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Relative digit lengths and testosterone levels in Guinea baboons

James R. Roney,[a,b,*] Jessica C. Whitham,[a,b] Marco Leoni,[a,b] Astrid Bellem,[c] Nadja Wielebnowski,[c] and Dario Maestripieri[a,b]

[a] *Animal Behavior Research Group, The University of Chicago, Chicago, IL 60637, USA*
[b] *Institute for Mind and Biology, The University of Chicago, Chicago, IL 60637, USA*
[c] *Brookfield Zoo, Brookfield, IL 60513, USA*

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Abstract

A growing body of literature suggests that the ratio of the lengths of the second to fourth digits (2D:4D) on human hands is sexually dimorphic and associated with prenatal exposure to gonadal hormones, circulating serum testosterone, and a number of psychological and behavioral measures. Little research has investigated digit ratios in nonhuman species. In the present study, we investigated sex differences in digit ratios and their possible association with serum testosterone in a captive group of Guinea baboons (*Papio papio*). Contrary to the sex difference typically reported in humans, male baboons exhibited a substantially larger 2D:4D than did female baboons. Consistent with the human data, however, lower 2D:4D was associated with higher serum testosterone among the males. The present findings suggest that the relationship between digit ratios and male gonadal hormones may be phylogenetically well-conserved, although they also suggest possible species differences in the causal relationships between developmental mechanisms and sex-differentiated digit length patterns.

Keywords: Digit ratios; Baboons; Sex differences; Testosterone

The relative lengths of the second and fourth digits on human hands are sexually dimorphic—men on average have fourth digits longer than their second digits, while in women, the ratio of these lengths is typically closer to 1:1 (Manning et al., 1998; McFadden and Shubel, 2002; Phelps, 1952). Recent research has shown that this ratio of the second digit length to fourth digit length (2D:4D) is significantly correlated with sperm counts, estrogen, and testosterone levels (Manning et al., 1998; but see also Neave et al., 2003), sexual orientation (McFadden and Shubel, 2002; Robinson and Manning, 2000; Williams et al., 2000), and a number of other variables related to health and fertility (for a review, see Manning, 2002). These relationships are thought to arise from the influence of prenatal androgens on 2D:4D, since adult phalangeal ratios are obtained by the 13th–14th week of gestation (Garn et al., 1975), and 2D:4D is significantly lower in individuals exposed to abnormally elevated prenatal androgen levels as a consequence of congenital adrenal hyperplasia (Brown et al., 2002b). The influence of specific homeobox genes on the development of both the genitals and digits (e.g., Kondo et al., 1997), furthermore, has inspired conjecture that prenatal androgens may produce correlated effects on digit ratios and hormone production through their modulation of the expression of such genes (Manning, 2002). The phylogenetic conservation of the *Hox* genes in turn raises the possibility that sex differences in digit ratios and their associations with hormone levels may extend to other species as well.

Little research has examined digit ratios in nonhuman species. One study reported lower 2D:4D in the right rear paw of male versus female mice (Brown et al., 2002a). Among nonhuman primates, McFadden and Bracht (2003, under review) have reported a number of interesting sex differences in the relative lengths and weights of metacarpals and metatarsals. The ratio of the second to fourth metacarpal did not show significant sex differences in baboons or gorillas, and this ratio was actually significantly higher in males versus females (i.e., a reversal of the 2D:4D sex difference in humans) in the left hand of chimpanzees. Because these studies did not measure

* Corresponding author. Institute for Mind and Biology, The University of Chicago, 940 East, 57th Street, Chicago, IL 60637.
E-mail address: jrroney@midway.uchicago.edu (J.R. Roney).

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lengths of phalanges, however, it is unclear how comparable the results are to the human literature that has focused specifically on finger length ratios. This comparability problem is compounded by the finding that the 2D:4D finger length ratio is not correlated with the ratio of the second to fourth metacarpals in humans (Phelps, 1952; reviewed in Manning, 2002). As such, it is possible that different developmental processes may determine relative lengths of phalanges and their associated soft tissues versus the relative lengths of metacarpals.

