

**Androgens in a female primate: relationships with reproductive status, age,
dominance rank, fetal sex and secondary sexual color**

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ABSTRACT

A comprehensive understanding of the role of androgens in reproduction, behavior and morphology requires the examination of female, as well as male, hormone profiles. However, we know far less about the biological significance of androgens in females than in males. We investigated the relationships between fecal androgen (immunoreactive testosterone) levels and reproductive status, age, dominance rank, fetal sex and a secondary sexual trait (facial color) in semi-free-ranging female mandrills, using samples collected from 19 reproductively mature females over 13 months. Fecal androgens varied with reproductive status, being highest during gestation. Fecal androgens began to increase at 3 months of gestation, and peaked at 5 months. This pattern is more similar to that found in a platyrrhine than in other cercopithecine species, suggesting that such patterns are not necessarily phylogenetically constrained. Fecal androgens did not vary systematically with rank, in contrast to the relationship we have reported for male mandrills, and in line with sex differences in how rank is acquired and maintained. Offspring sex was unrelated to fecal androgens, either prior to conception or during gestation, contrasting with studies of other primate species. Mean facial color was positively related to mean fecal androgens across females, reflecting the same relationship in male mandrills. However, the relationship between color and androgens was negative within females. Future studies of the relationship between female androgens and social behaviour, reproduction and secondary sexual traits will help to elucidate the factors underlying the similarities and differences found between the sexes and among studies.

KEYWORDS

Mandrills, *Mandrillus sphinx*, facial color, sexual ornaments, dominance rank, androgen, fetal sex

INTRODUCTION

Androgens are traditionally viewed as male hormones. The effects of androgens on male behavior, morphology and physiology are well studied, including the relationships with the expression of sexual behavior, stereotypically male behaviours such as aggression and display, and the development of secondary sexual characters (Dixon, 2012). Like males, females produce androgens in the gonads and adrenal glands, and the two sexes share many mechanisms of androgenic action (Staub and de Beer, 1997). Thus, a comprehensive understanding of the role of androgens in reproduction, behavior and morphology requires the examination of female, as well as male, hormone profiles. However, the biological significance of androgens has received far less attention in females than in males.

Reproductive status is probably the best-documented influence on female androgen levels. For example, testosterone is elevated during gestation in mammals (Weizenbaum et al., 1979; Chapman et al., 1998; Gudermuth et al., 1998; Nubbemeyer, 1999), including primates (e.g., Beehner et al., 2005; Fürtbauer et al., 2012). Testosterone also rises at ovulation in many mammal species (e.g., Gudermuth et al., 1998; Nubbemeyer, 1999), suggesting a possible link with sexual behavior and motivation, although this is less well understood (Dixon, 2012). Few studies, however, have investigated patterns of androgens across the conceptive period, gestation and lactation in detail for wild or semi-free-ranging primates (Fürtbauer et al., 2012).

The influence of age on female androgen levels is less well studied. In male primates, testosterone begins to increase at reproductive maturity and matches the age-profile of reproduction (e.g., Beehner et al. 2009). Testosterone decreases with age in women (*Homo sapiens*, e.g., Davison et al., 2005) and female baboons older than 16 years also had lower testosterone levels than younger females (Beehner et al., 2005).

Dominance rank has important implications for female primates, with higher-ranking females enjoying higher reproductive success than lower-ranking females in many studies (review in Pusey, 2013). In male primates, androgens increase in the winner of an aggressive encounter, but decrease in the loser (testosterone: Bernstein et al., 1974; Rose et al., 1975). If the same is true for females, then

higher-ranking females may have higher androgen levels than lower-ranking females. Studies of how testosterone relates to dominance rank among females in group-living primates show mixed findings. Higher-ranking females show higher serum testosterone and androstenedione levels than lower-ranking females in captive talapoin (*Miopithecus talapoin*, Batty et al., 1986), fecal testosterone is higher in dominant than in subordinate hybrid baboons (*Papio hamadryas hamadryas* x *P. h. anubis*, Beehner et al., 2005), and fecal 17b-OH-androgens are higher in dominant than in subordinate Barbary macaques (*Macaca sylvanus*, Grant et al., 2011). However, there is no relationship between rank and fecal testosterone in female yellow baboons (*Papio cynocephalus*, Altmann et al., 1995), between rank and fecal and salivary testosterone in ring-tailed lemurs (*Lemur catta*, von Engelhardt et al., 2000), or between rank and fecal 5a-androstan-17a-ol-3-one in bonobos (*Pan paniscus*, Sannen et al., 2004).

One possible explanation for the differences found across studies in the relationship between female rank and androgens is differences in the exact androgens measured, and in how androgens are metabolised and excreted (Möhle et al., 2002). However, species differences in how rank is attained, and maintained, may also influence the relationship between rank and androgens. Where male primates actively contest their rank, androgens are higher in higher-ranking males than in lower-ranking males (e.g., Beehner et al., 2006; Cavigelli and Pereira, 2000; Sapolsky, 2005; Setchell et al., 2008a). In contrast, where rank is stable, androgen levels often show no consistent relationship with rank (e.g., Barrett et al., 2002; Cavigelli and Pereira, 2000; Gordon et al., 1976). The same may also be true for females. For example, rank is inherited maternally in yellow baboons (Hausfater et al., 1982), where there is no relationship between rank and fecal testosterone, while the hybrid population may have more potential to contest their position, and show is a relationship between rank and fecal testosterone (Beehner et al., 2005). However, ring-tailed lemurs contest their rank position, yet there is no relationship between rank and testosterone, measured either in feces or in urine (von Engelhardt et al., 2000). Thus, there is as yet no comprehensive understanding of the link between dominance rank and androgens in female primates.

Female androgen levels may also relate to the sex of the offspring, both pre- and post-conception. For example, preconception androgens are higher in females that subsequently bear male offspring

than it is in those that subsequently bear females in ibex (*Capra nubiana*, Shargal et al., 2008), field voles (*Microtus agrestis*, Helle et al., 2008), and Barbary macaques (*Macaca sylvanus*, Grant et al., 2011), offering a possible mechanism by which females may influence offspring sex (Grant et al., 2011). Post-conception, gestational androgens are derived from multiple sources, both maternal (ovaries, corpus luteum, and adrenal glands) and fetal (testes and adrenals) (Smith et al., 2013). In rhesus macaques (*Macaca mulatta*) the fetal testes secrete androgens throughout gestation, peaking at days 40-75 (trimester 1-2) then declining, with another increase around day 140 (trimester 3) (Wallen, 2005). In line with this, females carrying male fetuses have higher androgen levels in some species, including humans (*Homo sapiens*, Meulenberg and Hofman, 1991), elephants (*Elephas maximus*, Duer et al., 2002), Assamese macaques (Fürtbauer et al., 2012), and yellow baboons (Altmann et al., 2004), although not in others, such as red-fronted lemurs (*Eulemur fulvus*, Ostner et al., 2003).

