

10

ABSTRACT

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12A comprehensive understanding of the role of androgens in reproduction, behavior and morphology
13requires the examination of female, as well as male, hormone profiles. However, we know far less
14about the biological significance of androgens in females than in males. We investigated the
15relationships between fecal androgen (immunoreactive testosterone) levels and reproductive status,
16age, dominance rank, fetal sex and a secondary sexual trait (facial color) in semi-free-ranging female
17mandrills, using samples collected from 19 reproductively mature females over 13 months. Fecal
18androgens varied with reproductive status, being highest during gestation. Fecal androgens began to
19increase at 3 months of gestation, and peaked at 5 months. This pattern is more similar to that found
20in a platyrrhine than in other cercopithecine species, suggesting that such patterns are not necessarily
21phylogenetically constrained. Fecal androgens did not vary systematically with rank, in contrast to the
22relationship we have reported for male mandrills, and in line with sex differences in how rank is
23acquired and maintained. Offspring sex was unrelated to fecal androgens, either prior to conception or
24during gestation, contrasting with studies of other primate species. Mean facial color was positively
25related to mean fecal androgens across females, reflecting the same relationship in male mandrills.
26However, the relationship between color and androgens was negative within females. Future studies
27of the relationship between female androgens and social behaviour, reproduction and secondary
28sexual traits will help to elucidate the factors underlying the similarities and differences found between
29the sexes and among studies.

30

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KEYWORDS

32Mandrills, *Mandrillus sphinx*, facial color, sexual ornaments, dominance rank, androgen, fetal sex

33

INTRODUCTION

34

35Androgens are traditionally viewed as male hormones. The effects of androgens on male behavior,
36morphology and physiology are well studied, including the relationships with the expression of sexual
37behavior, stereotypically male behaviours such as aggression and display, and the development of
38secondary sexual characters . Like males, females produce androgens in the gonads and adrenal
39glands, and the two sexes share many mechanisms of androgenic action . Thus, a comprehensive
40understanding of the role of androgens in reproduction, behavior and morphology requires the
41examination of female, as well as male, hormone profiles. However, the biological significance of
42androgens has received far less attention in females than in males.

43

44Reproductive status is probably the best-documented influence on female androgen levels. For
45example, testosterone is elevated during gestation in mammals , including primates . Testosterone
46also rises at ovulation in many mammal species , suggesting a possible link with sexual behavior and
47motivation, although this is less well understood . Few studies, however, have investigated patterns of
48androgens across the conceptive period, gestation and lactation in detail for wild or semi-free-ranging
49primates .

50

51The influence of age on female androgen levels is less well studied. In male primates, testosterone
52begins to increase at reproductive maturity and matches the age-profile of reproduction (e.g., Beehner
53et al. 2009). Testosterone decreases with age in women (*Homo sapiens*, and female baboons older
54than 16 years also had lower testosterone levels than younger females (Beehner et al., 2005).

55

56Dominance rank has important implications for female primates, with higher-ranking females enjoying
57higher reproductive success than lower-ranking females in many studies . In male primates,
58androgens increase in the winner of an aggressive encounter, but decrease in the loser . If the same
59is true for females, then higher-ranking females may have higher androgen levels than lower-ranking
60females. Studies of how testosterone relates to dominance rank among females in group-living
61primates show mixed findings. Higher-ranking females show higher serum testosterone and
62androstenedione levels than lower-ranking females in captive talapoins , fecal testosterone is higher

63in dominant than in subordinate hybrid baboons , and fecal 17b-OH-androgens are higher in dominant
64than in subordinate Barbary macaques . However, there is no relationship between rank and fecal
65testosterone in female yellow baboons , between rank and fecal and salivary testosterone in ring-
66tailed lemurs , or between rank and fecal 5a-androstan-17a-ol-3-one in bonobos .

67

68One possible explanation for the differences found across studies in the relationship between female
69rank and androgens is differences in the exact androgens measured, and in how androgens are
70metabolised and excreted . However, species differences in how rank is attained, and maintained,
71may also influence the relationship between rank and androgens. Where male primates actively
72contest their rank, androgens are higher in higher-ranking males than in lower-ranking males . In
73contrast, where rank is stable, androgen levels often show no consistent relationship with rank . The
74same may also be true for females. For example, rank is inherited maternally in yellow baboons ,
75where there is no relationship between rank and fecal testosterone, while the hybrid population may
76have more potential to contest their position, and show is a relationship between rank and fecal
77testosterone . However, ring-tailed lemurs contest their rank position, yet there is no relationship
78between rank and testosterone, measured either in feces or in urine . Thus, there is as yet no
79comprehensive understanding of the link between dominance rank and androgens in female primates.

80

81Female androgen levels may also relate to the sex of the offspring, both pre- and post-conception. For
82example, preconception androgens are higher in females that subsequently bear male offspring than
83it is in those that subsequently bear females in ibex , field voles , and Barbary macaques , offering a
84possible mechanism by which females may influence offspring sex . Post-conception, gestational
85androgens are derived from multiple sources, both maternal (ovaries, corpus luteum, and adrenal
86glands) and fetal (testes and adrenals) . In rhesus macaques (*Macaca mulatta*) the fetal testes
87secrete androgens throughout gestation, peaking at days 40-75 (trimester 1-2) then declining, with
88another increase around day 140 (trimester 3) . In line with this, females carrying male fetuses have
89higher androgen levels in some species, including humans , elephants , Assamese macaques , and
90yellow baboons , although not in others, such as red-fronted lemurs .

