and we hope that additional
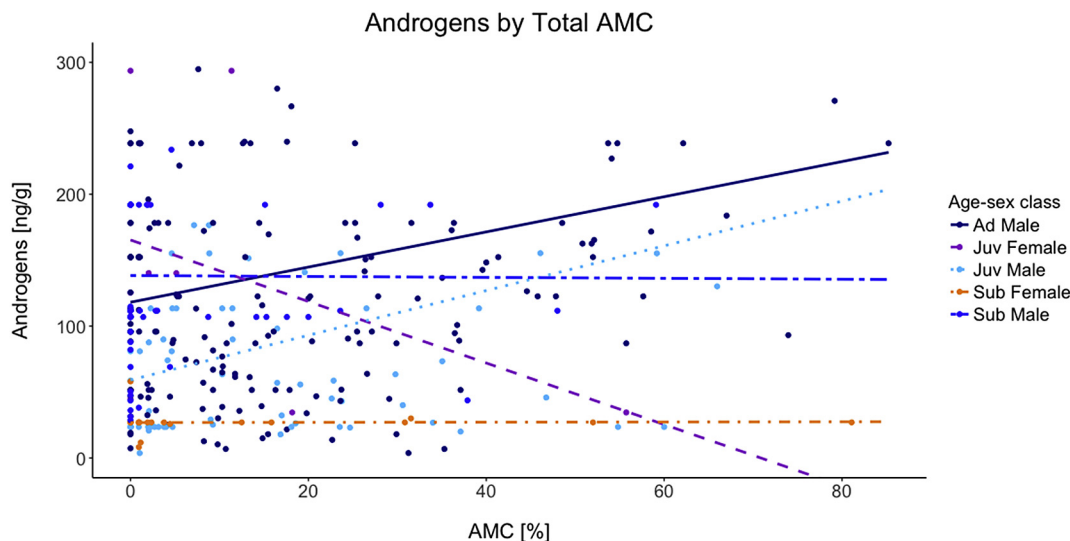
Table 3

Top ten models of fixed effects on log transformed fecal androgen levels as determined by infant age, helper age/sex class, weekly Total AMC or weekly AMC type, affiliation (% AFF), and the interactions of helper age-sex class with Total AMC and % AFF, respectively.

Model	Fixed factors	df	logLik	AICc	ΔAICc	weight
Total AMC Models						
1	**Age-sex + infant age + % Total AMC**	**11**	**− 381.93**	**786.7**	**0.00**	**0.23**
2	**Age-sex + % Total AMC**	**10**	**− 383.22**	**787.13**	**0.44**	**0.18**
3	Age-sex + infant age	10	− 384.05	788.81	2.11	0.08
4	Age-sex + infant age + % Total AMC + age-sex * % Total AMC	15	− 378.63	788.81	2.12	0.08
5	Age-sex + infant age + % AFF + % Total AMC	12	− 381.92	788.85	2.15	0.08
6	Age-sex + % AFF + % AMC	11	− 383.2	789.25	2.55	0.06
7	Age-sex + % Total AMC + age-sex * % AMC	14	− 380.21	789.78	3.08	0.05
8	Age-sex	9	− 385.9	790.37	3.68	0.04
9	Age-sex + infant age + % AFF	11	− 383.85	790.55	3.86	0.03
10	Infant age + % AMC	7	− 388.12	790.59	3.89	0.03
AMC Type Models						
1	**Age-sex + carry + groom + huddle**	**12**	**− 377.74**	**780.49**	**0**	**0.07**
2	**Age-sex + carry + groom + huddle + infant age**	**13**	**− 376.73**	**780.63**	**0.14**	**0.07**
3	**Age-sex + carry + groom + hold + huddle + infant age + % AFF**	**14**	**− 376.12**	**781.59**	**1.1**	**0.04**
4	**Age-sex + carry + groom + hold + huddle**	**13**	**− 377.23**	**781.63**	**1.14**	**0.04**
5	**Age-sex + carry + groom + infant age**	**12**	**− 378.64**	**782.28**	**1.8**	**0.03**
6	Age-sex + carry + groom + huddle + play	13	− 377.67	782.51	2.02	0.03
7	Age-sex + carry + groom + huddle + % AFF	13	− 377.69	782.54	2.06	0.03
8	Age-sex + carry + groom + huddle + play + infant age	14	− 376.71	782.77	2.28	0.02
9	Age-sex + carry + groom + huddle + infant age + % AFF	14	− 376.72	782.8	2.31	0.02
10	Age-sex + groom + huddle + infant age	12	− 378.93	782.87	2.38	0.02