Finally, the expression of many secondary sexual traits is related to androgens in males (Andersson, 1994), such that these traits act as 'badges of status' or signals of competitive ability (Rohwer and Ewald, 1981). Androgen-dependent traits may also signal the ability to withstand the costs of high testosterone to potential mates (Folstad and Karter, 1992). Females also possess secondary sexual traits, but these are less understood than those of males (Clutton-Brock, 2007). Female traits may represent a by-product, or correlated response, of selection for ornaments in males, particularly where females are muted by comparison to males (Darwin, 1871; Lande, 1980; Kraaijeveld et al., 2007). However, they may also have adaptive explanations in their own right, such as a role in contest competition or mate choice (Amundsen, 2000; Clutton-Brock, 2007, 2009). Studies of the similarities and differences between hormone profiles and secondary sexual traits in males and females can shed light on the evolution of ornaments. Experimental administration of testosterone increases the expression of sexual ornaments in female birds (e.g., de Ridder et al., 2002; Johns, 1964) and lizards (Rand, 1992; Hews and Moore, 1995). However, such experiments involve androgen levels greater than the levels females naturally experience. Very few studies have examined the relationship between natural variation in androgens and female ornamentation (Jawor et al., 2004; Muck and Goymann, 2011; Moreno et al., 2014), with none, to our knowledge, in female mammals.

In this study, we investigated the relationships between female androgens and reproductive status, dominance rank, fetal sex and secondary sexual color in mandrills (*Mandrillus sphinx*), a large, group-living, primate found in the rainforests of central Africa (Grubb, 1973). We investigated the correlates of fecal measures of immunoreactive testosterone over 13 months in 19 female mandrills living in a large, semi-free ranging group in Gabon. As in other non-invasive studies, it is very likely that our testosterone assay cross-reacts with metabolites of dehydroepiandrosterone, so we discuss our results in terms of female androgens, rather than testosterone specifically. Mandrills in our study population breed moderately seasonally, with 63 % of peri-ovulatory periods occurring between July and September, and only 6 % between December and April (Setchell and Wickings, 2004) and a corresponding birth peak in January to March (Setchell et al., 2002). Females show inherited, stable dominance relationships, and higher-ranking females have a reproductive advantage relative to lower-ranking females, experiencing their first sexual cycles on average 6 months earlier, giving birth for the first time at a younger age and undergoing shorter inter-birth intervals (Setchell and Wickings, 2004; Setchell et al., 2005a). Like male mandrills, females exhibit bright pink and red facial coloration, which varies extensively between females, and across the female reproductive cycle, peaking post-parturition (Setchell et al., 2006). Female coloration is more muted than in adult males, although ranges overlap. Male coloration is linked to fecal testosterone (Setchell et al., 2008a), but this relationship has not yet been investigated in females.

Based on the results of previous studies, we predicted that:

1. Reproductive condition would influence female androgen levels, as in other cercopithecines. Specifically, pregnant females would experience higher fecal androgen levels than either cycling or lactating females.
2. Androgen levels would decrease with age, as female baboons show a decrease in fecal testosterone measures with age (Beehner et al., 2005).
3. Fecal androgens would show no consistent relationship with rank, as rank is inherited and stable in female mandrills.
4. Fecal androgens would be higher in females that subsequently bear male offspring than those that subsequently bear female offspring, as in Barbary macaques (Grant et al., 2011).

5. Fetal sex would be significantly related to maternal androgens in late pregnancy, as in other cercopithecines (Altmann et al., 2004; Fürtbauer et al., 2012).
6. Female facial coloration would be positively related to fecal androgens, if female facial color reflects circulating androgen levels, as it does in male mandrills (Setchell and Dixson, 2001; Setchell et al., 2008).

METHODS

Ethical statement

This research was approved by the Comité Régional d’Ethique Ile de France Sud (the committee responsible for research on animals at the Centre International de Recherches Medicales, Franceville (CIRMF), Gabon, and adhered to Gabonese legal requirements.

Study population and subjects

Mandrills are found in the dense rainforests of Gabon, Congo, mainland Equatorial Guinea and southern Cameroon to the south of the Sanaga river (Grubb, 1973). No study has yet succeeded in habituating mandrills in the wild, but the semi-free-ranging mandrill colony housed at the CIRMF provides an opportunity to study the reproduction and behavior of known individuals under relatively natural conditions. CIRMF established its mandrill colony in 1983/4, by releasing 15 animals (7 males, 8 females) into a 6.5 ha naturally forested enclosure (E1). A second semi-free-ranging group was established in 1994 in a smaller enclosure (E2, 3.5 ha) by transferring 17 mandrills (including 6 adult females and 4 adult males) from the first enclosure. No subsequent additions were made to the colony, other than through breeding, until March 2005, although animals were removed occasionally. The mandrills forage freely and receive daily supplements of monkey chow, fruit and vegetables. Water is always available from a stream.

We conducted this study between February 2004 and March 2005 as part of a larger study of non-invasive endocrinology in mandrills. The 18 study subjects included 16 parous females living in E1 during the study period, and two females that gave birth for the first time in March 2004 (month 2 of

the study), and were aged 4.7 - 26.1 years (**Table 1**). We excluded one parous female who gave birth to an infant that appeared to be one month premature (based on swelling records) and died at two days and who subsequently cycled and showed a pregnancy swelling but never produced an infant, suggesting fetal loss at some stage. The size and age-sex composition of the study groups during the study period is summarized elsewhere (Table 1 in Setchell et al., 2008a) and corresponded to the smaller end of group sizes observed in the wild (Rogers et al., 1996). 19 infants were born into E1 during the study and one infant and one adult male died. The male dominance hierarchy was stable from five months before the beginning of the study until 31 July 2004, when the alpha male died of injuries probably sustained when falling out of a tree. The previously second-ranking male took-over as alpha male for 40 days, before being injured and deposed by a third male on September 20th 2004. The male hierarchy then remained stable until the end of the study (March 2005).

Table 1: Female mandrills at CIRMF included in this study, with age and parity at the beginning of the study (February 2004) and the number of samples contributed by reproductive status

ID ^a	Age	Parity	Dominance rank	Number of samples by reproductive status				Total
				quiescent	cycling	pregnant	lactating	
5	26.1 ^b	14	7	1	32	0	0	33
10	25.1 ^b	15	12	0	10	2	14	26
12	25.1 ^b	18	18	0	5	10	9	24
10E	15.0	6	15	0	1	4	4	9
10F	12.9	6	15	0	1	1	1	3
12A	20.6	14	26	0	0	9	13	22
12C3B	5.1	0	25	10	0	2	18	30
12D3	11.3	5	21	0	2	3	4	9
12O	5.1	1	20	2	1	0	5	9
2D	16.8	10	1	0	1	4	2	7
2D4 (12A8)	8.8	4	28	0	1	3	15	19
2D7	5.2	1	2	0	4	9	13	26
5D	15.9	7	9	0	2	2	3	7
5D3	7.1	2	10	3	5	5	4	17
N (12C4)	9.1	3	23	0	5	9	16	30
P (6B1)	8.9	3	28	10	0	0	17	27
U2	9.0	4	3	0	0	1	4	5
U2A	4.7	1	4	3	2	0	1	6
Total				29	72	64	143	309

^a Individuals with a second ID in parentheses were initially assigned to the incorrect matriline in colony records

^b Founder females, age estimated from dental records

Female age

The age of founder females was estimated using dental estimates when the animals arrived at CIRMF and their previous history (Wickings and Dixson, 1992). The date of birth is recorded for all individuals born into the colony. Female mandrills attain their full adult body mass and length at 7 yr (Setchell et al., 2001); by 20 yr they appear physically elderly, and their rate of reproduction decreases (Setchell et al., 2005a).