91

92 Finally, the expression of many secondary sexual traits is related to androgens in males , such that
93 these traits act as 'badges of status' or signals of competitive ability . Androgen-dependent traits may
94 also signal the ability to withstand the costs of high testosterone to potential mates . Females also
95 possess secondary sexual traits, but these are less understood than those of males . Female traits
96 may represent a by-product, or correlated response, of selection for ornaments in males, particularly
97 where females are muted by comparison to males . However, they may also have adaptive
98 explanations in their own right, such as a role in contest competition or mate choice . Studies of the
99 similarities and differences between hormone profiles and secondary sexual traits in males and
100 females can shed light on the evolution of ornaments. Experimental administration of testosterone
101 increases the expression of sexual ornaments in female birds and lizards . However, such
102 experiments involve androgen levels greater than the levels females naturally experience. Very few
103 studies have examined the relationship between natural variation in androgens and female
104 ornamentation , with none, to our knowledge, in female mammals.

105

106 In this study, we investigated the relationships between female androgens and reproductive status,
107 dominance rank, fetal sex and secondary sexual color in mandrills (*Mandrillus sphinx*), a large, group-
108 living, primate found in the rainforests of central Africa . We investigated the correlates of fecal
109 measures of immunoreactive testosterone over 13 months in 19 female mandrills living in a large,
110 semi-free ranging group in Gabon. As in other non-invasive studies, it is very likely that our
111 testosterone assay cross-reacts with metabolites of dehydroepiandrosterone, so we discuss our
112 results in terms of female androgens, rather than testosterone specifically. Mandrills in our study
113 population breed moderately seasonally, with 63 % of peri-ovulatory periods occurring between July
114 and September, and only 6 % between December and April and a corresponding birth peak in
115 January to March . Females show inherited, stable dominance relationships, and higher-ranking
116 females have a reproductive advantage relative to lower-ranking females, experiencing their first
117 sexual cycles on average 6 months earlier, giving birth for the first time at a younger age and
118 undergoing shorter inter-birth intervals . Like male mandrills, females exhibit bright pink and red facial
119 coloration, which varies extensively between females, and across the female reproductive cycle,
120 peaking post-parturition . Female coloration is more muted than in adult males, although ranges

121overlap. Male coloration is linked to fecal testosterone , but this relationship has not yet been
122investigated in females.

123

124Based on the results of previous studies, we predicted that:

- 125 1. Reproductive condition would influence female androgen levels, as in other cercopithecines.
126 Specifically, pregnant females would experience higher fecal androgen levels than either
127 cycling or lactating females.
- 128 2. Androgen levels would decrease with age, as female baboons show a decrease in fecal
129 testosterone measures with age .
- 130 3. Fecal androgens would show no consistent relationship with rank, as rank is inherited and
131 stable in female mandrills.
- 132 4. Fecal androgens would be higher in females that subsequently bear male offspring than those
133 that subsequently bear female offspring, as in Barbary macaques .
- 134 5. Fetal sex would be significantly related to maternal androgens in late pregnancy, as in other
135 cercopithecines .
- 136 6. Female facial coloration would be positively related to fecal androgens, if female facial color
137 reflects circulating androgen levels, as it does in male mandrills .

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METHODS

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Ethical statement

143This research was approved by the Comité Régional d'Ethique Ile de France Sud (the committee
144responsible for research on animals at the Centre International de Recherches Medicales, Franceville
145(CIRMF), Gabon, and adhered to Gabonese legal requirements.

146

147

Study population and subjects

148Mandrills are found in the dense rainforests of Gabon, Congo, mainland Equatorial Guinea and
149southern Cameroon to the south of the Sanaga river . No study has yet succeeded in habituating
150mandrills in the wild, but the semi-free-ranging mandrill colony housed at the CIRMF provides an

151 opportunity to study the reproduction and behavior of known individuals under relatively natural
 152 conditions. CIRMF established its mandrill colony in 1983/4, by releasing 15 animals (7 males, 8
 153 females) into a 6.5 ha naturally forested enclosure (E1). A second semi-free-ranging group was
 154 established in 1994 in a smaller enclosure (E2, 3.5 ha) by transferring 17 mandrills (including 6 adult
 155 females and 4 adult males) from the first enclosure. No subsequent additions were made to the
 156 colony, other than through breeding, until March 2005, although animals were removed occasionally.
 157 The mandrills forage freely and receive daily supplements of monkey chow, fruit and vegetables.
 158 Water is always available from a stream.

159

160 We conducted this study between February 2004 and March 2005 as part of a larger study of non-
 161 invasive endocrinology in mandrills. The 18 study subjects included 16 parous females living in E1
 162 during the study period, and two females that gave birth for the first time in March 2004 (month 2 of
 163 the study), and were aged 4.7 - 26.1 years (**Table 1**). We excluded one parous female who gave birth
 164 to an infant that appeared to be one month premature (based on swelling records) and died at two
 165 days and who subsequently cycled and showed a pregnancy swelling but never produced an infant,
 166 suggesting fetal loss at some stage. The size and age-sex composition of the study groups during the
 167 study period is summarized elsewhere and corresponded to the smaller end of group sizes observed
 168 in the wild . 19 infants were born into E1 during the study and one infant and one adult male died. The
 169 male dominance hierarchy was stable from five months before the beginning of the study until 31 July
 170 2004, when the alpha male died of injuries probably sustained when falling out of a tree. The
 171 previously second-ranking male took-over as alpha male for 40 days, before being injured and
 172 deposited by a third male on September 20th 2004. The male hierarchy then remained stable until the
 173 end of the study (March 2005).

174

175 **Table 1: Female mandrills at CIRMF included in this study, with age and parity at the beginning**
 176 **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

177

178^a Individuals with a second ID in parentheses were initially assigned to the incorrect matriline in colony
179records

180^b Founder females, age estimated from dental records

181

182

Female age

183The age of founder females was estimated using dental estimates when the animals arrived at CIRMF
184and their previous history . The date of birth is recorded for all individuals born into the colony. Female
185mandrills attain their full adult body mass and length at 7 yr ; by 20 yr they appear physically elderly,
186and their rate of reproduction decreases .