In this study, we investigated sex differences in relative digit lengths in Guinea baboons (*Papio papio*) and their possible association with plasma testosterone levels. Based on the human data (e.g., Manning et al., 1998), we predicted that baboon males would have lower 2D:4D than females and that males with lower 2D:4D would have higher serum testosterone than males with higher 2D:4D. Since male testosterone may be associated with age, body weight, and dominance rank in baboons (Bercovitch, 1999), these variables were also measured and included within the data analyses. Finally, available data on number of copulations observed per animal also allowed for an exploratory examination of possible relationships between digit ratios, testosterone levels, and sexual behavior.

Methods

Subjects

The study was conducted with a group of Guinea baboons at Brookfield Zoo in Brookfield, IL. This group is housed in an outdoor, multilevel grotto composed of artificial rocks and measuring 57.3 × 47.2 m. The animals are provisioned ad libitum with monkey chow and water. A total of 21 adult females (ages 11–22, Mean = 14.95 ± 0.56) and 11 adult males (ages 10–18, Mean = 13.27 ± 0.72) were measured for the present study. All adult males were vasectomized, and all adult females were intact and cycling. There were no subadults, juveniles, or infants in the group. Information on sexual activity within the group is reported elsewhere (Maestripieri et al., under review); for the present paper, we examined total number of copulations per individual over 3 months of observations that occurred 5 days per week for 5–10 h per day. Information on male dominance rank was available from previous studies and derived from data on dyadic aggression, submission, and displacements. This information was sufficient to classify the adult males as either top ranking (n = 5) or bottom ranking (n = 6) but insufficient to establish precise rankings within these categories. No information on female dominance ranks was available. The present research was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Procedure

The measurements used in this study were obtained when the animals were captured and anesthetized for their annual physical examination. On the day of testing, all animals were herded through an indoor capture and housing area and transferred one by one into a squeeze cage where they were anesthetized with a 5 mg/kg IM injection of Telazol, supplemented with ketamine whenever necessary. Within 10–20 min from the first injection, blood samples were collected from the femoral vein in heparinized vials and stored on ice. The samples from all the males and those from most of the females were collected between 9 AM and noon. A few females were sampled between 1 and 3 PM. Blood samples were later centrifuged, and plasma was stored in heparinized vials at −20°C until the time of assay.

While the animals were under anesthesia, they were placed on an electronic digital scale for weight measurements (to the nearest 0.01 kg), and their body length (crown–rump length) was measured with a caliper (to the nearest millimeter). The second to fifth digits of the right hands of the animals were measured (to the nearest millimeter) directly with a ruler. Measurements were made by retracting the flesh of the palm and placing the zero point of the ruler on the basal crease proximal to the palm and then measuring to the tip of the digit. Due to time constraints associated with the demands of the physical examination, only one measurement was possible for each digit. The experimenter who performed the digit measurements was not blind to the animals' sex but was blind to their testosterone levels.

Hormonal assays

Five hundred-microliter aliquots of serum were extracted with 1000 μl 100% EtOH in 12 × 75 borosilicate glass test tubes, vortexed for 15 s, and centrifuged at 1500 × g at room temperature for 15 min (Fisher Scientific Marathon 3000R). Five hundred microliters of the supernatant were pipetted into a second 12 × 75 borosilicate glass test tube and dried down in a 37°C incubator. The dried extract was reconstituted in 500 μl of assay buffer and then assayed via EIA.

All hormones and conjugates were prepared and supplied by Coralie Munro at University of California Davis, CA. Flat bottom 96-well microtiter plates (Nunc maxisorb) were coated with 50 μl antibody in coating buffer (50 mM sodium bicarbonate, pH 9.6) overnight at 4°C. The first column of the plates was not used due to high variability in antibody binding. After overnight incubation to allow the antibody to bind to the plastic, the plates were washed five times with wash solution (0.15 M NaCl containing 0.05% Tween 20) using a Dynatech Ultrawash Plus to remove unbound antibody. After washing, standards, samples, and controls diluted in assay buffer were

added to each well according to plate setup, followed immediately by addition of 50 μl per well of diluted horseradish peroxidase (HRP). Plates were then covered and incubated at room temperature (RT). After incubation, plates were washed five times to remove unbound antigen and blotted dry, and 100 μl of substrate solution (1.6 mM hydrogen peroxide, 125 μl 0.4 mM azino-bis[3-ethylbenzthiazoline-6-sulfonic acid] in 0.05 M citrate buffer, pH 4.0) was added to each well. Plates were then incubated at RT with shaking (Lab-Line plate shaker set at 2.5) for 0.5 to 2 h until maximum binding was approximately 1.0. Plates were read on a Dynatech MRX Revelation at 405 nm.