Female reproductive status

We noted the reproductive status of females daily as one of:

- cycling (females in any stage of the menstrual cycle, assigned based on the conspicuous perineal swellings females show during the follicular phase, Setchell and Wickings, 2004)
- pregnant (assigned post hoc from the birth of an infant, using the mean gestation length in the colony is 175 days (Setchell et al., 2002) and assigning conception to the day on which the perineal skin began to detumescence in the last sexual swelling cycle).
- lactating (the period following the birth of an infant during which the female has a dependent infant and does not cycle). The mean duration of post-partum amenorrhea following the birth of a live infant in the CIRMF colony is 7 months (Setchell and Wickings, 2004). During this study one female resumed cycling at 5 months; all others did so after ≥ 7 months.
- quiescent (not pregnant or cycling and with no dependent infant).

We noted the sex of the offspring produced following pregnancies (except in one case where we were unable to determine the sex of an infant born in February 2005).

We also subdivided cycling into follicular and luteal phases for some analyses, based on whether the sexual skin was inflating (follicular phase) or deflating (luteal) (Dixson, 2012).

Dominance rank

We noted submissive behavior by study subjects *ad libitum* during twice daily observation periods (approx. 10:00-11:30 h and 15:30-17:30 h). We calculated dominance rank using dyadic interaction matrices based on all interactions where one female avoided or fled when another female approached (Setchell and Wickings, 2004). There were no unresolved relationships and we observed no reversals. Dominance ranks were identical to those previously recorded for the females (Setchell and Wickings, 2004) with the only changes being those expected due to births and deaths. As in previous studies (Setchell et al., 2008b), we classified females as high- (upper-quartile), mid- (inter-quartile range) or low- (lower quartile) ranking, depending upon the proportion of females that they dominated.

Female color

We attempted to measure female facial color every two weeks. We obtained close-up digital images of female faces using a Nikon Coolpix 5700 digital camera with an 8x optical zoom and saved them as 'fine' quality JPEGs. We took all images when females were in either an open grassy area or in an open feeding pen. Images required calibration, to account for exposure and light drift (Gerald et al., 2001). It was impossible either to get animals in the same frame as a photographic white and black standard, or to place a standard in the same position as the animal and capture a second image immediately following that of the subject. Instead, we used only images where color ranged the full spectrum from white (the white ventral pelage) to black (all animals are black around the eyes), and used the 'Autolevels' command in Adobe Photoshop v. 6.0 (Image Mode set to RGB) to define the lightest and darkest pixels in each color channel as white and black. We then analysed images using Adobe Photoshop v. 6.0. We outlined the midnasal strip in a standard fashion using the polygonal lasso tool and measured the mean luminosity and the red intensity value (the number of pixels at each intensity value) for the highlighted area using the 'ImageO histogram' command. We found that the gray score from the red channel, divided by luminosity, produced color measures that correlated best with quantified color chart assessments of the same colors (Setchell et al., 2006a; b). Although we are aware that this method introduces scatter to the data set, there is no reason to believe that it introduces systematic bias.

Fecal sampling

We collected fecal samples when females defecated in the feeding area during morning and afternoon observation periods. We collected samples immediately after defecation and noted the identity of the individual, date, time and consistency of the sample. We homogenized the feces and stored a portion (mean \pm SEM = 6.6 \pm 0.1 g) at -20C in 40 ml of 90 % ethanol until extraction. We did not use diarrheic samples. We attempted to collect three samples per month for each female, achieving a mean of 2.0 \pm 0.1 samples per female per month, a total of 17 \pm 2 samples per female, and an overall total of 309 samples (Table 1).

Steroid extraction and assay methods

We used the same extraction and assay methods we have previously reported for male samples (Setchell et al., 2008a). We homogenized fecal samples in their storage ethanol, vortexed them for 1 minute, and shook them for three hours. We then centrifuged samples for 15 minutes at 2000 rpm, dried a 2 ml aliquot of the supernatant under N₂ at 40 °C and resuspended it in 1 ml EIA Phosphate buffered saline (0.1 M phosphate buffered saline, pH 7.0, with 0.1% bovine serum albumin). We dried the fecal pellet to constant mass at 60 °C to determine the dry mass of the sample. We determined extraction efficiency by measuring recovery of ³H–testosterone (3000 counts per minute) added to ten fecal samples prior to extraction. Mean extraction efficiency was 77.6 % (SEM 2.4 %). We corrected hormone concentrations for this extraction loss and expressed them as ng of hormone per g of dry feces. Mean dry fecal mass was 1.65 \pm 0.03 g.

We measured androgens in fecal extracts using microtitreplate enzyme immunoassay (EIA), using an antiserum and labeled testosterone conjugate (horseradish peroxidase: HRP) provided by Coralie Munro (University of California, Davis). **The antibody crossreacts with 5 α -dihydrotestosterone 57.37%, and less than 1 % with other steroids (Walker, 1999).** We refer to the results as fecal androgens. We validated the assay immunologically by demonstrating parallelism, accuracy, and sensitivity, and biologically by comparing fIT values with serum free testosterone, and comparing young males with adult male conspecifics with results that suggest that the assay may reasonably be regarded as indicative of testicular androgen secretion in mandrills (Setchell et al., 2008a).

We diluted fecal extracts 1:6 in assay buffer (0.1 M phosphate buffered saline, pH 7.0, with 0.1% bovine serum albumin) and assayed 50 µl aliquots along with 50 µl aliquots of reference standard in doubling dilutions (range 1.95-1000 pg/well). We re-ran samples if duplicates had coefficients of variation greater than 5 % and re-diluted and re-assayed samples binding >90 % or <20 %. The intra-assay coefficient of variation was 2.19 %. Inter-assay variation for pooled quality controls was 10.4 % for the high quality control, and 16.3 % for the low quality control.