187

188

Female reproductive status

189We noted the reproductive status of females daily as one of:

- 190 - cycling
- 191 - pregnant (assigned post hoc from the birth of an infant, using the mean gestation length in the
192 colony is 175 days and assigning conception to the day on which the perineal skin began to
193 detumescence in the last sexual swelling cycle).
- 194 - lactating (the period following the birth of an infant during which the female has a dependent
195 infant and does not cycle). The mean duration of post-partum amenorrhea following the birth
196 of a live infant in the CIRMF colony is 7 months . During this study one female resumed
197 cycling at 5 months; all others did so after ≥ 7 months.
- 198 - quiescent (not pregnant or cycling and with no dependent infant).

199

200We noted the sex of the offspring produced following pregnancies (except in one case where we were
201unable to determine the sex of an infant born in February 2005).

202

203We also subdivided cycling into follicular and luteal phases for some analyses, based on whether the
204sexual skin was inflating (follicular phase) or deflating (luteal) .

205

206

Dominance rank

207We noted submissive behavior by study subjects ad libitum during twice daily observation periods
208(approx. 10:00-11:30 h and 15:30-17:30 h). We calculated dominance rank using dyadic interaction
209matrices based on all interactions where one female avoided or fled when another female approached
210. There were no unresolved relationships and we observed no reversals. Dominance ranks were
211identical to those previously recorded for the females with the only changes being those expected
212due to births and deaths. As in previous studies , we classified females as high- (upper-quartile), mid-
213(inter-quartile range) or low- (lower quartile) ranking, depending upon the proportion of females that
214they dominated.

215

216

Female color

217We attempted to measure female facial color every two weeks. We obtained close-up digital images
218of female faces using a Nikon Coolpix 5700 digital camera with an 8x optical zoom and saved them as
219'fine' quality JPEGs. We took all images when females were in either an open grassy area or in an
220open feeding pen. Images required calibration, to account for exposure and light drift . It was
221impossible either to get animals in the same frame as a photographic white and black standard, or to
222place a standard in the same position as the animal and capture a second image immediately
223following that of the subject. Instead, we used only images where color ranged the full spectrum from
224white (the white ventral pelage) to black (all animals are black around the eyes), and used the
225'Autolevels' command in Adobe Photoshop v. 6.0 (Image Mode set to RGB) to define the lightest and
226darkest pixels in each color channel as white and black. We then analysed images using Adobe
227Photoshop v. 6.0. We outlined the midnasal strip in a standard fashion using the polygonal lasso tool
228and measured the mean luminosity and the red intensity value (the number of pixels at each intensity
229value) for the highlighted area using the 'ImageO histogram' command. We found that the gray score

230from the red channel, divided by luminosity, produced color measures that correlated best with
231quantified color chart assessments of the same colors . Although we are aware that this method
232introduces scatter to the data set, there is no reason to believe that it introduces systematic bias.

233

234

Fecal sampling

235We collected fecal samples when females defecated in the feeding area during morning and
236afternoon observation periods. We collected samples immediately after defecation and noted the
237identity of the individual, date, time and consistency of the sample. We homogenized the feces and
238stored a portion (mean \pm SEM = 6.6 \pm 0.1 g) at -20C in 40 ml of 90 % ethanol until extraction. We did
239not use diarrheic samples. We attempted to collect three samples per month for each female,
240achieving a mean of 2.0 \pm 0.1 samples per female per month, a total of 17 \pm 2 samples per female, and
241an overall total of 309 samples (Table 1).

242

243

Steroid extraction and assay methods

244We used the same extraction and assay methods we have previously reported for male samples . We
245homogenized fecal samples in their storage ethanol, vortexed them for 1 minute, and shook them for
246three hours. We then centrifuged sampled for 15 minutes at 2000 rpm, dried a 2 ml aliquot of the
247supernatant under N₂ at 40 °C and resuspended it in 1 ml EIA Phosphate buffered saline (0.1 M
248phosphate buffered saline, pH 7.0, with 0.1% bovine serum albumin). We dried the fecal pellet to
249constant mass at 60 °C to determine the dry mass of the sample. We determined extraction efficiency
250by measuring recovery of ³H–testosterone (3000 counts per minute) added to ten fecal samples prior
251to extraction. Mean extraction efficiency was 77.6 % (SEM 2.4 %). We corrected hormone
252concentrations for this extraction loss and expressed them as ng of hormone per g of dry feces. Mean
253dry fecal mass was 1.65 \pm 0.03 g.

254

255We measured androgens in fecal extracts using microtitreplate enzyme immunoassay (EIA), using an
256antiserum and labeled testosterone conjugate (horseradish peroxidase: HRP) provided by Coralie
257Munro (University of California, Davis). The antibody crossreacts with 5 α -dihydrotestosterone
25857.37%, and less than 1 % with other steroids . We refer to the results as fecal androgens. We
259validated the assay immunologically by demonstrating parallelism, accuracy, and sensitivity, and

260biologically by comparing fiT values with serum free testosterone, and comparing young males with
261adult male conspecifics with results that suggest that the assay may reasonably be regarded as
262indicative of testicular androgen secretion in mandrills .

263

264We diluted fecal extracts 1:6 in assay buffer (0.1 M phosphate buffered saline, pH 7.0, with 0.1%
265bovine serum albumin) and assayed 50 µl aliquots along with 50 µl aliquots of reference standard in
266doubling dilutions (range 1.95-1000 pg/well). We re-ran samples if duplicates had coefficients of
267variation greater than 5 % and re-diluted and re-assayed samples binding >90 % or <20 %. The intra-
268assay coefficient of variation was 2.19 %. Inter-assay variation for pooled quality controls was 10.4 %
269for the high quality control, and 16.3 % for the low quality control.

270

271

Statistical analysis

272We normalized fecal androgen levels via log transformation. We used a general linear mixed model
273(GLMM) to assess the effects of predictor variables on fiT levels. We included female identity as a
274random factor, to account for the fact that we sampled the same individuals repeatedly, and tested for
275main effects of the following predictor variables:

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

282

283We also included whether samples were collected during morning or afternoon observation periods as
284a categorical variable, to detect any influence of circadian rhythms . We have previously shown that
285the number of months for which samples were stored prior to extraction does not influence fecal
286androgens significantly .