Interaction terms are indicated with an asterisk. All models include Season, Group, and helper identity as random effects. Rows shown in bold should be considered equivalent to the best model (i.e., Δi < 2).

Fig. 4. Relationship between fecal androgen levels (ng/g) and weekly *Total AMC* (%) in each age/sex class. Each point represents a weekly sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Generalized linear mixed models predicting log transformed fecal androgen levels within age- classes as determined by infant age, weekly AMC type, and affiliation (% AFF).

Model	Fixed factor	Factor level	Estimate	s.e.	z	p-value
Weekly AMC Type - Adult males	Infant age	–	0.015	0.010	1.464	0.143
	Carry	–	− **0.042**	**0.021**	**1.953**	**0.051**
	Groom	–	**0.192**	**0.077**	**2.467**	**0.014**
	Hold	–	0.029	0.018	1.629	0.103
	Huddle	–	**0.010**	**0.004**	**2.465**	**0.014**
	Play	–	− 0.030	0.073	0.414	0.679
	% AFF	–	0.003	0.004	0.691	0.490
Weekly AMC Type - Subadults	Infant age	–	0.016	0.008	1.832	0.067
	Sex	**Male**	**1.350**	**0.154**	**8.581**	**< 0.001**
	Carry	–	0.331	0.547	0.594	0.552
	Groom	–	0.077	0.236	0.322	0.747
	Hold	–	− 0.029	0.126	0.222	0.825
	Huddle	–	− 0.001	0.004	0.240	0.810
	Play	–	0.021	0.069	0.304	0.761
	% AFF	–	− 0.002	0.003	0.586	0.558
Weekly AMC Type - Juveniles	(intercept)		4.2496	0.143	29.740	

Bold text indicates statistical significance at the level of < 0.05.

studies of primates will be conducted to help determine what behavioral and physiological mechanisms regulate caregiving throughout the Order.

4.1. AMC behavior in red-bellied lemurs

Overdorff [69] first reported the presence of paternal care in red-bellied lemurs. She found that presumed fathers carried and held infants, and rested and played with infants more than did adult females [69]. Building on this research, we found that all mothers received help caring for their infants, though this help varied to a great extent. All infants received AMC from fathers and siblings, if present. Fathers and subadult males and females contributed AMC in the form of carrying, grooming, holding, huddling, and playing with infants. No observations of food sharing nor allomaternal nursing were recorded, the latter of which would be highly unlikely since this species lives in family groups

with a single breeding pair [70,71].

Red-bellied lemurs provided a substantial amount of AMC to infants, though it was quite variable across individuals and groups. Ross and MacLarnon [19] compiled a list of primate species with AMC, and assigned them to categories based upon the percentage of time that infants were carried and held by helpers. This list provides context for assessing how biologically meaningful AMC is in red-bellied lemurs. A review of the cited literature in [19] indicates that some of these calculations included other care behaviors (e.g., contact) because of inconsistency of methods across studies. Based on the proportion of observations in which infants were carried, held, groomed, and huddled by helpers, red-bellied lemurs rank relatively high for a non-cooperatively breeding primate species ("category 3" out of a maximum of 4 according to [19]; mean 31.71% ± 9.86). According to Lamb and colleagues [90], contact time in adult male lemurs is on par with men in the United States (men: 15–90 min/day, 25–35% as much time as mothers, on average; lemur adult males: 27% as often as mothers, on average). Moreover, lemur infants play more with fathers than with mothers, as do humans [90]. Overdorff [69] found that, once red-bellied lemur fathers began carrying infants, mothers and fathers cared equally for their offspring (as measured in time) until they were approximately 55 days old. From day 55–100, only fathers cared for infants, a pattern similar to carrying behavior in cooperatively breeding golden lion tamarins, *Leontopithecus rosalia* [91].