Statistical analysis

We normalized fecal androgen levels via log transformation. We used a general linear mixed model (GLMM) to assess the effects of predictor variables on fIT levels. We included female identity as a random factor, to account for the fact that we sampled the same individuals repeatedly, and tested for main effects of the following predictor variables:

- age (covariate)
- reproductive status (categorical variable: cycling, pregnant, lactating, quiescent, see above for definitions). We matched fecal samples with the reproductive condition of a female on the previous day, to allow for the time lag to peak steroid excretion, based on excretion data for other non-human primates (Bahr et al., 2000).
- dominance rank (covariate)

We also included whether samples were collected during morning or afternoon observation periods as a categorical variable, to detect any influence of circadian rhythms (Perachio et al., 1977; Beattie and Bullock, 1978; Plant, 1981). We have previously shown that the number of months for which samples were stored prior to extraction does not influence fecal androgens significantly (Setchell et al., 2008a).

To explore the effects of reproductive status on fecal androgen levels further we divided gestation and lactation into 6 and 7 monthly intervals, respectively, with the day of parturition termed day 0, to examine changes in fecal androgens across the reproductive cycle in more detail.

We did not investigate the influence of seasonality on fecal androgen levels because this was confounded with the seasonality of reproduction in the colony (Setchell and Wickings, 2004). The

daily provisioning of the colony should compensate for any influence of seasonality in calorie intake, and the monkey chow does not vary across the year, although the types of fruits and vegetables provided do vary and seasonal cues may also be provided by changes in daylength (albeit small in equatorial Africa), temperature, humidity, or rainfall.

To determine whether androgen levels predict fetal sex we compared fecal androgens in mothers of daughters and sons during (i) the follicular phase of the conceptive cycle using a Mann-Whitney exact test, given the small sample size, and (ii) during the two months prior to conception using a GLMM with female identity as a random effect, androgen level as the covariate and fetal sex as the dependent variable and a binomial error structure (cf. Grant et al., 2011). As the sample sizes were small, we also compared the mean value for females bearing a male and a female offspring during the two months prior to conception using an exact Mann-Whitney test.

To examine the relationship between maternal fecal androgens during pregnancy and fetal sex we used a GLMM with female identity as a random effect and included pregnancy trimester (days 1-58, 59-117 and 118-175) to account for changes in fecal androgens across gestation.

Finally, to examine the relationship between facial color and fecal androgens, we assigned the closest fecal androgen value to each color value, using a 1 day excretion lag (as above) and only values obtained within 15 days (mean \pm SEM timelag = -0.8 \pm 0.3 days, n = 370). We examined the relationship between fecal androgen levels and red color in two ways: first by comparing mean values of red color and fecal androgens for each female using a Pearson's correlation, then using a GLMM with red coloration as the dependent variable, female identity as a random factor, and fecal androgens as a covariate.

Where we had a directional prediction concerning the relationships between fecal androgens and fetal sex we used a one-tailed tests, and state this in the results. Otherwise we used two-tailed tests. We set the statistical threshold set at $P = 0.05$ and conducted all tests using SPSS version 20.0. Figures present means and SEMs calculated directly from the logged fecal androgen data.

RESULTS

Whether a sample was collected during the morning or afternoon observation sessions did not predict fecal androgen levels in our study subjects (**Table 2**). Reproductive status was significantly related to fecal androgen levels (**Table 2**). Post-hoc comparisons showed that fecal androgen levels were significantly higher in pregnant females than in females at any other stage (**Table 3, Fig. 1**). Cycling females also showed higher fecal androgen levels than those who were pregnant or lactating. There was no significant difference in fecal androgen level between quiescent and either cycling or lactating females. Closer inspection of the data revealed that fecal androgens rose from low levels in early pregnancy, and peaked two months before parturition (**Fig. 2**).

Table 2 Results of a GLMM assessing the effects of age, whether samples were collected during morning or afternoon observation periods, reproductive status and dominance rank on log₁₀T levels in female mandrills

Predictor	Numerator df	Denominator df	F	P
Intercept	1	17.73	303.56	<0.001
Observation period	1	293.64	1.86	0.174
Reproductive status	3	299.49	51.30	<0.001
Age	1	19.35	1.11	0.306
Dominance rank	1	18.16	1.68	0.211

Table 3: Results of Least Significant Difference pairwise comparisons comparing fecal androgens in female mandrills in different reproductive states

	mean +/- SE difference (p)		
	Cycling	Pregnant	Lactating
Quiescent	-0.050 +/- 0.076 (p = 0.513)	-0.457 +/- 0.072 (p < 0.001)	0.083 +/- 0.062 (p = 0.184)
Cycling		-0.407 +/- 0.056 (p < 0.001)	0.133 +/- 0.052 (p = 0.011)
Pregnant			0.540 +/- 0.044 (p < 0.001)