287

288To explore the effects of reproductive status on fecal androgen levels further we divided gestation and
289lactation into 6 and 7 monthly intervals, respectively, with the day of parturition termed day 0, to
290examine changes in fecal androgens across the reproductive cycle in more detail.

291

292We did not investigate the influence of seasonality on fecal androgen levels because this was
293confounded with the seasonality of reproduction in the colony . The daily provisioning of the colony
294should compensate for any influence of seasonality in calorie intake, and the monkey chow does not
295vary across the year, although the types of fruits and vegetables provided do vary and seasonal cues
296may also be provided by changes in daylength (albeit small in equatorial Africa), temperature,
297humidity, or rainfall.

298

299To determine whether androgen levels predict fetal sex we compared fecal androgens in mothers of
300daughters and sons during (i) the follicular phase of the conceptive cycle using a Mann-Whitney exact
301test, given the small sample size, and (ii) during the two months prior to conception using a GLMM
302with female identity as a random effect, androgen level as the covariate and fetal sex as the
303dependent variable and a binomial error structure . As the sample sizes were small, we also
304compared the mean value for females bearing a male and a female offspring during the two months
305prior to conception using an exact Mann-Whitney test.

306

307To examine the relationship between maternal fecal androgens during pregnancy and fetal sex we
308used a GLMM with female identity as a random effect and included pregnancy trimester (days 1-58,
30959-117 and 118-175) to account for changes in fecal androgens across gestation.

310

311Finally, to examine the relationship between facial color and fecal androgens, we assigned the closest
312fecal androgen value to each color value, using a 1 day excretion lag (as above) and only values
313obtained within 15 days (mean +/- SEM timelag = -0.8 +/- 0.3 days, n = 370). We examined the
314relationship between fecal androgen levels and red color in two ways: first by comparing mean values
315of red color and fecal androgens for each female using a Pearson's correlation, then using a GLMM
316with red coloration as the dependent variable, female identity as a random factor, and fecal androgens
317as a covariate.

318

319Where we had a directional prediction concerning the relationships between fecal androgens and fetal
320sex we used a one-tailed tests, and state this in the results. Otherwise we used two-tailed tests. We
321set the statistical threshold set at P = 0.05 and conducted all tests using SPSS version 20.0. Figures
322present means and SEMs calculated directly from the logged fecal androgen data.

323

324

325**RESULTS**

326

327Whether a sample was collected during the morning or afternoon observation sessions did not predict
328fecal androgen levels in our study subjects (**Table 2**). Reproductive status was significantly related to
329fecal androgen levels (**Table 2**). Post-hoc comparisons showed that fecal androgen levels were
330significantly higher in pregnant females than in females at any other stage (**Table 3, Fig. 1**). Cycling
331females also showed higher fecal androgen levels than those who were pregnant or lactating. There
332was no significant difference in fecal androgen level between quiescent and either cycling or lactating
333females. Closer inspection of the data revealed that fecal androgens rose from low levels in early
334pregnancy, and peaked two months before parturition (**Fig. 2**).

335

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

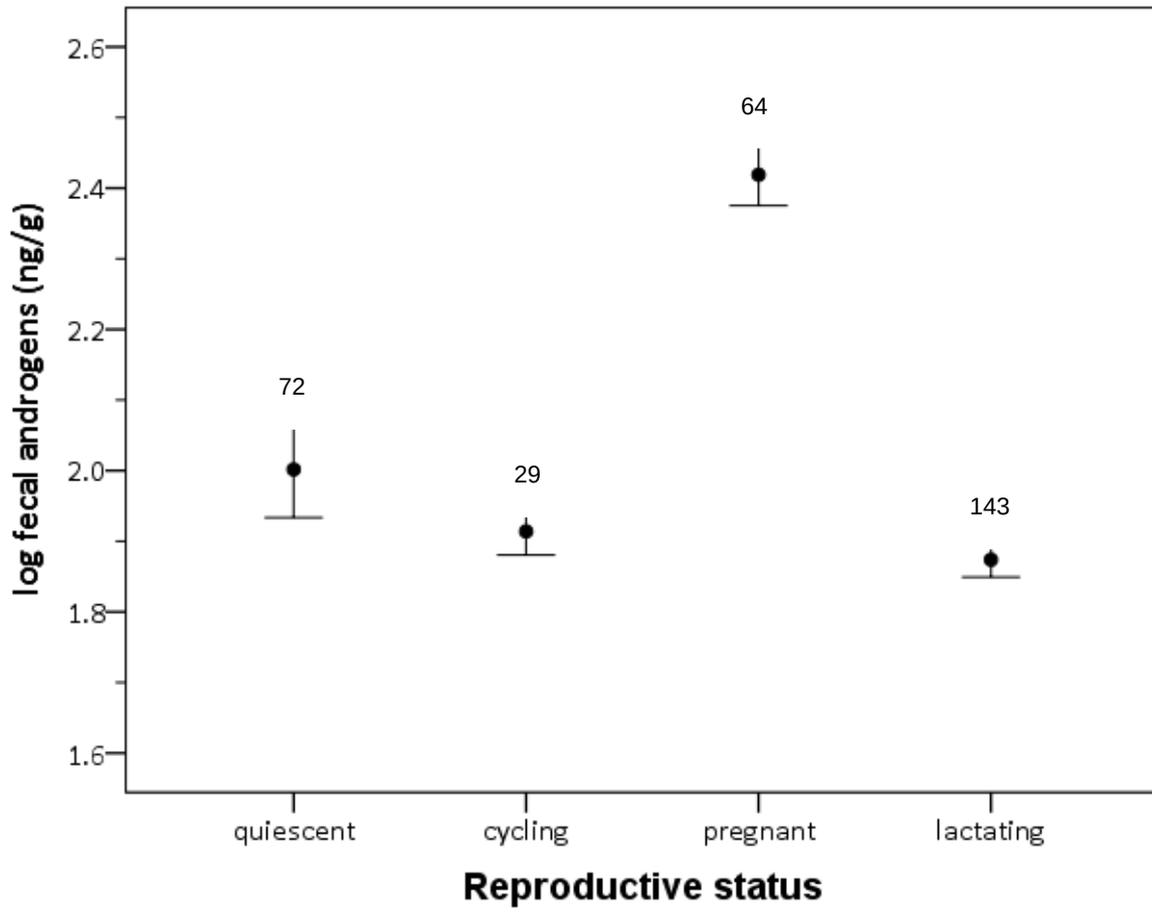
Predictor	Numerator df	Denominator df	F	P
Intercept	1	17.73	303.56	<0.001
Observation period	1	293.64	1.86	0.174
Reproductive status	3	299.49	51.30	<0.001
Age	1	19.35	1.11	0.306
Dominance rank	1	18.16	1.68	0.211