We did not find any differences in the amount of *Total AMC* provided by male and female helpers, but fathers provided the most AMC, followed by juveniles and then subadults, potentially demonstrating developmental constraints on AMC behavior. Fathers carried, groomed, and held infants more than subadults and juveniles, and huddled infants more than subadults. Infant carrying is an energetically expensive behavior [92,93], it can make travel more difficult [94], and it may put the carrier at greater risk of predation [95]. The smaller body sizes of juveniles and subadults may preclude them from carrying infants. In fact, owl (*Aotus*) and titi (*Callicebus*) monkey fathers are the primary caregivers [96–98], and siblings provide less [99,100] or no infant care at all [56,101].

Adult males themselves varied greatly in how much they carried infants. This was also observed in callitrichines [36,91,102], and may be explained by different degrees of relatedness to the infant. Since red-bellied lemurs are monogamous [68], and we did not observe group takeovers when infants were present or during the period of the study that preceded their birth, relatedness does not likely explain individual variation in paternal carrying. Variation in red-bellied lemurs may be

related to the energetic status of the mother (the need for help), the energetic status of the father (the ability to help), or perhaps the strength of the pair-bond (investment in future reproduction with a pair-mate), which remain to be tested. If huddling, grooming, and holding serve to protect infants (see below), the larger body size [Morelli and Tecot, unpublished data] and prior experience of fathers with infants may be most effective. Juveniles and subadults did hold, groom, and huddle infants, but these bouts sometimes involved other group members. In fact, juveniles huddled with infants significantly more than did subadults, but this result likely stems from the fact that huddling between juveniles and infants often occurred with fathers and/or mothers as well, and could serve to protect the juveniles in addition to the infants. Finally, juveniles played with infants more than fathers and subadults. Juveniles may be more likely to play with infants because they are still quite young and potentially benefit from play themselves, in preparation for adulthood [103]. If no juveniles were present in a group, infants spent less of their observed time playing with a partner (mean percent of observations in which infants played with juvenile females present in group: 5.74%; with juvenile males in group: 3.62%, without juveniles in group: 1.15%), which may have effects on their social and physical development [103,104].

4.2. Androgens and AMC

Fecal androgen levels significantly increased with *Total AMC*, as well as with grooming and huddling, but only in adult males. Because high androgen levels are associated with mate competition [36], infanticide [105], and aggression ([30,106] (see [107]), they are thought to inhibit infant care. Experimental studies have led some to suggest that there is a causal relationship between low androgen levels and paternal care [33]. Castrated Mongolian gerbil adult males spent more time caring for infants, and chose to be with infants more, while testosterone implants diminished paternal care [33]. Our results do not support this interpretation. Indeed, there is disagreement over this assertion [49], and in fact castration did not diminish paternal care in biparental dwarf hamsters [108], and it increased paternal care in the California mouse [48,51]. Others hypothesize instead that lowered androgen levels set the stage to allow paternal care to occur [28], or that they fine-tune it [109], allowing for plasticity in individual responses. As Ketterson and Nolan ([29], S35) write, "it is important to recall that the effect of steroids is not the direct production of behavior; rather, steroids affect the likelihood that behavior will be expressed" [110,111].