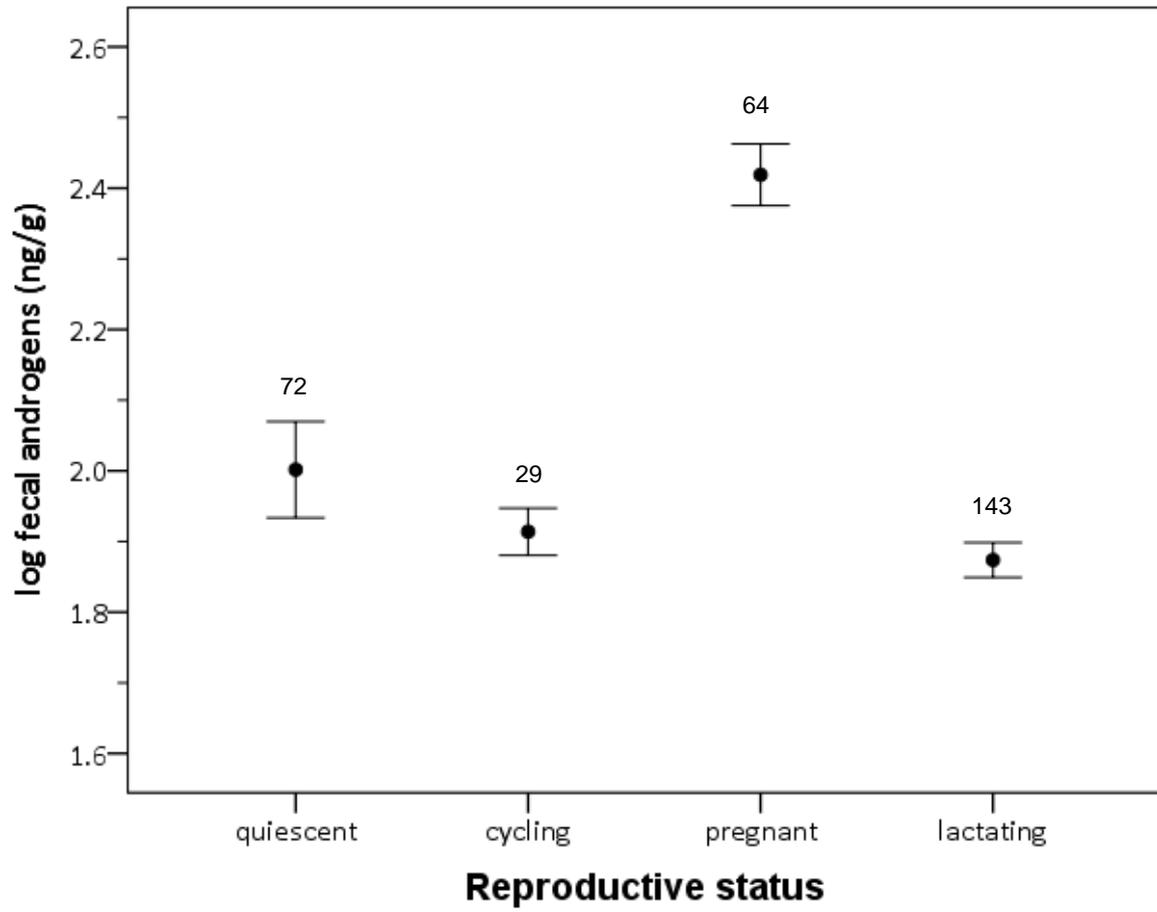
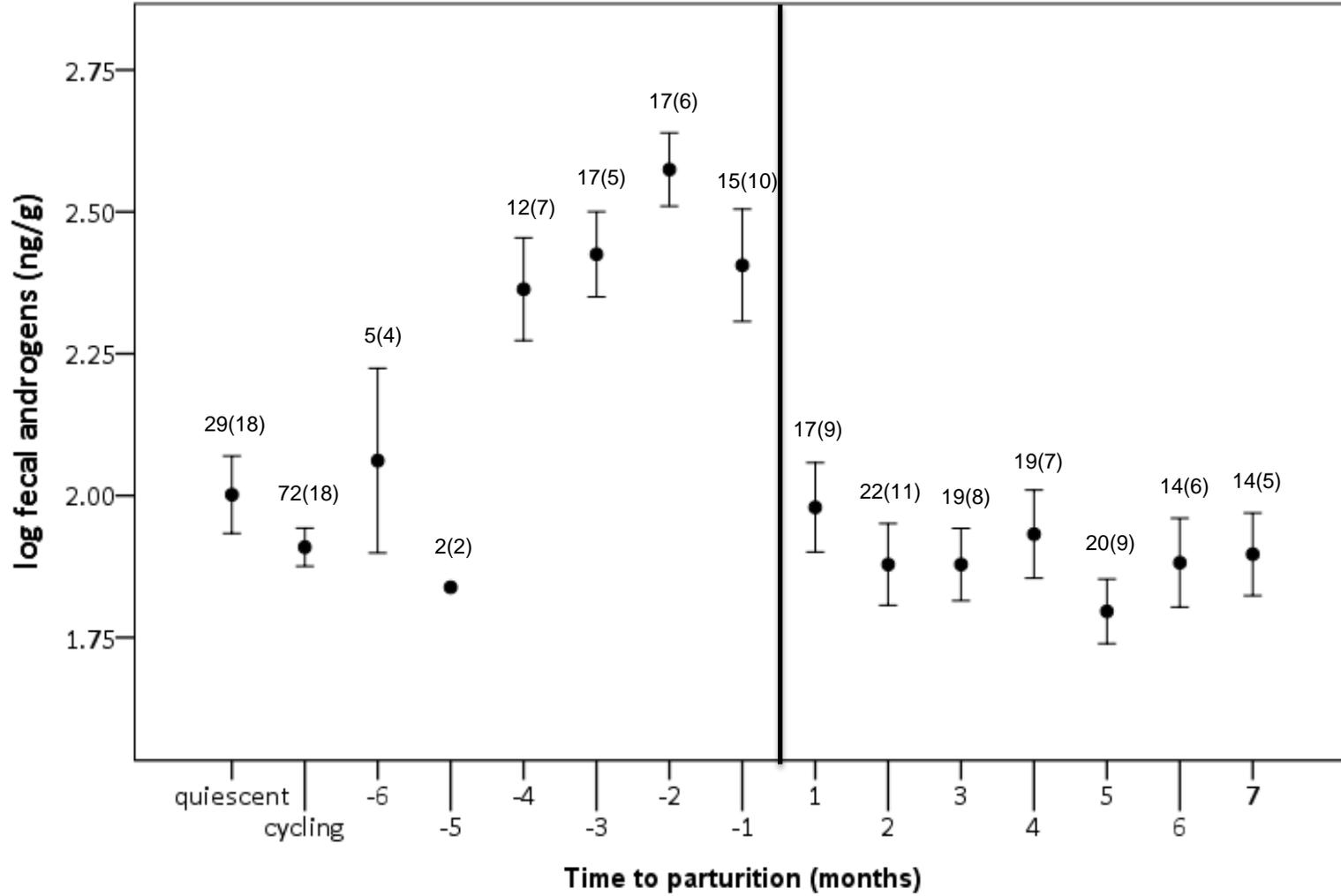


Fig. 1: Mean +/- SEM fecal androgens in 18 female mandrills by reproductive status. Numbers above bars indicate sample size.



1
2
3
4 **Fig. 2: Mean +/- SEM fecal androgens across the reproductive cycle in 18 female mandrills. Vertical line indicates parturition. Numbers above bars**
5 **indicate sample size, with number of females contributing in parentheses.**

Neither female age nor dominance rank significantly predicted fecal androgen levels (Table 2).

We found no significant difference in maternal fecal androgens in follicular phase samples for females that went on to conceive a male ($n = 4$) or female ($n = 4$) offspring (Mann-Whitney $U = 13.0$, exact $p = 0.200$). Similarly we found no relationship between fetal sex and maternal fecal androgens when we examined all samples collected during the two months prior to conception (13 female offspring, 5 mothers, 17 male offspring, 7 mothers, GLMM $F_{1,28} = 0.786$, one-tailed $p = 0.192$; using mean values for females bearing female and male offspring Mann-Whitney $U = 22.0$, exact $p = 0.530$). We found no significant relationship between fecal androgens and fetal sex during pregnancy ($F_{2, 48} = 0.206$, $p = 0.815$). We had very few samples for trimester 1 (Table 4).

Table 4: Number of samples available for the three trimesters of pregnancy

Trimester	Number of samples available (number of females contributing)	
	Female offspring	Male offspring
1	4 (3)	3 (2)
2*	7 (4)	22 (6)
3*	10 (6)	20 (7)

* one female contributed one male and one female offspring

Finally, we found a significant, positive relationship between female red color and fecal androgens when we compared the mean of each across females ($n = 18$, $r = 0.501$, $p = 0.034$, Fig 3). However, using all the data available, with red coloration as the dependent variable and female identity as a random factor revealed a negative relationship between red and fecal androgens ($\beta \pm SE = -0.94 \pm 0.018$, $t_{367.82} = -5.09$, $p < 0.001$).

