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Different ontogenetic patterns of testosterone production reflect divergent male reproductive strategies in chimpanzees and bonobos

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HIGHLIGHTS

- Bonobos exhibit minimal increases in testosterone during puberty, unlike chimpanzees.
- Increases in testosterone with age are clear among chimpanzee, but not bonobo, males.
- Bonobos' stable testosterone levels are likely tied to reduced mating competition.
- Testosterone production across development may be shaped by adult mating strategies.

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ABSTRACT

Male reproductive effort is often strongly related to levels of the steroid hormone testosterone. However, little research has examined whether levels of testosterone throughout development might be tied to individual or species differences in the reproductive strategies pursued by adult males. Here, we tested the hypothesis that inter-specific differences in male reproductive strategy are associated with differences in the pattern of testosterone production throughout early life and puberty. We compared testosterone levels from infancy to adulthood in two closely related species where levels of mating competition and male–male aggression differ significantly, bonobos (*Pan paniscus*) and chimpanzees (*Pan troglodytes*). We predicted that the reduction in male mating competition found in bonobos would be accompanied by a lesser developmental increase in testosterone production. We performed radioimmunoassay of salivary testosterone levels in a mixed-longitudinal sample of both species, collected from individuals living in semi free-ranging populations. This allowed us to examine the effects of development in a more naturalistic setting than possible in a zoo or laboratory. We found that among chimpanzees, testosterone levels declined slightly from infancy to juvenility, then remained low until increasing markedly during adolescence (with pubertal increases most pronounced among males). In contrast, there was little change in testosterone production with age in bonobos of either sex, with levels of testosterone consistent throughout infancy, juvenility, and the transition to adulthood. Our data are therefore consistent with the hypothesis that the ontogenetic pattern of testosterone production can be subject to rapid evolutionary change, shifting in association with species differences in male reproductive strategy.

1. Introduction

Investment in reproduction among males can be divided into both the production of gametes and the allocation of energy towards somatic and behavioral strategies that facilitate mating opportunities [1,2]. The steroid hormone testosterone (abbreviated as T) is particularly important in influencing these latter two elements of male reproductive strategy, increasing muscle mass, enhancing libido, and stimulating aggressive and dominance behaviors in a given season or situation [3–6].

While the association between testosterone and male reproductive effort has been well-documented in adults of numerous taxa, our understanding of how development mediates this relationship is less clear. According to life history theory, the production of testosterone across ontogeny should differ between species or individuals to facilitate the optimal allocation of energy toward growth, maintenance, and reproduction across the lifespan [1,7]. Since high levels of testosterone can have a deleterious effect on the immune system [2,8], production of testosterone may be minimized in situations or life stages where it is not sufficiently advantageous [5,9]. Accordingly, testosterone levels typically remain low during juvenility, only beginning to increase at puberty in conjunction with reproductive maturation [10–12]. Despite this general developmental pattern being present across

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mammals, there may be important species differences in the precise patterns of testosterone production throughout development that reflect divergent male reproductive strategies in adulthood. This possibility is particularly compelling in light of the growing body of evidence that phenotypic changes between species commonly arise through evolutionary shifts in developmental trajectories [13–15].

Several studies have begun to investigate whether individual and species-level variation in the ontogeny of androgen production exist in association with differing adult reproductive strategies, using non-human primate models to examine these effects over an extended period of ontogeny. Within mandrills (*Mandrillus sphinx*) and chacma baboons (*Papio hamadryas ursinus*), individual differences in the production of testosterone during puberty have been found to correlate with dominance ranks among adult males [16,17]. Similarly, in orangutans (*Pongo pygmaeus*), males who retained subadult body size into adulthood (a viable strategy in this species to obtain sneaky mating opportunities without overt physical competition) were found to show smaller increases in testosterone during adolescence than males who developed their body size fully [18]. In addition, differences between baboon species in the timing and magnitude of the pubertal testosterone increase have been found to reflect inter-specific variation in the length of alpha male tenure and the association between rank and mating success [19]. These results support the hypothesis that within and across species, variation in the developmental trajectory of androgen production is central to the relationship between testosterone and reproductive effort among adult males.

These prior studies of testosterone production throughout development have largely focused on the pubertal increase in testosterone levels, since the period of adolescence represents an important transition between an individual's focus on growth and its focus on reproduction. However, individuals or species may also vary in their production of testosterone even before puberty. In a number of species, from humans to yellow baboons (*Papio cynocephalus*) and cotton-top tamarins (*Saguinus oedipus*), males and females show a neonatal elevation in testosterone that lasts for the first few weeks or even months after birth [11,20,21]. Though there is considerable debate about the function of this neonatal testosterone elevation [22,23], one possibility is that variation in its duration or magnitude contributes to differences in reproductive capabilities among adult males [20,24].

Here we test the hypothesis that species differences in male mating strategy are associated with variation in the ontogenetic patterns of testosterone production across the entire lifespan. We do so by comparing testosterone levels from infancy into adulthood in two closely-related ape species, bonobos (*Pan paniscus*) and chimpanzees (*Pan troglodytes*). Chimpanzees and bonobos provide an ideal test case for this hypothesis, as they have been found to differ in both their reproductive strategy and in broader aspects of their ontogeny, despite having diverged from one another as recently as 850 kya [25].