Androgens can be positively, negatively, or unrelated to paternal care. One explanation for a positive relationship is that elevated androgen levels actually reflect investment in mating rather than infant care, as would be possible in biparental species with fast life histories and a postpartum estrus [34]. Cotton-top tamarin fathers have elevated testosterone levels 5 days after the birth of their infant, and those whose mates ovulated within 15 days had the largest responses [46]. Because some males can manage paternal care while maintaining high androgen levels, aggressive behavior, and mating behavior (e.g., Mongolian gerbils, *Meriones unguiculatus*, [106]), some have proposed that paternal care coincident with high androgen levels reflects "behavioral insensitivity" to androgens, which may be necessary in species with high paternal investment in infant care during the mating period [112]. Reinvestment in mating and/or insensitivity to androgens cannot explain the elevated fA levels observed in red-bellied lemur helpers. First, red-bellied lemurs are highly seasonal breeders, generally reproducing at the same time each year, once every 1–2 years [72]. Gestation is approximately five months and infants are weaned 2–5 months prior to the next mating season, depending on time of birth [71,72,113]. Red-bellied lemurs are not known to experience a postpartum estrus, so there is no overlap between the mating and infant care periods. They are also rarely ever aggressive [69,70]. Postpartum estrus, though present in moustached tamarins (*Saguinus mystax*), also could not

explain elevated fA levels after the birth of infants in that species [114]. Second, if red-bellied lemurs were insensitive to androgens, we would not observe individual-level associations between androgen levels and infant care.

As a monogamous species with long-term bonds [68,71], it is possible that pair-bonded red-bellied lemurs maintain low androgen levels year-round, which could facilitate AMC behaviors like carrying, and that acute elevations occur with investment in certain AMC behaviors. Most studies finding support for an inverse relationship between paternal care and androgens are correlative, and show that androgen concentrations decline with the transition to paternity (testosterone: [41,42]), during infant care periods (testosterone: [30,35,86], androgens: [87]), and with infant care and interaction (testosterone: [32,58]). Testosterone levels are also lower in pair-bonded men if they are fathers [115], and if they invest more in their children [39,40]. However, these results are not consistent among closely related species: male black tufted-ear marmosets (*Callithrix kuhlii*), but not white-faced marmosets (*Callithrix geoffroyi*), have lower testosterone levels after an infant's birth, if they carry infants more, and during the peak infant carrying season ([32,35,59]; see also [57] for *Callithrix jacchus*). Nor are results consistent within species. Some human studies show no acute or prolonged decline in testosterone after interacting with offspring (e.g., [58,61]; see [116]). While cultural differences in paternal care can explain some of this variation in men [40,115], there is also evidence to suggest that other factors moderate the relationship between androgens and paternal care [117]. For example, men experience an acute reduction in serum testosterone levels in response to interacting with their children, but this response is dependent upon how much contact they have, as mediated by mothers, or how much contact they have had in the recent past [58]. The pattern observed in lemurs might be similar to that observed in men. First, several studies report lower testosterone levels in married or committed men (e.g., [38,118]). Future research is necessary to determine year-round androgen profiles for red-bellied lemur males, but we might predict that they are low and stable in this pair-bonded species. Second, new fathers' testosterone levels have been found to decrease after their child's birth, and increase in response to holding their infant and hearing their cries [21,119]. Our results show that acute elevations in red-bellied lemur fA levels are also associated with specific AMC behaviors.

There is mounting evidence that elevated androgen levels during infant care do not necessarily reflect a trade-off between paternal care and competitive, aggressive behaviors (in fact, aggression can be disassociated with androgens; see [25,107]), though their relationship with sexual behavior is quite clear. On the contrary, elevated androgen levels can be directly associated with paternal care itself, rather than be inhibitory or incompatible with it. As noted above, testosterone levels in men are higher after holding infants [21,119]. In mice, testosterone increases with huddling behavior five days after birth (volcano mice, *Neotomodon alstoni*, [120]), and testosterone implants increase huddling behavior and responsiveness (African striped mice, *Rhabdomys pumilio*, [60]; California mice, *Peromyscus californicus*, [48,51]). In California mice, and perhaps cotton-top tamarins (*Saguinus oedipus*), associations between testosterone and paternal behavior are mediated by the conversion of testosterone into estradiol and the activation of estradiol receptors, which has been found to increase paternal behavior [23,48]. We conducted a preliminary study of fecal estradiol levels in fathers. We found that, during the four weeks prior to the infant's birth, estradiol levels were higher in those who later contributed a substantial amount of AMC, suggesting that helpers respond to the female's gestation and that estradiol levels may be related to AMC in this species [121]. This result should be used with caution however, as our sample size was very small (n = 6 adult males) and we did not investigate estradiol in relation to fA concentrations.