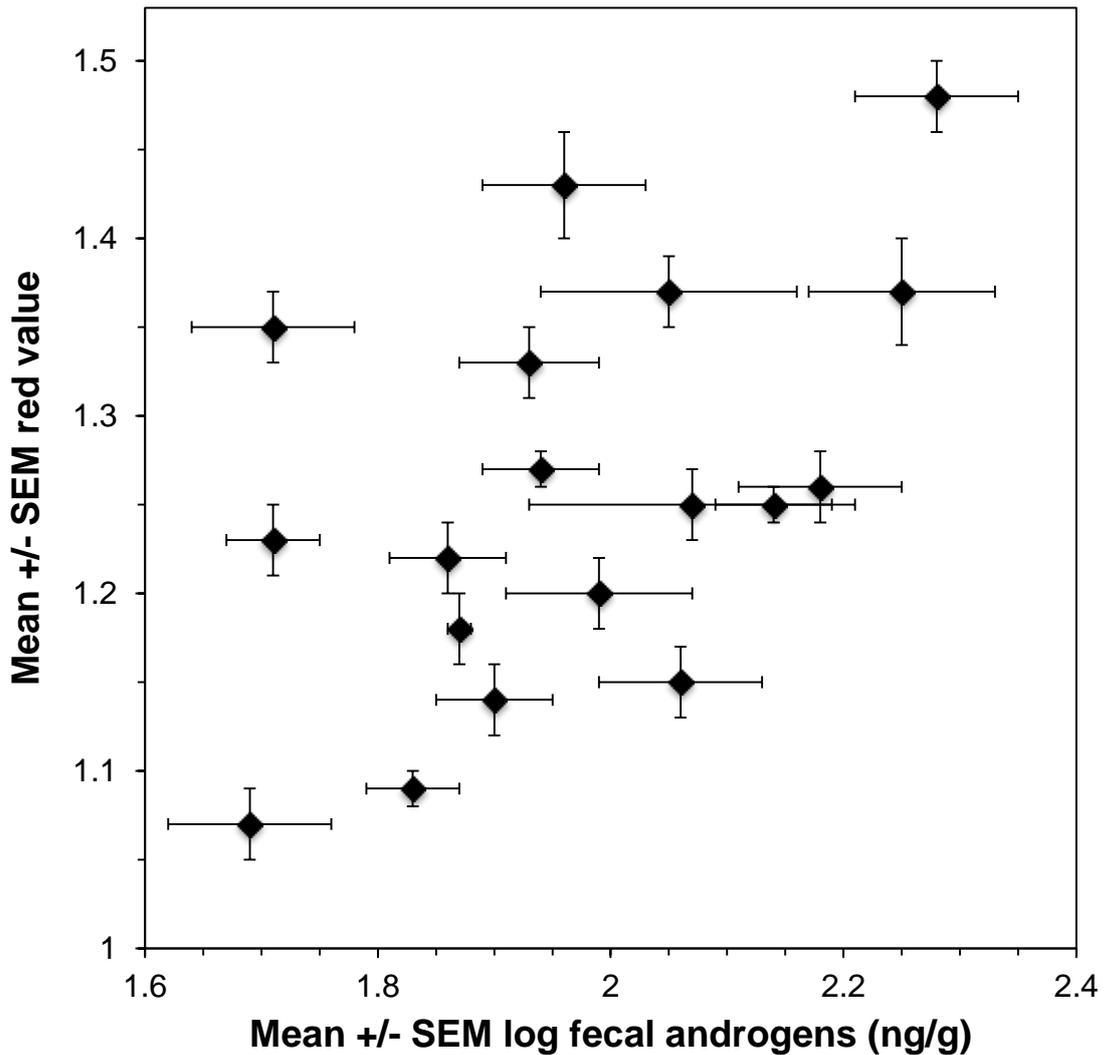


Figure 3: Relationship between fecal androgens and facial red coloration in 18 female mandrills

DISCUSSION

We found that female fecal androgen levels varied with reproductive status, as predicted. However, the specific patterns we found across gestation differed from those reported for other cercopithecine species. Fecal androgens were unrelated to age, rank or the sex of an offspring, either prior to conception or during gestation. Our analyses revealed a complex relationship between androgens and female secondary sexual coloration. Androgen metabolism in primates is highly variable between species and no one method can be used across species for the assessment of fecal androgens

(Möhle et al., 2002), complicating comparisons among studies. Nevertheless, these findings are both similar to, and differ from, those for male mandrills, and for females of other species.

Fecal androgens and female reproductive status

The overall pattern of fecal androgens across female reproductive status in mandrills was similar to that found in hybrid baboons (Beehner et al., 2005), being highest during gestation, although we also found that cycling females had higher fecal androgens than lactating females, a pattern not found in the baboons (Beehner et al., 2005). During gestation, fecal androgens began to increase in the third month of gestation (4 months prior to parturition, in trimester 2), and peaked at 5 months (2 months before parturition, in trimester 3). This contrasts with patterns in other cercopithecines, where the peak in androgens occurs earlier. For example, fecal androgens rise in week 2 of gestation, peak at week 4, and decline to pre-conception levels in weeks 7-9 in Assamese macaques (Fürtbauer et al., 2012). In baboons, fecal testosterone rise significantly on day 21 of pregnancy, peak around week 5-6, drop back to early pregnancy levels for weeks 8-10, then rise again to a plateau lower than the initial peak (Gesquiere et al., 2014). Similar patterns are seen in other primate species (review in Smith et al., 2013). Although we had relatively few samples for early pregnancy, and it is possible that we missed a transient, early peak in fecal androgens, we found no indication of this in our data. We also estimated the onset of gestation from patterns of sexual swelling, rather than hormonally, which might result in small errors in the date of conception. However, such errors are also unlikely to explain the difference between mandrills and other species.

Patterns of gestational fecal androgens also differed from those in other cercopithecines later in gestation, when we had more samples, and can be more confident in our findings. Fecal androgen levels were high in trimesters 2 and 3 (the 4 months prior to parturition), and did not decrease to pre-conception levels until parturition. This pattern differs from that reported for Assamese macaques, where androgen levels during trimesters 2 and 3 were similar to pre-conception levels (Fürtbauer et al., 2012). Fecal androgens during the last 2 months of gestation were markedly higher than any lactation values in mandrills, a pattern which contrasts with that found in peripartum baboons, in which androgen levels during the last 8 weeks of gestation were similar to those during lactation (Altmann et al., 2004). The overall pattern of fecal androgens across gestation in mandrills is more similar that

reported for a platyrrhine, the white-faced marmoset (*Callithrix geoffroyi*), in which urinary androgen levels rose significantly during the first trimester, peaked in the middle of the second trimester, then declined gradually to parturition (French et al., 2010), although the mandrill peak occurs slightly later during gestation. Overall, these results suggest that patterns of gestational androgen levels are variable across species, and are not necessarily constrained by phylogeny, as mandrill gestational androgen patterns are more similar to marmosets than to macaques and baboons.

Mandrills at CIRMF tend to conceive during the dry season, and gestation continues into the wet season, with births occurring in the wet season (Setchell et al., 2002). Any effect of ecological seasonality on fecal androgens might therefore confound the influence of reproductive status on fecal androgens. For example, fecal testosterone levels are higher during the wet season in hybrid baboons, when contest competition also increases (Beehner et al., 2005). However, as in the study of Assamese macaques, inspection of the data for individual female mandrills showed the same general patterns, suggesting that gestational stage, rather than season, underlies the patterns observed (Fürtbauer et al., 2012).