Differences in male reproductive strategy between chimpanzees and bonobos appear to derive largely from the increased social gregariousness and sexual receptivity of bonobo females relative to chimpanzee females, presuming that the last common ancestor of the two species was chimpanzee-like [26,27]. Bonobo females associate with males and engage in extensive non-conceptive sexual behavior throughout their menstrual cycle, while associations between male and female chimpanzees peak during the period of female sexual swelling, with copulations largely limited to this period [28–31]. Correspondingly, it has been argued that competition for dominance rank and coercive aggression are less effective means of obtaining conceptive mating opportunities for bonobo males than for male chimpanzees [27,32–34]. In support of this argument, bonobo males exhibit less frequent and less severe displays of aggressive behavior of all types than chimpanzee males, be it intra-group aggression, inter-group aggression, or even inter-specific predation [26,35–40]. Bonobos have also been found to show a lesser sex difference in androgen production relative to chimpanzees, lesser increases in male androgen levels when females are peri-ovulatory than found among male chimpanzees, and a weaker correlation on the whole between basal testosterone level and dominance rank among adult males [41–44]. These two species therefore provide an excellent opportunity to test whether divergent male reproductive strategies in closely-related taxa are associated with broader differences in their endocrine maturation.

In addition to their divergent reproductive strategies, bonobos and chimpanzees have been found to differ in numerous aspects of their development. In particular, bonobos exhibit delays in development relative to chimpanzees in features of their morphology [45,46], behavior [14,15,47], and cognition [14]. These distinctions provide support for the possibility that the ontogenetic pattern of testosterone production has also shifted between these two species, given the evidence from numerous taxa that hormones are a central mechanism in facilitating the pace of developmental transitions [48,49].

No evidence exists at present to compare endocrine maturation between bonobos and chimpanzees, since to our knowledge no prior study of bonobo endocrine ontogeny has been performed. Existing studies of endocrine maturation in chimpanzees have consistently found that male testosterone levels increase during puberty, with these increases coinciding with growth in body weight and testicular volume [50–57]. In captive populations, male chimpanzees begin to show elevations in testosterone between 6 and 7 years of age [50–52,54,57], with the onset of spermatogenesis occurring between 7 and 9 years of age [58,59]. The only existing study of testosterone development among a small sample of wild chimpanzees indicates a similar developmental increase, occurring at a slightly later age [56]. Despite the relatively large number of studies documenting patterns of testosterone production throughout chimpanzee development, few have incorporated individuals from a broad developmental window (encompassing infancy, juvenility, adolescence, and adulthood). Moreover, these studies have primarily been conducted in laboratory environments, where asocial or minimally social housing conditions may have diminished any effects of dominance rank or social behavior on testosterone production [60]. This study represents one of the first opportunities to investigate testosterone production in chimpanzees ranging from infancy into adulthood, utilizing semi free-ranging study populations where individuals live in mixed-age and sex groups closely resembling those found in the wild [61].

Our major prediction was that bonobos would show a lesser developmental increase in testosterone production than chimpanzees, given their lesser mating competition as adults and their maintenance of numerous juvenile characteristics in adulthood [15,27]. Our alternative hypothesis was that bonobos and chimpanzees would differ little in their ontogeny of testosterone production, given their genetic similarity [25]. We tested these predictions by measuring salivary testosterone levels from infancy into adulthood among bonobos and chimpanzees, making it possible for us to examine the contributions of both neonatal and pubertal testosterone elevations to the overall trajectory of testosterone production in each species. We examined testosterone in both sexes to assess the degree to which male patterns of development diverged from those of females.

2. Materials and methods

2.1. Subjects

Subjects for this research were chimpanzees living at the Tchimpounga Chimpanzee Sanctuary in Pointe Noire, Congo Republic and bonobos living at Lola ya Bonobo in Kinshasa, Democratic Republic of Congo. Both facilities house semi free-ranging ape populations living in mixed age and sex groups that have access to forest enclosures during the day and sleep in dormitories at night. Apes at these sites are provisioned but have access to natural food items in their primary forest enclosures. Although these apes are largely orphans of the bushmeat trade, there is little evidence for any deficits in their behavior

or cognition [61]. In addition, we have demonstrated that orphans at Tchimpounga and Lola ya Bonobo show comparable baseline cortisol levels to mother-reared individuals born at these sites, suggesting minimal physiological impacts of potential early life stress in the orphan individuals [61]. Moreover, any such effects of early life stress would be controlled for in our cross-species comparison, since individuals of both species arrive at these sites at a comparable age and are reared in similar circumstances upon arrival according to guidelines of the Pan-African Sanctuary Alliance, of which both sites are members [61,62]. Because subjects' exact ages were not known (other than for those individuals born on-site), estimates were made based on comparisons of weight and dental emergence patterns to published values both at the time of the individual's arrival at the sanctuary and at the time of data collection [63–65]. These estimates allowed us to be confident of subjects' ages to the year; we also placed individuals in wider age categories (see below), which conferred an even greater degree of certainty in the assignment of individuals to a particular age group.