The working antibody was R156 and was diluted 1:20,000. Standard values were 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 ng/ml. Working HRP dilution was 1:20,000, and sample volume was 100 μl per well. Sample incubation time was 2 h. Assay sensitivity was 0.039 ng/ml, and intraassay and interassay coefficients of variation were 7.9% and 19.6% at 34.2% binding and 9.5% and 4.1% at 63.3% binding, respectively. Recovery of exogenous testosterone (0.312–5 ng/ml) was 89.9% ± 18.1% ($y = 0.26 + 0.64x$, $r^2 = 0.99$).

Statistical analyses

Descriptive statistics were computed by sex for all possible ratios among the measured digits. Following McFadden and Bracht (2003), effect sizes for sex differences in digit ratios were calculated by dividing the difference between the female and male means by the weighted mean of the variances (cf. Cohen, 1977). Sex differences in digit ratios were also compared by *t* tests, although the involvement of the same digit in the calculation of multiple ratios indicates the nonindependence of tests. As such, the results of these tests should be interpreted as additional descriptive information only. Both the Pearson *r* and Spearman *ρ* were computed (separately by sex) to test the a priori hypothesis that 2D:4D may be correlated with testosterone levels as evidence suggests it is in humans; exploratory analyses also tested for associations between testosterone and the other digit ratios. Multiple regression analysis was used to test for an association between 2D:4D and testosterone after the influence of age, size, and rank on testosterone had been held constant. Finally, parametric and nonparametric correlations were employed to test for possible associations between digit ratios, testosterone, and number of copulations. Reported significance levels are all two-tailed.

Results

Data from one female were excluded due to a digit measurement that was nearly three standard deviations below the female mean (although inclusion of this individual would not change any conclusions presented below). Among the remaining animals, each digit was significantly larger in males than in females ($P < 0.001$), a result expected from the known sexual dimorphism in this species (Napier and Napier, 1967). Of greater interest in the present report are the relative lengths of digits compared across the sexes.

Table 1 presents all of the possible digit length ratios by sex, as well as effect sizes for sex differences in mean ratios. Large effect sizes were found for three specific ratios—males had a substantially smaller 4D:5D ratio but substantially larger 2D:4D and 3D:4D ratios. These ratios all involved the fourth digit, and the sex differences were all in the direction of relatively longer 4D in the females of this sample.

Testosterone levels were next correlated with 2D:4D, separately by sex. Among females, there was no evidence for a significant relationship, whether the association was tested using parametric or nonparametric statistics. Among males, there was a trend toward a significant negative relationship using Pearson *r* ($r = -0.58$, $P < 0.07$) and a significant negative relationship using Spearman *ρ* ($\rho = -0.78$, $P < 0.01$). Fig. 1 plots this relationship between 2D:4D and testosterone in the male animals. If the outlier indicated by the asterisk is removed from the data set, the product moment correlation more closely agrees with the rank-order correlation, $r = -0.88$, $P < 0.01$. Since the outlier from the regression line is also an outlier for 2D:4D, it will be excluded from subsequent analyses (this exclusion reduced the effect size for the sex difference in 2D:4D to 0.79, $t\ (28) = -2.11$, $P < 0.05$).