Another possibility is that elevated androgen levels during infant care reflect territorial aggression and/or infant protection. Androgen levels are hypothesized to be elevated in other primate species in

association with protection against infanticidal intruders (ursine colobus monkeys, *Colobus vellerosus*, [44]), even including some species that lack allomaternal care (Verreaux's sifaka, *Propithecus verreauxi*, [37]; red-fronted brown lemurs, *Eulemur rufifrons*, [122]). Red-bellied lemurs have never been observed committing infanticide, but they are territorial. They do not interact aggressively with other groups very often, but males and females monitor and scent-mark their territories, over which they maintain nearly exclusive access [70,71,123]. Marking occurs throughout the year, and males scent-mark more often with their anogenital glands during the food abundance season, which corresponds to the birth season [70]. Scent-marking is accompanied by elevated testosterone in Mongolian gerbils [124], and it could mediate the correlated relationship between fA levels and AMC behavior in red-bellied lemurs. We think it is unlikely that territorial marking alone can explain our results, as grooming and huddling were most strongly associated with fA levels, and androgen levels increased through time with infant development. Additionally, it is thought that territorial species have evolved to maintain relatively low androgen levels [125]. Therefore, higher androgen levels should not be related to territorial aggression in red-bellied lemurs, but this needs to be tested. It is possible that elevated androgens are related to infant protection. While infant mortality is most common when infants are born out of season, all infants are vulnerable to predation and falling from trees [72]. What does infant protection look like in red-bellied lemurs, aside from territorial defense? While it is not something that is obvious in this species, there are behaviors that might be considered protective.

Infant care includes a diversity of behaviors that can include both direct support (e.g., carrying infants) and indirect support (e.g., providing resources for infants; [126]). Direct and indirect support can fall into one of two behaviorally meaningful categories: nurturing and competitive [89,127]. van Anders [89] hypothesized that lower androgen levels are associated with nurturing behaviors, while elevated androgen levels are associated with competitive behaviors, which can be both protective and antagonistic [88]. One example of a competitive infant care behavior is infant defense, where males protect infants while androgen levels are high. This has been observed in both fathers and helpers in a cooperatively breeding cichlid fish, *Neolamprologus pulcher* [128], as well as primate species with facultative AMC (savanna baboon, *Papio cynocephalus* fathers [86]; ursine colobus monkey, *Colobus vellerosus*, adult males [44]; see also [122]). Indeed, carrying was the only behavior that was negatively associated with fA levels in red-bellied lemurs, and testosterone was lowest in male black tufted-ear marmosets (*Callithrix kuhlii*) who carried infants the most [32] (but see [57,59]). The behaviors most strongly (and positively) associated with fA levels in red-bellied lemurs—grooming and huddling—may seem nurturing, but they may also serve a protective role. Adult males allogroom offspring more than females do [70]. Grooming is done with the mouth, using a toothcomb, and the hands. It is an intensely focused behavior, and often includes holding and huddling as well (recorded in this study as grooming). These results support the idea that androgen levels decrease with supportive infant care behaviors such as carrying, and elevate with protective infant care behaviors such as hearing infant cries, huddling, or maintaining proximity, as others have suggested for a variety of species (e.g., [21,86,119,120]).