339

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

	Cycling	mean +/- SE difference (p) 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)

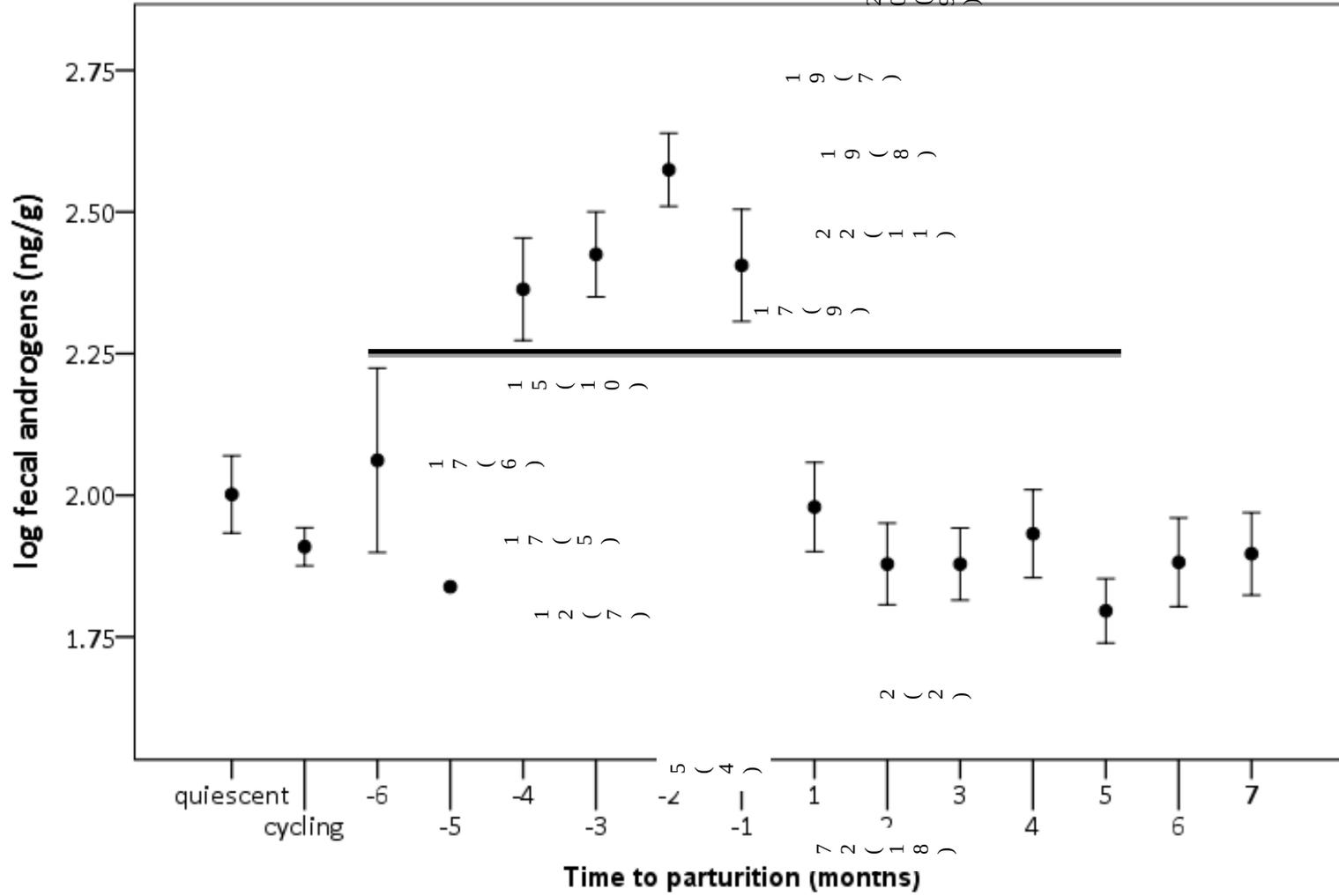
342



343

344

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



347
 348
 349
 350 **Fig. 2:** Mean +/- SEM fecal androgens across the reproductive cycle in 18 female mandrills. Vertical line indicates parturition. Numbers above bars
 351 indicate sample size, with number of females contributing in parentheses.

352

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

354

355 We found no significant difference in maternal fecal androgens in follicular phase samples for females
356 that went on to conceive a male ($n = 4$) or female ($n = 4$) offspring (Mann-Whitney $U = 13.0$, exact $p =$
357 0.200). Similarly we found no relationship between fetal sex and maternal fecal androgens when we
358 examined all samples collected during the two months prior to conception (13 female offspring, 5
359 mothers, 17 male offspring, 7 mothers, GLMM $F_{1,28} = 0.786$, one-tailed $p = 0.192$; using mean values
360 for females bearing female and male offspring Mann-Whitney $U = 22.0$, exact $p = 0.530$). We found no
361 significant relationship between fecal androgens and fetal sex during pregnancy ($F_{2, 48} = 0.206$, $p =$
362 0.815). We had very few samples for trimester 1 (Table 4).