Fecal androgens, age and dominance rank

Outside the human medical literature, very little known is about the relationship between hormones and aging or senescence. Our study subjects included females aged 4.7-26.1 yr. Females at the top end of this range are clearly elderly, and the limited data available suggest a decrease in reproductive output in elderly females (Setchell et al., 2005a). However, we found no relationship between age and female androgens. This contrasts with findings for female baboons, where, females older than 16 years had significantly lower fecal testosterone levels compared to other females (Beehner et al., 2005). Adult male baboons also show age-related decreases in testosterone (e.g., Beehner et al. 2009).

The lack of a relationship between fecal androgens and rank in female mandrills contrasts with findings for males, where fecal androgen levels are significantly positively related to dominance rank (Setchell et al., 2008a). This sex difference is likely to relate to contrasts in the way in which rank is acquired in the two sexes. Aggressive encounters increase testosterone in the winner, but decrease it

in the loser (Bernstein et al., 1974; Rose et al., 1975). This is likely to explain the relationship between rank and fecal testosterone in males, which contest their rank position physically, and appear to live in a permanently aggressive context (Setchell et al., 2008a). Male rank can change dramatically from one day to the next, as a result of fights, and males can suffer serious injury or death (Setchell et al., 2006b). In contrast, serious dyadic conflict is rare in female mandrills (we have observed it only once, when two groups came into contact when a wall fell down). Females in the CIRMF colony inherit their mothers' rank in classical youngest ascendancy fashion and the hierarchy has been stable across generations, with the only changes being due to births, deaths or removal of individuals (Setchell et al., 2008b). This stability suggests that females do not experience the effects of winning or losing aggressive encounters on androgen levels, which therefore remain undifferentiated with respect to rank.

Our results for the relationship between fecal androgens and rank in female mandrills are similar to those reported for female yellow baboons (fecal testosterone, Altmann et al., 1995), ring-tailed lemurs (fecal and salivary testosterone, von Engelhardt et al., 2000), and bonobos (fecal 5 α -androstano-17 α -ol-3-one, Sannen et al., 2004), in which rank is also not related to androgens. However, our findings differ from those for captive talapoin (serum testosterone, Batty et al., 1986), hybrid baboons (fecal testosterone, Beehner et al., 2005), and Barbary macaques (fecal 17 β -OH-androgens, Grant et al., 2011), in which dominant females show higher androgen levels than subordinates. As we note in the introduction, these differences in the relationship between androgens and rank in females may relate to how females acquire their rank, although this does not explain all the findings documented. Findings may also differ according to the stability of the hierarchy at the time of the study. For example, it is possible that rank is particularly stable in our study population, where inter-birth intervals are short and mortality is low, conditions conducive to rank stability (Datta and Beauchamp, 1991). This may not be the case in wild primate groups, where inter-birth intervals are longer and survival is lower, and the hierarchy may be less stable, even where it is inherited.

An alternative, but not mutually exclusive, explanation for differences between species lies in variation in the importance of aggressive competition for rank, or dominance style. More 'despotic' species, where social interactions are asymmetrical and dominance hierarchies are steep, may show a

stronger relationship between rank and androgens than 'egalitarian' species, where interactions are more symmetrical and hierarchies less steep. However, Barbary macaques are rated as 'egalitarian' in dominance style (Thierry, 2000), yet females show rank-related differences in fecal androgens (Grant et al., 2011), arguing against this hypothesis. Studies of dominance style have concentrated mainly on the macaques (Thierry, 2000; Balasubramaniam et al., 2012), and as yet we have no detailed data on dominance style in female mandrills to determine where they fall on the spectrum.

Maternal fecal androgens and fetal sex

We found no significant relationship between preconception maternal fecal androgens and fetal sex in mandrills. However, this finding should be viewed as preliminary, as we obtained only a small number of fecal samples during the preconception period, and may have missed any preconception peaks in androgens. A study of Barbary macaques found that offspring sex is related to pre-conceptive fecal androgens, measured both 2-3 weeks prior to mating in three zoo-housed females (similar in nature and sample size to our follicular phase comparison) and 1-2 months prior to conception in 9 females who produced 13 offspring in a group of provisioned macaques (similar to our 2 month prior to conception comparison) (Grant et al., 2011). High-ranking females in the Barbary macaque study also had higher androgens than low-ranking females, providing a possible mechanism by which females are able to adjust the sex of their offspring prior to conception based on the social environment (Grant et al., 2011), and supporting both the Trivers-Willard maternal condition hypothesis (Trivers and Willard, 1973), and the maternal dominance hypothesis (Grant, 2007) of sex allocation.

Mandrills are extremely sexually dimorphic (Setchell et al., 2001), with very high reproductive skew among males (Charpentier et al., 2005; Setchell et al., 2005b), both of which suggest that a mechanism that links female dominance to offspring sex would be advantageous, as high-ranking females produce larger offspring (Setchell et al., 2001). As large size is more beneficial for males than for females, sex ratio theory (Trivers and Willard, 1973) predicts that females in good condition (i.e., high-ranking females) should produce more male offspring than those in poor condition (low-ranking females). However, birth sex ratios are not related to maternal dominance rank in either our study population of mandrills (Setchell et al., 2002), nor in primates in general (Brown and Silk, 2002). Thus, while our analyses of preconception fecal androgens in mothers and offspring sex are based on a

small sample size, in combination with a lack of a relationship between female androgens and rank (this study), or between female rank and offspring sex (Setchell et al., 2002), they suggest that the maternal dominance hypothesis (Grant, 2007) does not apply in this species.

Our sample of mandrills in the first trimester of gestation is very small, making it difficult to draw conclusions about the influence of offspring sex during early pregnancy. However, we obtained more samples during trimesters 2 and 3, but found no relationship between maternal fecal androgens and fetal sex. A recent review noted inconsistency about whether fetally-derived androgens contribute to elevated maternal androgens in primates (Smith et al., 2013). Two studies report that female non-human primates carrying a male fetus show higher androgen levels while others do not. A study of a small number of red-fronted lemurs also found no link between maternal androgens (fecal testosterone-3-(carboxymethyl)oxime-BSA) and fetal sex (Ostner et al., 2003). Similarly, there were no differences between gestation levels of urinary testosterone in male-biased and female-biased litters in white-faced marmosets, nor were levels of maternal androgens associated with the number of males in the litter, or with the proportion of the litter that was male (French et al., 2010). However, other studies report relationships between female androgens and fetal sex. There is a sex difference in maternal androgens in trimester 3, but not in trimesters 1 and 2, in Assamese macaques (fecal epiandrosterone, Fürtbauer et al., 2012), during the 3rd trimester in yellow baboons (fecal testosterone, Altmann et al., 2004), in late pregnancy in ring-tailed lemurs (serum androgens, Drea, 2011), and in trimesters 2 and 3, but not trimester 1, in elephants (serum testosterone, Duer et al., 2002). It is not clear why species differ in the relationship between fetal sex and maternal androgens, but it may be that high levels of maternal androgens overwhelm the contribution of fetal androgens in mandrills, as they maintain high levels of fecal androgens in trimesters 2 and 3 regardless of fetal sex. It is also possible that assay variation obscures small effects of fetal sex in our study.