4.3. Androgen concentrations in non-breeding helpers

There was an effect of age/sex category on the relationship between fA levels and *Total AMC* and grooming behavior. Juvenile female fA levels did not differ from adult males, but caution should be used when extrapolating from this result, as our study only included three juvenile females. Moreover, because females generally have lower testosterone levels than males, androgen assays may be more likely to pick up on cross-reacting metabolites that may be of adrenal, rather than gonadal, origin [129]. Fecal androgen levels were lowest in subadult females and juvenile males, which was to be expected. Of the males, fA levels were

significantly lower in juveniles, with no difference between subadults and adults. This result is similar to what has been found in callitrichines and humans, with male testosterone levels surging in infancy, lowering during juvenility/childhood, and then increasing with the onset of sexual maturity [76,130–132]. Red-bellied lemur offspring disperse to new groups at 2.5–4 years of age [69,71], when they are visibly indistinguishable in body size from adults, and presumably sexually mature. Penile spines, which are known to be sensitive to testosterone [133], begin to resemble that of adults at this stage (Tecot, unpublished data). Juveniles in our study were approximately 12–18 months old, had not yet reached adult body size, and were presumed to be sexually immature. Subadults in our study were > 2 years old, at or approaching adult body size and sexual maturity. They did not occupy adult breeding positions in their groups, and were presumed to be the offspring of the adult bonded pair.

Non-paternal helpers are hypothesized to have been important in our own evolution, providing necessary AMC that might be facilitated by hormonal mechanisms [14]. We know very little of the hormonal changes associated with AMC in non-breeding helpers. Meerkat (*Suricata suricatta*) helpers undergo changes in prolactin and cortisol levels prior to babysitting [134], vole helpers increase pup retrieval and huddling with endogenous oxytocin [135], and cichlid helpers have increased testosterone levels with nest defense and brood care [128]. Here we found that fA levels were not significantly associated with helping behavior from juveniles and subadults. Our sample of juvenile and subadult females was very small, so additional data are necessary to determine the relationship between female helper androgens and AMC behavior. It seems as though androgen levels increase through time, but helping is quite variable between individuals, perhaps because parents regulate their interactions with infants. For example, the majority (94.07%) of huddling by juveniles and subadults with infants also included an adult male or female. Therefore, huddling with infants may have been an unintended consequence of huddling with a parent, which could then explain why there was not a strong association between huddling and fA levels in non-adults. Prior experience with infants can impact the hormonal response to infants or their scent, such that androgen levels have a stronger positive or negative relationship with infant care in adults [23,32,35,136,137], but some studies have found no such relationship [59,119,138]. Experience level may also interact with the age differences we found in red-bellied lemurs, but for this study we were unable to determine whether adult males and subadults older than two years had prior experience with infants. Finally, Schradin and colleagues [139] hypothesize that, in cooperative breeders, the neurobiological circuitry for providing infant care is present during pre-adolescence, deactivated through endocrine regulation during adolescence, and reactivated at sexual maturity. Our findings suggest that this hypothesis should also be tested in species with facultative AMC.

4.4. Limitations

We sampled each group weekly to maximize our sample size while collecting data with sufficient resolution to answer our research questions. However, weekly sampling may underestimate AMC if certain care behaviors are highly context-specific and the need for AMC differs greatly from day-to-day. For example, plant phenology impacts traveling time and path use (Tecot, unpublished data), so daily feeding choices can potentially impact allomaternal carrying. For those interested in conducting similar studies, we recommend rotating focal groups more frequently if possible, depending on the frequency with which certain AMC behaviors occur.

Our noninvasive method of hormone sampling (fecal collection) is beneficial to determine seasonal and long-term changes in androgen concentrations. We focused on androgen changes when infants were present in groups, but sampling outside of this period would allow us to better characterize androgen profiles in this species. Such data will help

us determine whether red-bellied lemurs maintain relatively low levels outside of the infant care period. One limitation of fecal sampling is that it precluded the measurement of immediate, short-term changes, and it also limits our ability to determine how androgens are related to AMC in red-bellied lemurs. We were only able to establish correlated changes in fA levels and AMC behavior and were thus unable to determine 1) whether androgens regulate AMC, AMC impacts hormonal levels, or both; 2) what might mediate this relationship (e.g., conversion to estradiol, [48]); and 3) whether AMC is possible when androgens are inhibited (e.g. as in castration studies, [33]).