363

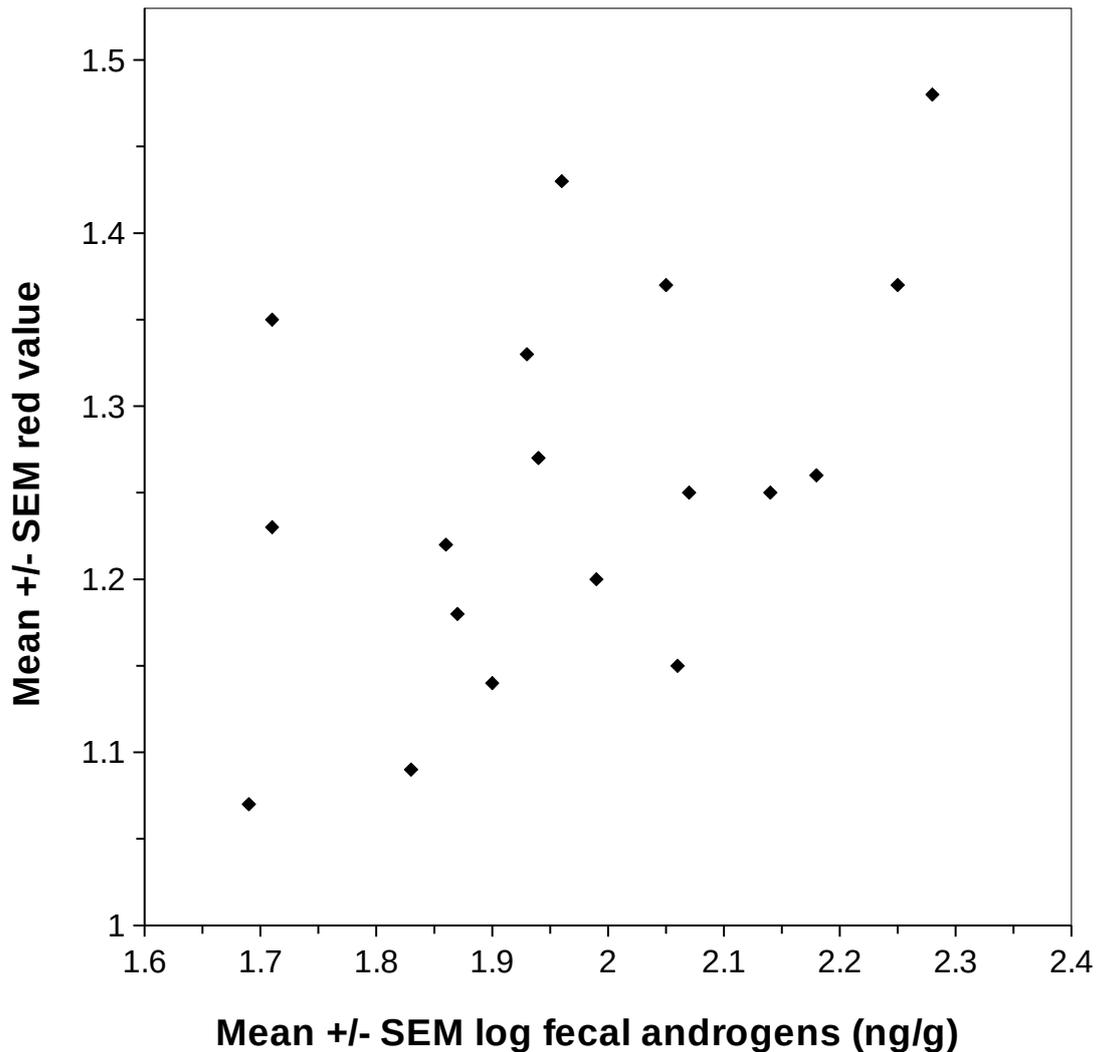
364 **Table 4: Number of samples available for the three trimesters of pregnancy**

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)

365* one female contributed one male and one female offspring

366

367 Finally, we found a significant, positive relationship between female red color and fecal androgens
368 when we compared the mean of each across females ($n = 18$, $r = 0.501$, $p = 0.034$, Fig 3). However,
369 using all the data available, with red coloration as the dependent variable and female identity as a
370 random factor revealed a negative relationship between red and fecal androgens ($\beta \pm SE = -0.94 \pm$
371 0.018 , $t_{367.82} = -5.09$, $p < 0.001$).



372

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

374 **mandrills**

375

376

377

DISCUSSION

378

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

385complicating comparisons among studies. Nevertheless, these findings are both similar to, and differ
386from, those for male mandrills, and for females of other species.

387

388

Fecal androgens and female reproductive status

389The overall pattern of fecal androgens across female reproductive status in mandrills was similar to
390that found in hybrid baboons , being highest during gestation, although we also found that cycling
391females had higher fecal androgens than lactating females, a pattern not found in the baboons .
392During gestation, fecal androgens began to increase in the third month of gestation (4 months prior to
393parturition, in trimester 2), and peaked at 5 months (2 months before parturition, in trimester 3). This
394contrasts with patterns in other cercopithecines, where the peak in androgens occurs earlier. For
395example, fecal androgens rise in week 2 of gestation, peak at week 4, and decline to pre-conception
396levels in weeks 7-9 in Assamese macaques . In baboons, fecal testosterone rise significantly on day
39721 of pregnancy, peak around week 5-6, drop back to early pregnancy levels for weeks 8-10, then rise
398again to a plateau lower than the initial peak . Similar patterns are seen in other primate species .
399Although we had relatively few samples for early pregnancy, and it is possible that we missed a
400transient, early peak in fecal androgens, we found no indication of this in our data. We also estimated
401the onset of gestation from patterns of sexual swelling, rather than hormonally, which might result in
402small errors in the date of conception. However, such errors are also unlikely to explain the difference
403between mandrills and other species.