In total, samples were collected from 77 chimpanzees (41 male, 36 female) and 53 bonobos (29 male, 24 female) (Table 1). Individuals ranged in age from 1 to 24 years over the three years of sampling (chimpanzees: mean age 8.6 years, median age 7.0 years; bonobos: mean age 8.4 years, median age 7.0 years; there was no species difference in the ages sampled, independent samples t-test). These ages spanned infancy and adulthood in both species but did not include any individuals that could be considered geriatric. For our statistical analyses, we created a categorical factor for age (Age Category) so that we could perform post-hoc comparisons between age groups to examine the precise timing of increases in testosterone. The Age Category factor had four levels, in line with general patterns of aging observed in chimpanzees and bonobos [66–69]: infant (1 to 4 years, $n = 39$), juvenile (5 to 8 years, $n = 84$), subadult (9 to 12 years, $n = 50$), and adult (13 years and above, $n = 38$).

2.2. Saliva sampling

Samples for endocrine analysis were collected during the summers of 2007, 2008, and 2009. Each individual was represented by at least one sample, with a range of 1 to 25 samples collected per individual in a given year (chimpanzees: mean 8.0 samples per year, median 8.0 samples, range 1 to 25; bonobos: mean 4.7 samples per year, median 4.0 samples, range 1 to 9), and a total of 1392 samples collected (Table 1). Individual chimpanzees were sampled more frequently than individual bonobos in any given year (independent samples t-test on the number of samples for each individual in each year, $t(209) = 6.59$, $p < 0.001$). Procedures were taken in the statistical analysis to account for this unbalanced sampling (see below).

Samples for a given individual in a given year were all collected within a 2-month period. Certain individuals were sampled in multiple data collection seasons (29 of the 77 chimpanzees and 34 of the 53 bonobos), with this repeated sampling controlled for in our statistical analysis (see below). Saliva samples were collected throughout the day (chimpanzees: mean and median hour of sampling 12:00, range 7:42 to 17:10; bonobos: mean and median hour of sampling 11:00, range 6:24 to 16:01). Previous research has shown circadian variation in androgen production among chimpanzees [70,71]. To control for any potential effects of circadian variation on androgen levels, we entered hour of sample as an effect in our statistical analyses. Note that samples were collected significantly earlier in the day for bonobos than for chimpanzees (independent samples t-test across all samples collected, $t(1390) = 5.99$, $p < 0.001$), necessitating caution in any comparison of absolute testosterone level between the two species.

Identical procedures were followed for collection and storage of the saliva samples in all three data collection seasons, as described previously [72]. Saliva collection protocols and radioimmunoassay of testosterone also followed previously published methods [72]. In brief, 50 μl of 0.1% sodium azide solution was added to each saliva sample immediately after collection to prevent contamination and to allow samples to be kept at room temperature until being returned to the laboratory [73]. Salivary testosterone measurements were made in the Reproductive Ecology Laboratory at Harvard University using an I-125 based radioimmunoassay kit (#4100, Diagnostic Systems Laboratories, Webster, TX, USA) with the following modifications: standards were prepared in assay buffer and run at six concentrations from 2 to 375 pg/ml. Samples were added in 100 μl amounts together with 300 μl of assay buffer. First antibody (20 μl) and labeled steroid (50 μl) were added to each tube to yield a total reaction volume of 470 μl per tube. After overnight incubation at 4 °C, 500 μl of second antibody was added to each reaction tube. Reaction tubes were subsequently centrifuged for 45 min; after aspiration of the supernatant, tubes were counted in a gamma counter for 2 min. In pilot assays using the standard human assay protocol, the ape testosterone values were too high to be readable in the assay range. Therefore, we reduced the sample aliquot to 100 μl of ape saliva (from 200 μl for human saliva) in order to be able to read the values on the same standard curve as employed in the human testosterone radioimmunoassay protocol. Assays were counterbalanced according to species, sex, and age. Cross-reactivity of the testosterone RIA kit with other steroids is as follows: 6.6% with 5α-dihydrotestosterone, 2.2% with 5-androstane-3β,17β-diol, 1.8% with 11-oxotestosterone, 0.9% with androstenedione, and 0.6% with 5β-dihydrotestosterone. Cross-reactivity with all other steroids is 0.5% or less.

It is important to emphasize that the RIA we utilized is highly specific for testosterone, showing extremely low levels of cross-reactivity with other androgens. Unlike analyses of urine, which rely on measurements of steroid metabolites, free (unbound) steroids diffuse directly from the blood into the saliva, causing salivary and serum steroid measurements to be highly correlated both in humans and in other species [71,74–76]. Because the RIA is sensitive to the steroid itself and steroids are identical in structure across mammals, published cross-reactivities of a particular antiserum are the same across species. Moreover, in specifically validating the use of a commercially-available testosterone RIA kit with chimpanzee saliva, a previous study [71] demonstrated that 1) measurements of testosterone from salivary RIA strongly correlate with those obtained from serum, and 2) measurements of salivary testosterone from RIA strongly correlate with those obtained by liquid chromatography–tandem mass spectrometry (LC–MS) [71]. It can thus be concluded that RIA selectively measures testosterone in

Table 1
Characteristics of the subjects that participated in saliva sampling, divided by species and sex. The number of individuals that contributed saliva samples in at least one year and those that contributed samples for multiple (two or three years) are shown for each subgroup. We also show, for each subgroup, the mean age (along with the age range) and the mean number of samples per individual per year (along with the range of samples collected per individual in a given year).