Multiple regression analyses were undertaken to test whether the relationship between 2D:4D and testosterone persisted exclusive of the influence of age, body size, and dominance rank. Age and rank were highly correlated in this sample (Maestripieri et al., under review), with younger males being higher ranking. Likewise, the two measures of body size—crown–rump length and weight—were highly correlated ($r = 0.78$, $P < 0.05$). To avoid problems of

Table 1
Mean digit length ratios presented by sex

	Male		Female		Effect size	*t*
	Mean	SEM	Mean	SEM		
2D:3D	0.82	0.01	0.83	0.01	0.11	0.30
2D:4D	0.88	0.01	0.83	0.01	1.00	−2.57*
2D:5D	1.00	0.02	1.04	0.02	0.35	0.90
3D:4D	1.07	0.02	1.00	0.01	1.09	−2.81**
3D:5D	1.22	0.02	1.25	0.03	0.34	0.87
4D:5D	1.15	0.03	1.25	0.02	1.12	2.89**

Note. The second to fifth digits are denoted 2D, 3D, 4D, and 5D, respectively, and ratios represent the length of the first digit listed divided by the length of the second (e.g., 2D:3D denotes the length of the second digit divided by the length of the third digit).
* $P < 0.05$.
** $P < 0.01$.

multicolinearity, the correlated measures were never entered into the same regression model. None of the above variables was significantly correlated with 2D:4D.

Table 2 presents the results of one such regression analysis. It can be seen that 2D:4D continued to predict large amounts of variance in male testosterone after the effects of dominance rank and weight had been held constant. Rank and weight, furthermore, accounted for no variance at all. The null effect of rank suggests that testosterone levels were not distorted by differential endocrine responses to capture by animals of differing social status (see Sapolsky, 1982). The effect of 2D:4D on testosterone remains if age is substituted for rank or crown–rump length for weight and persists regardless of which combination of independent variables is entered in prior steps (all Ps < 0.01). Finally, an exploratory analysis found no evidence for significant correlations in either sex between testosterone and digit ratios other than 2D:4D.

Testosterone levels were not significantly associated with number of copulations among animals of either sex. Among females, there was likewise no evidence for a significant association between 2D:4D and number of copulations. Among males, number of copulations was highly skewed (range from 0 to 40), with two of the high-ranking animals having performed the majority of the copulations (Maestripieri et al., under review). Although such skew makes interpretation of inferential statistics difficult, there was a significant and positive rank-order correlation between male 2D:4D and number of copulations, $\rho = 0.64$, $P < 0.05$ (the Pearson product moment correlation did not approach significance). Unlike the relationship between 2D:4D and testosterone, however, this correlation did not persist exclusive of the effects of rank or age insofar as there were no significant rank-order correlations between 2D:4D and the residuals from models that had regressed number of copulations on either rank or age.

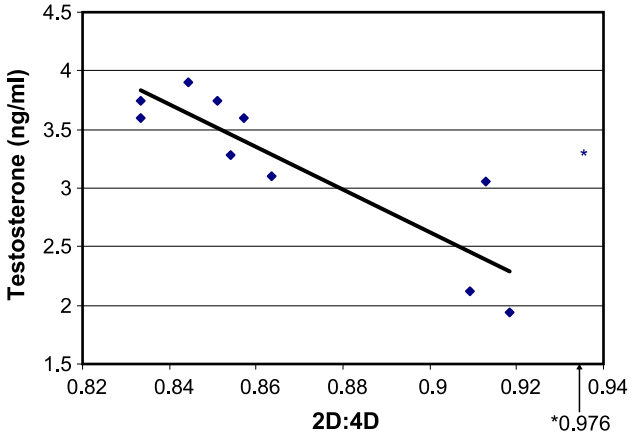

Fig. 1. Relationship between 2D:4D and serum testosterone among male Guinea baboons. The least-squares regression line excludes the outlier indicated by the asterisk.

Table 2
Multiple regression model predicting testosterone levels in the male Guinea baboons

Step	Variable	Beta	F (Δr^2)	r^2 (adj.)
1	Dominance rank	0.115	0.108	0.000
2	Weight	−0.253	0.483	0.000
3	2D:4D	−0.857	19.01**	0.668

Note. Each model includes all of the variables entered on the previous steps.
** $P < 0.01$.

Discussion

Substantial sex differences in finger length ratios were found in the right hands of a group of captive Guinea baboons. Contrary to the pattern reported in humans (e.g., McFadden and Shubel, 2002) and to our prediction, however, the digit ratios showing the largest sex differences were higher in males than in females. The sex difference in 2D:4D was among the ratios showing this reversal of the human pattern. Such a reversal was also found in the ratio of the second to fourth metacarpals in the left hands of chimpanzees (McFadden and Bracht, under review), although other metacarpal ratios have generally been lower in males than in females in analyses of primate skeletons (McFadden and Bracht, 2003). The present work thus complements existing evidence that digit ratios may serve as markers for developmental sex differences in nonhuman primates as well as in humans. The variable directions of the sex differences, however, suggest potential species differences in the causal relationships between developmental mechanisms and sex-differentiated digit length patterns.