404

405Patterns of gestational fecal androgens also differed from those in other cercopithecines later in
406gestation, when we had more samples, and can be more confident in our findings. Fecal androgen
407levels were high in trimesters 2 and 3 (the 4 months prior to parturition), and did not decrease to pre-
408conception levels until parturition. This pattern differs from that reported for Assamese macaques,
409where androgen levels during trimesters 2 and 3 were similar to pre-conception levels . Fecal
410androgens during the last 2 months of gestation were markedly higher than any lactation values in
411mandrills, a pattern which contrasts with that found in peripartum baboons, in which androgen levels
412during the last 8 weeks of gestation were similar to those during lactation . The overall pattern of fecal
413androgens across gestation in mandrills is more similar that reported for a platyrrhine, the white-faced
414marmoset (*Callithrix geoffroyi*), in which urinary androgen levels rose significantly during the first

415trimester, peaked in the middle of the second trimester, then declined gradually to parturition ,
416although the mandrill peak occurs slightly later during gestation. Overall, these results suggest that
417patterns of gestational androgen levels are variable across species, and are not necessarily
418constrained by phylogeny, as mandrill gestational androgen patterns are more similar to marmosets
419than to macaques and baboons.

420

421Mandrills at CIRMF tend to conceive during the dry season, and gestation continues into the wet
422season, with births occurring in the wet season . Any effect of ecological seasonality on fecal
423androgens might therefore confound the influence of reproductive status on fecal androgens. For
424example, fecal testosterone levels are higher during the wet season in hybrid baboons, when contest
425competition also increases . However, as in the study of Assamese macaques, inspection of the data
426for individual female mandrills showed the same general patterns, suggesting that gestational stage,
427rather than season, underlies the patterns observed .

428

429

Fecal androgens, age and dominance rank

430Outside the human medical literature, very little known is about the relationship between hormones
431and aging or senescence. Our study subjects included females aged 4.7-26.1 yr. Females at the top
432end of this range are clearly elderly, and the limited data available suggest a decrease in reproductive
433output in elderly females . However, we found no relationship between age and female androgens.
434This contrasts with findings for female baboons, where, females older than 16 years had significantly
435lower fecal testosterone levels compared to other females . Adult male baboons also show age-
436related decreases in testosterone (e.g., Beehner et al. 2009).

437

438The lack of a relationship between fecal androgens and rank in female mandrills contrasts with
439findings for males, where fecal androgen levels are significantly positively related to dominance rank .
440This sex difference is likely to relate to contrasts in the way in which rank is acquired in the two sexes.
441Aggressive encounters increase testosterone in the winner, but decrease it in the loser . This is likely
442to explain the relationship between rank and fecal testosterone in males, which contest their rank
443position physically, and appear to live in a permanently aggressive context . Male rank can change
444dramatically from one day to the next, as a result of fights, and males can suffer serious injury or

445death . In contrast, serious dyadic conflict is rare in female mandrills (we have observed it only once,
446when two groups came into contact when a wall fell down). Females in the CIRMF colony inherit their
447mothers' rank in classical youngest ascendancy fashion and the hierarchy has been stable across
448generations, with the only changes being due to births, deaths or removal of individuals . This stability
449suggests that females do not experience the effects of winning or losing aggressive encounters on
450androgen levels, which therefore remain undifferentiated with respect to rank.

451

452Our results for the relationship between fecal androgens and rank in female mandrills are similar to
453those reported for female yellow baboons , ring-tailed lemurs , and bonobos , in which rank is also not
454related to androgens. However, our findings differ from those for captive talapoins , hybrid baboons ,
455and Barbary macaques , in which dominant females show higher androgen levels than subordinates.
456As we note in the introduction, these differences in the relationship between androgens and rank in
457females may relate to how females acquire their rank, although this does not explain all the findings
458documented. Findings may also differ according to the stability of the hierarchy at the time of the
459study. For example, it is possible that rank is particularly stable in our study population, where inter-
460birth intervals are short and mortality is low, conditions conducive to rank stability . This may not be
461the case in wild primate groups, where inter-birth intervals are longer and survival is lower, and the
462hierarchy may be less stable, even where it is inherited.

463

464An alternative, but not mutually exclusive, explanation for differences between species lies in variation
465in the importance of aggressive competition for rank, or dominance style. More 'despotic' species,
466where social interactions are asymmetrical and dominance hierarchies are steep, may show a
467stronger relationship between rank and androgens than 'egalitarian' species, where interactions are
468more symmetrical and hierarchies less steep. However, Barbary macaques are rated as 'egalitarian'
469in dominance style , yet females show rank-related differences in fecal androgens , arguing against
470this hypothesis. Studies of dominance style have concentrated mainly on the macaques , and as yet
471we have no detailed data on dominance style in female mandrills to determine where they fall on the
472spectrum.

473

474

Maternal fecal androgens and fetal sex

475We found no significant relationship between preconception maternal fecal androgens and fetal sex in
476mandrills. However, this finding should be viewed as preliminary, as we obtained only a small number
477of fecal samples during the preconception period, and may have missed any preconception peaks in
478androgens. A study of Barbary macaques found that offspring sex is related to pre-conceptive fecal
479androgens, measured both 2-3 weeks prior to mating in three zoo-housed females (similar in nature
480and sample size to our follicular phase comparison) and 1-2 months prior to conception in 9 females
481who produced 13 offspring in a group of provisioned macaques (similar to our 2 month prior to
482conception comparison) . High-ranking females in the Barbary macaque study also had higher
483androgens than low-ranking females, providing a possible mechanism by which females are able to
484adjust the sex of their offspring prior to conception based on the social environment , and supporting
485both the Trivers-Willard maternal condition hypothesis , and the maternal dominance hypothesis of
486sex allocation.