Group	Number of individuals sampled		Age range	Samples per individual in each year
	In at least one year	In multiple years		
Chimpanzee males	41	18	2 to 21 years (mean 9.0 years)	1 to 20 samples (mean 7.3 samples/year)
Chimpanzee females	36	11	2 to 18 years (mean 8.1 years)	1 to 25 samples (mean 9.0 samples/year)
Bonobo males	29	18	3 to 24 years (mean 8.5 years)	1 to 9 samples (mean 5.1 samples/year)
Bonobo females	24	16	1 to 23 years (mean 8.3 years)	1 to 8 samples (mean 4.1 samples/year)

non-human ape saliva, and does not bind significant fractions of other androgens or their metabolites. Salivary methods have now been successfully used to quantify steroid levels in numerous non-human primate species [77–80], making this an exciting direction for future research.

The quality control samples (QC) used for the assays were changed after the data from 2007 were analyzed, so coefficients of variation (CV) are reported separately for that year and for the two subsequent years (2008 and 2009). For assays run in 2007, the average intra-assay CV was 8% and the average inter-assay CV was 16%. For assays run in 2008 and 2009 combined, the average intra-assay CV was 10% and the average inter-assay CV was 15%. There were no significant differences in the QC values between 2008 and 2009 for either the low QC (Mann–Whitney U test given the small sample size, $Z = 0.47$, $p = 0.6$) or the high QC ($Z = 0.78$, $p = 0.4$), suggesting that assay characteristics did not vary significantly between years.

2.3. Sampling of body weight

To provide an empirical measure of growth to complement our Age Category measure, we also examined the relationship between testosterone and body weight. For this analysis, we were able to obtain weights taken in the same month as saliva sampling for a number of individuals who were younger than 9 years ($n = 55$ weights across the three years of data collection, taken from 42 individuals). However, individuals who were 9 years and older could only be weighed when anesthetized. Thus for these individuals, we utilized weights obtained from an annual health check performed within 6 months of saliva sampling ($n = 23$ weights from 23 individuals). Because individuals who were 9 and older were likely growing less rapidly than the younger age group, this 6-month weight estimate provided the best available proxy for their weights at the time of saliva sampling.

2.4. Sampling of dental emergence

To provide yet another independent measure of general maturation, we examined the relationship between testosterone and an individual's level of dental development. Namely, we performed a visual inspection of subjects' tooth emergence, recording the emergence of permanent teeth for the majority of individuals sampled in the hormone analysis ($n = 155$ dental category measures, taken from 99 individuals). Based on previously-documented patterns of dental emergence, which are identical in sequence between chimpanzees and bonobos [64,81,82], we created 6 dental categories: no permanent dentition ($n = 11$), first molar (M1) only ($n = 30$), permanent incisors only ($n = 13$), second molar (M2) only ($n = 34$), permanent canine only ($n = 2$), and third molar (M3) emergence/complete adult dentition ($n = 65$). We treated the presence of either a mandibular or maxillary tooth as sufficient for placement of the individual into a given category, and we grouped together both permanent incisors (I1 and I2) into our "incisors only" category. Because only two individuals who were possible to sample for dental emergence fell into the "permanent canine only" category, we grouped these individuals together with the "M3" category for our statistical analysis. Note that while these dental categories provided numerous classifications for young individuals, they did not provide a way to distinguish between age groups of individuals that were fully dentally developed (e.g., a 10-year-old and a 20-year-old would both be classified as dentally mature).

2.5. Statistical analysis

As is typical with hormone values, our raw testosterone data exhibited significant skew. We log-transformed the steroid values to normalize the data, enabling the use of parametric statistics (a histogram of log testosterone values is provided in Supplemental Fig. 1). We analyzed the data using Linear Mixed Models (LMM), performing these analyses with the nlme package [83] in R v 2.13.2 [84].

We performed statistical analyses separately by species, for two main reasons. First, comparisons of absolute steroid level across species have limited significance without information on receptor density in each species. Second, performing the analyses separately by species allowed us to control for differential sampling patterns between the two. As mentioned above, chimpanzee individuals were sampled more frequently in any given year than were individual bonobos. Moreover, bonobos tended to be sampled earlier on in the day relative to chimpanzees. Our comparisons thus focused instead on the patterns of development within each species.

Our linear mixed model was constructed as follows. Within each species, we were primarily interested in the effect of age on testosterone values, entering a fixed effect of Age Category (with the groupings for this measure described above). We also needed to take into account the predicted effect of sex on testosterone values, and so we entered Sex as a fixed effect in our model. Finally, to account for any circadian effects on testosterone, we also entered Hour of Sample (denoting the hour of the day at which each sample was taken) as a fixed effect. To take into account the fact that repeated samples were collected from the same individuals and certain individuals were sampled over multiple years, we added a random effect to the model of individual nested within year. In addition, given the significant autocorrelation present in the data (again, since individuals were sampled repeatedly), we used a first-order autoregressive-moving-average (ARMA) term in the model to account for this structure of the variance. We used Akaike's Information Criteria (AIC) values and comparisons of log-likelihood ratios to evaluate these predicted models relative to other credible models, as described below. For the predicted model in each species, we report the parameter estimates and their standard errors, t-values, and p-values. We used maximum likelihood estimates to compare models, but report values calculated with restricted estimate maximum likelihood in tables describing model parameter estimates [83]. To investigate differences between specific age categories post-hoc, we used Tukey's Honestly Significant Difference tests.