Female fecal androgens and facial red coloration

Our most intriguing results concern the relationship between female facial color and fecal androgens. The overall positive relationship between mean color and mean fecal androgens reflects that found in males (Setchell et al., 2008a). Experimental administration of both testosterone and estrogen also increases red skin color in male rhesus macaques (*Macaca mulatta*, Vandenberg, 1965), due to

increased epidermal blood flow (Rhodes et al., 1997). However, when we accounted for female identity, we found a negative relationship between facial color and androgens in female mandrills. Both variables are also related to reproductive status, although the patterns differ: androgens peak in the second half of pregnancy, but are low post-parturition (this study), while color peaks post-parturition during lactation (Setchell et al., 2006a). It is possible that the negative relationship we detected between facial color and androgens within individuals is due to a time-lagged positive relationship between hormone levels and color due to changes in hormone receptors in the skin, where testosterone is aromatised to estrogen (Rhodes et al., 1997). However, this hypothesis is not supported by temporal relationships between male color and testosterone, where current testosterone predicts color during the current month, but not during future months (Setchell et al., 2008a).

The evolution of female secondary sexual traits is far less understood than that of males, particularly where females possess traits similar to those of males, like facial coloration in mandrills (Setchell et al., 2006a). Without strong selection pressure against the expression of traits in females, then females will share these traits with males because males and females share most of their genome (Kraaijeveld et al., 2007; Lande, 1980). However, females cannot be assumed to be a control condition for males, and hormone-linked color may also play an adaptive role in females (Amundsen, 2000). Our findings that facial color in female mandrills is linked to androgens contribute to the small literature on the relationship between naturally occurring variation in female androgens and female secondary sexual traits. Studies also report positive correlations between circulating testosterone levels and female ornaments in birds. Testosterone is positively linked to plumage coloration in female Northern cardinals (*Cardinalis cardinalis*, Jawor et al., 2004), the size and color of the throat patch in females of the polyandrous, sex-role reversed barred button quail (*Turnix suscitator*, Muck and Goymann, 2011) and the size of the white wing patch but not the presence of a forehead patch during the incubation phase in female pied flycatchers (*Ficedula hypoleuca*, Moreno et al., 2014). The proximate and ultimate influences on variation in female color in mandrills merit further investigation. Selection pressures on male and female mandrills differ greatly, with females investing far more in gestation, lactation and infant care, while males invest in competition for access to receptive females (Trivers, 1972).

Conclusions and future directions

Our findings contribute to a more comprehensive view of androgens by improving our understanding of the relationship between female androgens and reproductive status, rank, and fetal sex. They reveal differences with studies of male mandrills. Fecal androgens are related to dominance rank in males, but not in females. Comparisons across species suggest that the relationship between androgens and rank in female primates deserves more detailed attention, including detailed studies of aggression and submission within and across species to elucidate the factors that underlie the patterns observed. Intriguingly, patterns of androgens across gestation in mandrills are more similar to those in a platyrrhine than those in other cercopithecines, suggesting that such patterns are not necessarily phylogenetically constrained. Further data, for additional species, will shed light on the correlates of these patterns. We did not detect any relationships between fetal sex and female androgens, either pre-conception or during gestation. Again, additional studies will help to clarify the question of how and why species vary in these relationships. Sample collection directed specifically at the peri-conceptual phase and early gestation would improve sample sizes in future studies, although these phases are identified much more easily post-hoc than during sample collection. We detected intriguing patterns in the relationship between female androgens and facial red color, which merit further attention. Other possibilities for the future include comparison of female fecal androgens with other endocrine factors, such as glucocorticoids (Setchell et al., 2008b), and with physical condition, and examination of the relationship between maternal androgen levels and morphological, physiological, and behavioural development in offspring. As examples of the latter relationship, endogenous variation in maternal gestational androgens affects offspring development in spotted hyenas (*Crocuta crocuta*, Dloniak et al., 2006); and white-faced marmosets (Birnie et al., 2012; Smith et al., 2010) suggesting a role for female androgens in adaptive environmental signaling from mother to offspring via effects of the maternal phenotype on offspring phenotype.

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ELECTRONIC SUPPLEMENTARY INFORMATION

Summary of fecal androgen levels (unlogged data) in 18 female mandrills, split by reproductive status

Female	Mean +/- SEM (n) fecal androgen levels in ng/g			
	cycling	lactating	pregnant	quiescent
10	120.7 +/- 24.2 (10)	79.4 +/- 10.5 (14)	313.9 +/- 75.2 (2)	(0)
10E	174.0 (1)	53.8 +/- 9.7 (1)	137.6 +/- 27.3 (4)	(0)
10F	45.1 (1)	54.7 (1)	87.1 (1)	(0)
12	187.7 +/- 26.3 (5)	146.3 +/- 21.0 (9)	527.2 +/- 108.7 (10)	(0)
12A	(0)	97.3 +/- 16.6 (13)	429.3 +/- 76.9 (9)	(0)
12C3B	(0)	153.6 +/- 24.3 (18)	655.9 +/- 168.3 (2)	169.2 +/- 39.2 (10)
12D3	81.1 +/- 48.4 (2)	44.3 +/- 6.6 (4)	593.9 +/- 168.1 (3)	(0)
12O	105.0 (1)	121.1 +/- 50.0 (5)	68.9 (1)	35.2 +/- 3.4 (2)
2D	49.7 (1)	55.8 +/- 17.6 (2)	271.5 +/- 91.4 (4)	(0)
2D4	48.4 (1)	68.6 +/- 15.2 (15)	353.5 +/- 74.1 (3)	(0)
2D7	136.0 +/- 9.9 (4)	149.5 +/- 54.3 (13)	370.5 +/- 82.2 (9)	(0)
5	84.4 +/- 9.9 (32)	(0)	(0)	56.2 (1)
5D	131.2 +/- 24.9 (2)	114.7 +/- 44.9 (3)	210.9 +/- 5.5 (2)	(0)
5D3	77.8 +/- 13.9 (5)	73.9 +/- 30.2 (4)	91.5 +/- 11.9 (5)	32.1 +/- 2.1 (3)
N	61.6 +/- 6.3 (5)	67.0 +/- 9.1 (16)	208.4 +/- 54.7 (9)	(0)
P	(0)	79.0 +/- 10.8 (17)	(0)	149.0 +/- 25.9 (10)
U2	(0)	44.0 +/- 6.0 (4)	216.7 (1)	(0)
U2A	69.7 +/- 0.7 (2)	80.0 (1)	(0)	74.0 +/- 2.7 (3)