What might account for individual variation in AMC behavior and fA levels? Our results indicate that androgens are associated with AMC behavior, but we are unable to answer the question of why some individuals provide little or no care. Storey and colleagues [21] hypothesized that variability in a helper's social environment can explain variability in whether or not changes occur in hormones that can then increase infant care behavior. It is possible that mothers modulate helper-infant relationships [58], and future research should determine who (helpers, infants, and/or mothers) initiates and mediates AMC behavior in order to best associate hormonal changes with helper effort [86]. Additionally, energetic status may impact androgen secretion (see [43,76,116]) as well as activity, and we might expect lemurs with poorer energetic status to be constrained in their ability to elevate androgen levels and provide AMC. Finally, AMC behavior may be inherited, through genetic and non-genetic means, across generations [25,29,140]. Variation in receptor distribution and affinity, enzyme activity, rates of hormone secretion and clearance from the body, and a multitude of other heritable factors can cause the individual variation in androgen levels that we observed. In this study, we collected fecal samples to determine the paternity of infants, but we were unable to genotype all infants. We used presumed relationships based on prior research that reported monogamous mating [68] and our own long-term observations of these groups. However, it is possible that some red-bellied lemur individuals reside in groups with unrelated individuals (Tecot and Baden, unpublished results; Jacobs et al. in review [70]). Definitive data on kinship would aid in interpreting our results, and genetic analyses are now underway.

4.5. Future research

Additional hormones may underlie paternal behavior (see [26]), and helpers appear to respond not only to infants themselves, but also to adult females while they are gestating ([138]; see [27]). We are currently analyzing changes in fecal cortisol and androgen levels that occur throughout gestation to determine whether coordinated hormonal changes occur in gravid females and future helpers ([21,23]; but see [141]), and whether helper hormone levels during a female's gestation can predict helping behavior after the infant's birth. We are also interested in further exploring sex and age differences in androgens detected by EIA. As observed in chimpanzees [129], samples with generally low gonadal androgen levels (e.g., from females) may result in high detection of adrenal androgens, despite these androgens having low cross-reactivity with the assay's antibody. However, red-bellied lemurs are egalitarian [70], so we might predict that their circulating levels of gonadal androgens such as testosterone do not differ, as observed in female-dominant Milne-Edwards' sifaka (*Propithecus edwardsi*, Tecot et al., in preparation; [142]). Following Preis and colleagues [129], we intend to use LC-MS to examine specific androgen metabolites in red-bellied lemur fecal extracts. We hope to collect longitudinal data that will allow us to determine if subadult responses predict their future parental profiles as well [50]. Additionally, it would help us better understand how flexible AMC phenotypes are in red-bellied lemurs. Our two-year study suggests that within-individual variation can be large, with fathers and siblings helping to a great extent with one infant and barely at all with another. For example, the three adult males studied in two consecutive years invested in AMC between 10.5 and

27.2% of mean weekly observations in Year 1, but only 6.2 to 9.9% of mean weekly observations in Year 2. Even more striking was that one juvenile male spent as much as 66.7% of his weekly observations performing AMC (weekly average % AMC, 24.9%), whereas he did not help at all in Year 2. What causes such variation remains unknown.

5. Conclusions

We found that red-bellied lemur helpers contribute a substantial amount of infant care, and that androgen levels in fathers are negatively associated with carrying, a supportive AMC behavior. We also found that androgen levels in fathers are positively associated with *Total AMC*, grooming, and huddling, perhaps because AMC serves a protective role. We lack a broader, year-round context of androgen profiles for this species within which to situate these patterns, but one possible interpretation is that these acute changes occur within a landscape of lowered androgen levels that occurs after pairing with a mate and having offspring.

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