We performed analyses first with the full data set and then with two subsets of the data. One subset encompassed only data from 2008 and 2009, excluding samples from 2007 since these were collected in conjunction with a behavioral experiment [72]; the 2008/2009 data were analyzed for both chimpanzees and bonobos. In addition, we noted in our preliminary examinations of the data that one of our female chimpanzee subjects had extremely high testosterone values in both of the years she was sampled (as can be seen in Fig. 1). To ensure that this individual did not bias our analyses, our second subset analysis examines the chimpanzee data excluding the values from this individual.

Finally, to ensure that we had adequate power to detect significant effects of age on testosterone in each species, we also performed a power analysis using the program GPower (Version 3.1.3).

3. Results

Though our analyses were performed with log-transformed testosterone (T) values, untransformed values are shown in Fig. 1 for illustrative purposes. For both chimpanzees and bonobos, the predicted models included the following terms: fixed effects of Age Category (4 levels, as described above), Sex (male/female), and Hour of Sample, and random effects of individual nested within year and a first-order ARMA term.

For chimpanzees ($n = 971$), analysis with the predicted model revealed significant effects of Age Category, Sex (with males having higher log T than females), and Hour of Sample (with log T values decreasing throughout the day) (Table 2). Post-hoc comparisons of the differences between age categories revealed that adults had higher log T than infants (Tukey's HSD, $p = 0.005$), and juveniles (Tukey's

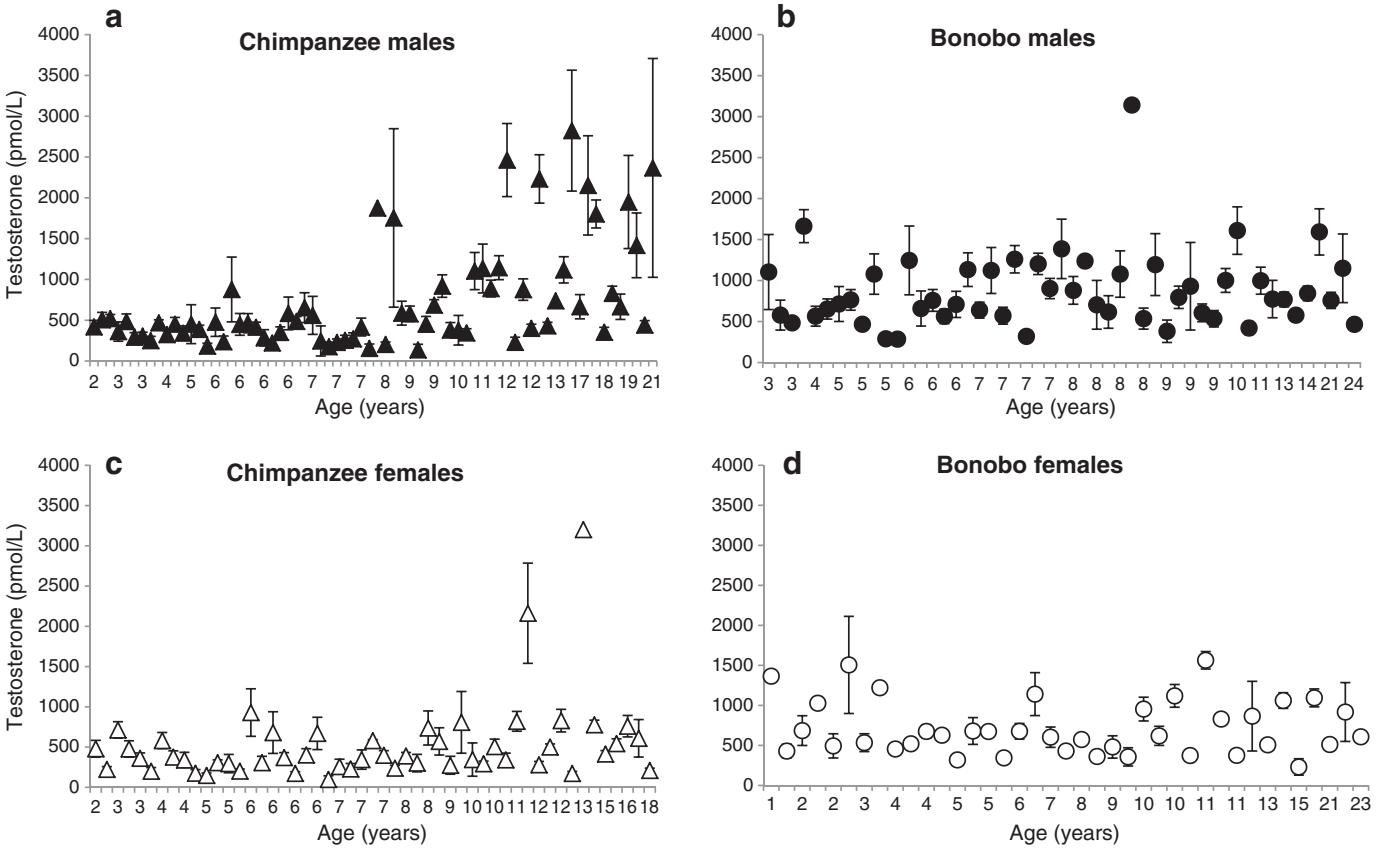

Fig. 1. Average testosterone levels according to age for a) chimpanzee males, b) bonobo males, c) chimpanzee females, and d) bonobo females. Yearly averages for each individual are shown, together with standard errors around those individual averages. Individuals are ordered according to increasing age in each species and sex. All graphs are shown on the same scale. Actual testosterone values (in pmol/L) are shown here, though log-transformed values were used for the statistical analyses. Bonobos changed little in testosterone with age in either sex, while in chimpanzees there was a slight increase in testosterone with age in females and a more dramatic increase in testosterone with age among males.