The present investigation provides the first evidence for associations between digit ratios and sex hormones in a nonhuman primate. Consistent with some findings for human males (Manning et al., 1998), 2D:4D was a significant negative predictor of testosterone levels in male baboons. The correlation between adult testosterone and 2D:4D in humans has been attributed to the effect of prenatal androgens on 2D:4D in conjunction with a presumed correlation between prenatal and adult testosterone levels (e.g., Manning, 2002). If the same causal scenario were applicable in baboons, our data would imply that prenatal androgen levels (as indexed by 2D:4D) are better predictors of adult testosterone than such factors as age (at least among adults), dominance rank, and body size. Alternatively, it is possible that circulating testosterone levels affect 2D:4D postnatally among males of this species.

The association between 2D:4D and testosterone should be considered a preliminary finding, due to both the small sample size and the fact that we were only able to collect one testosterone measure per individual. The known pulsa-

tility and diurnal variation in serum testosterone levels in male baboons (Castracane et al., 1981) suggest caution in the interpretation of single measurements as indicative of stable individual differences in testosterone. Likewise, testosterone responses to the stress of capture (e.g., Sapolsky, 1982) raise the possibility that 2D:4D may actually predict hormonal reactivity to stress more than stable individual differences in hormone levels. Future work will be necessary to both replicate the current finding and sort out issues related to differences in baseline hormone levels versus differences in hormonal reactivity.

Testosterone levels were not associated with number of copulations in either sex in this sample, which is consistent with previous research on baboons (Bercovitch, 1999). The significant rank-order correlation between 2D:4D and number of copulations among males was in the opposite direction from what might have been expected from human data (cf. Manning, 2002)—male baboons with lower 2D:4D were observed to participate in fewer copulations. The importance of this relationship, however, is called into question by the fact that 2D:4D did not explain significant variance in number of copulations beyond the influence of either age or dominance rank.

Although higher androgen levels were associated with lower 2D:4D in males, males nonetheless showed higher 2D:4D than females. Males of this species are almost certainly exposed to higher prenatal androgen levels than are females, and as such, if prenatal androgens reduce 2D:4D, one would expect the sex difference in this ratio to be the opposite of that which was found. Many explanations might account for this pattern. Genes on the Y chromosome, for instance, might increase 2D:4D even as androgens tend to reduce it (cf. Arnold, 1996). Likewise, other neurotransmitters or hormones that are potentially sexually dimorphic (e.g., growth hormone) might decrease 2D:4D in females and/or increase it in males. Alternative relationships between 2D:4D and testosterone are possible in this species, such as a positive association between perinatal androgens and 2D:4D combined with negative relationships between prenatal and adult testosterone levels among males. Nonlinear effects of prenatal androgens might also account for the pattern reported here if, for example, intermediate levels were associated with high 2D:4D and low and high androgen levels were associated with low 2D:4D. Such nonlinear effects of androgens are not unprecedented, as intermediate testosterone levels have been associated with higher levels of performance on spatial cognition tasks in humans (Kimura, 1999). Explanations such as these might be tested within studies that manipulate pre- and neonatal androgen levels in nonhuman primates (e.g., Herman et al., 2000). The correlation between 2D:4D and testosterone in the present study suggests that digit length measurements may be a profitable addition to ongoing studies that perform such manipulations.

In sum, our data begin to provide a comparative context for the human literature on digit ratios. On the one hand, the negative correlation between testosterone and 2D:4D in the male animals suggests the possibility that the relationship between this ratio and sex hormones is phylogenetically well-conserved. On the other hand, the reversal of the human sex difference in 2D:4D suggests interspecific diversity in the relationship between digit ratios and mechanisms of sexual differentiation. Future work on nonhuman species could enrich this comparative context and thus provide insights into the evolution of digit ratios as possible markers of human developmental processes.

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