487

488Mandrills are extremely sexually dimorphic , with very high reproductive skew among males , both of
489which suggest that a mechanism that links female dominance to offspring sex would be
490advantageous, as high-ranking females produce larger offspring . As large size is more beneficial for
491males than for females, sex ratio theory predicts that females in good condition (i.e., high-ranking
492females) should produce more male offspring than those in poor condition (low-ranking females).
493However, birth sex ratios are not related to maternal dominance rank in either our study population of
494mandrills , nor in primates in general . Thus, while our analyses of preconception fecal androgens in
495mothers and offspring sex are based on a small sample size, in combination with a lack of a
496relationship between female androgens and rank (this study), or between female rank and offspring
497sex , they suggest that the maternal dominance hypothesis does not apply in this species.

498

499Our sample of mandrills in the first trimester of gestation is very small, making it difficult to draw
500conclusions about the influence of offspring sex during early pregnancy. However, we obtained more
501samples during trimesters 2 and 3, but found no relationship between maternal fecal androgens and
502fetal sex. A recent review noted inconsistency about whether fetally-derived androgens contribute to
503elevated maternal androgens in primates . Two studies report that female non-human primates
504carrying a male fetus show higher androgen levels while others do not. A study of a small number of

505red-fronted lemurs also found no link between maternal androgens (fecal testosterone-3-
506(carboxymethyl)oxime-BSA) and fetal sex . Similarly, there were no differences between gestation
507levels of urinary testosterone in male-biased and female-biased litters in white-faced marmosets, nor
508were levels of maternal androgens associated with the number of males in the litter, or with the
509proportion of the litter that was male . However, other studies report relationships between female
510androgens and fetal sex. There is a sex difference in maternal androgens in trimester 3, but not in
511trimesters 1 and 2, in Assamese macaques , during the 3rd trimester in yellow baboons , in late
512pregnancy in ring-tailed lemurs , and in trimesters 2 and 3, but not trimester 1, in elephants . It is not
513clear why species differ in the relationship between fetal sex and maternal androgens, but it may be
514that high levels of maternal androgens overwhelm the contribution of fetal androgens in mandrills, as
515they maintain high levels of fecal androgens in trimesters 2 and 3 regardless of fetal sex. It is also
516possible that assay variation obscures small effects of fetal sex in our study.

517

518

Female fecal androgens and facial red coloration

519Our most intriguing results concern the relationship between female facial color and fecal androgens.
520The overall positive relationship between mean color and mean fecal androgens reflects that found in
521males . Experimental administration of both testosterone and estrogen also increases red skin color in
522male rhesus macaques , due to increased epidermal blood flow . However, when we accounted for
523female identity, we found a negative relationship between facial color and androgens in female
524mandrills. Both variables are also related to reproductive status, although the patterns differ:
525androgens peak in the second half of pregnancy, but are low post-parturition (this study), while color
526peaks post-parturition during lactation . It is possible that the negative relationship we detected
527between facial color and androgens within individuals is due to a time-lagged positive relationship
528between hormone levels and color due to changes in hormone receptors in the skin, where
529testosterone is aromatised to estrogen . However, this hypothesis is not supported by temporal
530relationships between male color and testosterone, where current testosterone predicts color during
531the current month, but not during future months .

532

533The evolution of female secondary sexual traits is far less understood than that of males, particularly
534where females possess traits similar to those of males, like facial coloration in mandrills . Without

535strong selection pressure against the expression of traits in females, then females will share these
536traits with males because males and females share most of their genome . However, females cannot
537be assumed to be a control condition for males, and hormone-linked color may also play an adaptive
538role in females . Our findings that facial color in female mandrills is linked to androgens contribute to
539the small literature on the relationship between naturally occurring variation in female androgens and
540female secondary sexual traits. Studies also report positive correlations between circulating
541testosterone levels and female ornaments in birds. Testosterone is positively linked to plumage
542coloration in female Northern cardinals , the size and color of the throat patch in females of the
543polyandrous, sex-role reversed barred button quail and the size of the white wing patch but not the
544presence of a forehead patch during the incubation phase in female pied flycatchers (*Ficedula*
545*hypoleuca*, . The proximate and ultimate influences on variation in female color in mandrills merit
546further investigation. Selection pressures on male and female mandrills differ greatly, with females
547investing far more in gestation, lactation and infant care, while males invest in competition for access
548to receptive females .

549

550

Conclusions and future directions

551Our findings contribute to a more comprehensive view of androgens by improving our understanding
552of the relationship between female androgens and reproductive status, rank, and fetal sex. They
553reveal differences with studies of male mandrills. Fecal androgens are related to dominance rank in
554males, but not in females. Comparisons across species suggest that the relationship between
555androgens and rank in female primates deserves more detailed attention, including detailed studies of
556aggression and submission within and across species to elucidate the factors that underlie the
557patterns observed. Intriguingly, patterns of androgens across gestation in mandrills are more similar to
558those in a platyrrhine than those in other cercopithecines, suggesting that such patterns are not
559necessarily phylogenetically constrained. Further data, for additional species, will shed light on the
560correlates of these patterns. We did not detect any relationships between fetal sex and female
561androgens, either pre-conception or during gestation. Again, additional studies will help to clarify the
562question of how and why species vary in these relationships. Sample collection directed specifically at
563the peri-conceptual phase and early gestation would improve sample sizes in future studies, although
564these phases are identified much more easily post-hoc than during sample collection. We detected

565intriguing patterns in the relationship between female androgens and facial red color, which merit
566further attention. Other possibilities for the future include comparison of female fecal androgens with
567other endocrine factors, such as glucocorticoids , and with physical condition, and examination of the
568relationship between maternal androgen levels and morphological, physiological, and behavioural
569development in offspring. As examples of the latter relationship, endogenous variation in maternal
570gestational androgens affects offspring development in spotted hyenas ; and white-faced marmosets
571suggesting a role for female androgens in adaptive environmental signaling from mother to offspring
572via effects of the maternal phenotype on offspring phenotype.

573

574

575

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576

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770 **ELECTRONIC SUPPLEMENTARY INFORMATION**

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

773 **status**

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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)

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