HSD, $p < 0.001$) while subadults also had higher log T than juveniles (Tukey's HSD, $p < 0.001$) (Fig. 2).

The main effects of Age Category and Hour of Sample were still present among chimpanzees when removing data obtained in 2007 (since data in this year were collected in conjunction with a behavioral experiment), though the main effect of Sex became non-significant in this subset of the data ($n = 546$, Supplemental Table 1). Meanwhile, all three main effects (Sex, Age Category, and Hour of Sample) were still present among chimpanzees ($n = 948$) after removing a female outlier with much higher testosterone values than other females (see Fig. 1), though removing this individual did increase the magnitude of the effect of Sex on log T (Supplemental Table 1).

In comparing the predicted model to other potential models for chimpanzees, we found that adding an interaction term between Age Category and Sex did not significantly improve model fit, despite the theoretical rationale that the sexes should increase differentially in their testosterone levels with age (the AIC values of the two models differed by less than 1 point, and a comparison of the two models' log-likelihood values was not significant). The predicted model fit the data from chimpanzees significantly better than a null model including only random effects (Likelihood ratio test = 50.546, $p < 0.001$), or models removing any one of the terms (Likelihood ratio tests, p values < 0.05).

For bonobos ($n = 421$), analysis with the predicted model revealed significant effects of Sex (with males having higher log T than females),

Table 2
Effects of predictor variables on log testosterone in chimpanzees and bonobos using age categories. Restricted estimate maximum likelihood models investigating patterns of log testosterone production were performed separately for chimpanzees ($n = 971$) and bonobos ($n = 421$) with Age Category, Sex, and Hour of Sample as fixed effects. Random effects took into account the fact that repeated samples were collected from individuals, and certain individuals were sampled across multiple years. Values for the Sex factor are shown relative to males as a reference category, while values for Age Category are shown relative to infants as a reference category. Significant effects are indicated as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Chimpanzees showed a main effect of Age Category, with adults having higher log testosterone values than younger individuals, while no effect of Age Category was present among bonobos.

	Chimpanzees				Bonobos			
	Estimate	SE	t-Value	p-Value	Estimate	SE	t-Value	p-Value
Intercept	2.791	0.090	31.007	<0.001***	2.676	0.131	20.397	<0.001***
Hour of Sample	−0.013	0.004	−3.442	<0.001***	0.016	0.006	2.857	0.005**
Sex: female	−0.109	0.048	−2.287	0.024*	−0.096	0.040	−2.430	0.017*
Age cat: juvenile	−0.107	0.074	−1.454	0.149	−0.004	0.053	−0.083	0.934
Age cat: subadult	0.122	0.078	1.553	0.123	0.053	0.059	0.899	0.371
Age cat: adult	0.256	0.085	3.022	0.003**	0.073	0.066	1.106	0.272

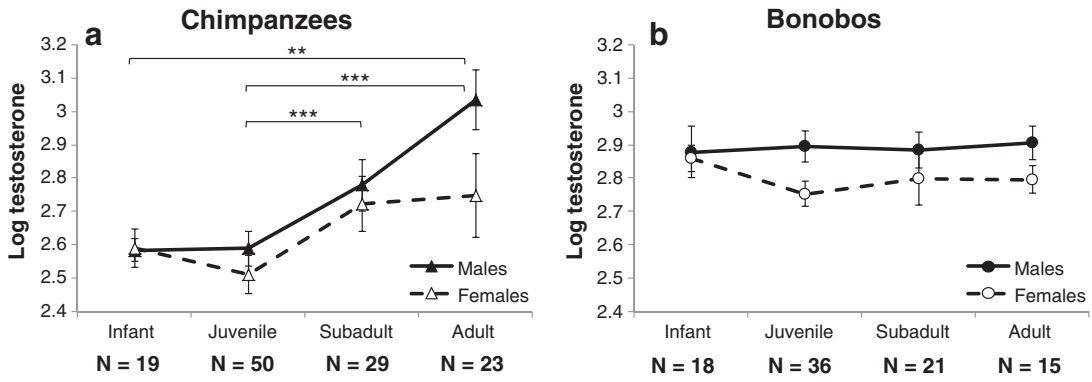

Fig. 2. Log testosterone levels across development in a) chimpanzees and b) bonobos. Average log testosterone values and sample sizes are shown for each age category, excluding one individual outlier within female chimpanzees. Significant post-hoc comparisons between age categories (corrected for multiple comparisons using Tukey's HSD) within chimpanzees are denoted as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. There were no significant differences in testosterone level between age groups in bonobos. Statistical comparisons were performed with both sexes pooled; values are shown separately by sex here for illustrative purposes.

and Hour of Sample (with a marginal increase in log T values throughout the day), but no significant effect of Age Category (Table 2). Post-hoc comparisons also revealed no significant differences in log testosterone between any age categories among bonobos. In line with previous work [43], the effect of Sex appeared to be stronger in chimpanzees than in bonobos (Fig. 2), but statistically the effects of Sex were comparable in each species (Table 2), even when removing the high-T female chimpanzee outlier (Supplemental Table 1).

The main effects of Sex and Hour of Sample remained significant among bonobos even when removing the data taken in 2007 from the analyses, while the effect of Age Category remained non-significant in this subset of the data ($n = 304$, Supplemental Table 1).

In comparing the predicted model to other potential models for bonobos, we found that adding an interaction term between Age Category and Sex resulted in poorer model fit (with a 6-point increase in AIC value upon adding the interaction term, though the comparison of the two models' log-likelihood values was not significant). The predicted model fit the data significantly better than a null model including only random effects (Likelihood ratio test $= 13.956$, $p = 0.016$), but when removing specific terms from the model the removal of the Age Category term resulted in improved model fit (with a 3-point decrease in AIC value, though the comparison of the two models' log-likelihood values was not significant). This indicated, as suggested by our analyses with the predicted model, that the Age Category term was unsuccessful in accounting for observed variance in bonobo log testosterone values. Meanwhile, similar to chimpanzees, the removal of Sex, Hour of Sample, or either of the random effect terms from the model resulted in poorer fit of the model to the bonobo data (Likelihood ratio tests, p values < 0.05).

To ensure that these findings did not result from our smaller bonobo sample size, we also performed analyses of effect size and power. To enable the use of a power analysis, we examined only the fixed effect of age category in a one-way ANOVA of log testosterone performed for each species. This ANOVA revealed the predicted effect of Age Category in chimpanzees ($F(3,967) = 57.633$, $p < 0.001$), with a high effect size and power ($f^2 = 0.423$, power $= 1.000$). Meanwhile, as predicted, there was no effect of Age Category in a one-way ANOVA on log T in bonobos ($n = 421$, $p > 0.1$). Assuming that bonobos were to show a similar effect size of Age Category to that found in chimpanzees, the sample size needed to obtain a significant ($p < 0.05$) result with reasonable power (>0.8) would have been 68, indicating that our sample of 421 data points from bonobos was large enough to have detected such an effect. In addition, even if bonobos were to show a weaker relationship between age and testosterone (as might be expected given the known differences in the male dominance rank–testosterone relationship between the two species), our sample size gave us the ability to detect any effect size with an f^2 greater than 0.16, or an effect less than half as strong as the one found in chimpanzees. We can thus conclude that our sample size from bonobos was more than adequate to detect even a weak relationship present between age and testosterone.

To ensure that our analyses were not biased by imprecise age estimates, we also analyzed the data using two empirical measures of growth — body weight and dental category. Because weights and dental categories were not available for all individuals sampled, this involved analyzing a subset of the data (see Materials and methods). When using body weight as the relevant developmental metric instead of Age Category (entering Bodyweight as a fixed effect in the model together with Sex and Hour of Sample), chimpanzees ($n = 422$) again showed a significant effect of Bodyweight on log T, in addition to the effects of Sex and Hour of Sample. Meanwhile, in bonobos ($n = 165$), there was no effect of Bodyweight on log T (Table 3). Similarly, when using dental category as the relevant developmental metric instead of Age Category, there was a main effect of Dental Category on log T in chimpanzees ($n = 830$) but not bonobos ($n = 296$). Notably, this analysis revealed that adult (dentally mature) chimpanzees had higher log testosterone levels than every other dental category except the youngest group (those with no permanent dentition) (Tukey's HSD, p values < 0.05), providing some evidence for a neonatal elevation in chimpanzee testosterone production (Table 4).

4. Discussion

Our results support the hypothesis that differences in male reproductive strategy between bonobos and chimpanzees are associated with important distinctions in the ontogeny of testosterone production between the two species. In chimpanzees, levels of testosterone increased in both males and females during the transition from juvenility to adulthood, doing so more markedly in males, in agreement with previous work [50,51]. In bonobos, by contrast, there was no evidence of maturational increases in testosterone production in either sex. Relative to chimpanzees, bonobos showed a lesser neonatal decline and a lesser pubertal elevation in testosterone, indicating that both developmental periods might be critical in shaping adult reproductive behavior.

It is important to emphasize that we cannot conclude definitely on the basis of our results that bonobos show no pubertal increase in testosterone production, given that this would strongly contradict the general mammalian pattern. Since these saliva samples were collected during the summers of subsequent years (rather than continuously throughout the year), it is possible that bonobo males showed transient testosterone increases (not represented in these samples) as part of their pubertal maturation. But even if such increases occurred, our results indicate that adolescent and adult bonobos did not sustain heightened levels of testosterone for more than a matter of months. We thus

Table 3
Effects of predictor variables on log testosterone in chimpanzees and bonobos using body weights. Restricted estimate maximum likelihood models investigating patterns of log testosterone production were performed separately for chimpanzees (n = 422) and bonobos (n = 165) with Bodyweight, Sex, and Hour of Sample as fixed effects. Random effects took into account the fact that repeated samples were collected from individuals, and certain individuals were sampled across multiple years. Values for Sex are shown relative to males as a reference category. Significant effects are indicated as follows: *p < 0.05, **p < 0.01, and ***p < 0.001. In line with the analyses of Age Category, chimpanzees showed a main effect of body weight on log testosterone, while bonobos did not.

	Chimpanzees				Bonobos			
	Estimate	SE	t-Value	p-Value	Estimate	SE	t-Value	p-Value
Intercept	2.752	0.114	24.227	<0.001***	2.587	0.168	15.378	<0.001***
Hour of Sample	−0.033	0.006	−5.269	<0.001***	0.009	0.011	0.794	0.429
Bodyweight	0.010	0.002	5.400	<0.001***	0.002	0.003	0.652	0.515
Sex female	−0.109	0.048	−2.287	0.024*	−0.006	0.065	−0.095	0.926

argue that bonobos show a lesser developmental increase in testosterone in association with their lesser degree of male mating competition relative to chimpanzees. Before elaborating on this point, we first review the quality of our data.

4.1. Strengths and limitations of the present data set

Limitations of our sample collection procedure were unlikely to have generated the present pattern of results. For example, the use of cotton as a saliva collection material has been suggested to lead to over-estimation of steroid concentrations, with the use of oral stimulants potentially elevating measurement values as well [85,86]. However, even if some bias were introduced into our data by cotton or oral stimulants, any impacts of these factors on the salivary steroid measures would have similarly influenced the results from all individuals of both species (since the same procedures were used for saliva collection throughout). This therefore could not account for our finding of a developmental transition in testosterone production among chimpanzees but not bonobos.

In regard to our finding that testosterone levels were comparable in adult and juvenile bonobos, it is important to note that this pattern is not unprecedented for non-human primates. In several seasonally breeding strepsirhines, adult male testosterone levels have been observed to drop into the juvenile range outside of the breeding season [87–89]. Similarly, such patterns have been documented outside of the breeding season in mandrills, with low-ranking males increasing little in their androgen levels during puberty [16]. Bonobos may thus represent the rare case of an aseasonally breeding species where testosterone levels are consistently low among adults. It is possible that this denoted a stable hierarchy among bonobos in our sample, similar to a group of baboons in which a positive relationship between male rank and testosterone was only present when the hierarchy was unstable [90]. However, male chimpanzees have been found to maintain rank–testosterone relationships even during periods of rank stability [42]. Moreover, a recent study found little association between basal testosterone and dominance rank in a group of wild bonobos [44]. Our results thus suggest that the reduced aggression and fluid dominance hierarchy present among bonobos may be accompanied by low, invariant testosterone levels in adult bonobo males.

While the species differences in maturational patterns of male testosterone production are easily interpreted in relation to reproductive strategies, the differences between bonobos and chimpanzees in absolute testosterone level cannot be evaluated without data on androgen receptor density. Qualitative comparisons indicate that absolute testosterone levels are higher among bonobo juveniles than chimpanzee juveniles. However, if bonobos have a lesser density of androgen receptors, they may need to produce a greater amount of testosterone relative to chimpanzees to obtain an equivalent metabolic effect. Their higher testosterone levels may therefore not be biologically meaningful. As in humans, there is considerable inter-individual variability in the expression of the androgen receptor gene in both chimpanzees and bonobos [91–93], so it is difficult even to characterize average receptor densities in each species. Further research is thus necessary to illuminate how individual differences in genotype may mediate the phenotypic differences observed between bonobos and chimpanzees.

4.2. Directions for future research

An intriguing aspect of our data was the lesser variability in testosterone production found among adult bonobos relative to adult chimpanzees, with this effect particularly strong among males (Fig. 1). This effect may partly have been due to a larger sample size of adult chimpanzee males, though we did sample all adult bonobo males living at our study site except for one individual that had a history of biting caretakers. It is worth noting that even if this male bonobo had exhibited higher testosterone levels than those of the other adult bonobo males, the degree of inter-individual variability would not match that found among male chimpanzees, nor would the relationship between age and testosterone have become statistically significant in bonobo males unless he was an extreme outlier. We propose that this reduction in

Table 4
Effects of predictor variables on log testosterone in chimpanzees and bonobos using dental categories. Restricted estimate maximum likelihood models investigating patterns of log testosterone production were performed separately for chimpanzees (n = 830) and bonobos (n = 296) with Dental Category, Sex, and Hour of Sample as fixed effects. Random effects took into account the fact that repeated samples were collected from individuals, and certain individuals were sampled across multiple years. Values for Sex are shown relative to males as a reference category, and values for Dental Category are shown relative to fully dentally mature individuals (the oldest group) as a reference category. Significant effects are indicated as follows: *p < 0.05, **p < 0.01, and ***p < 0.001. Similar to the analyses for Age Category, chimpanzees showed a main effect of Dental Category, with dentally mature individuals showing higher log testosterone values than dentally immature individuals. Meanwhile, there was no effect of Dental Category on log testosterone values in bonobos.

	Chimpanzees				Bonobos			
	Estimate	SE	t-Value	p-Value	Estimate	SE	t-Value	p-Value
Intercept	2.897	0.134	21.634	<0.001***	2.750	1.632	16.854	<0.001***
Hour of Sample	−0.013	0.004	−3.166	0.002**	0.011	0.008	1.305	0.193
Sex: female	−0.119	0.050	−2.371	0.020*	−0.111	0.040	−2.743	0.008**
Dental cat: no perm	−0.158	0.109	−1.447	0.151	−0.026	0.076	−0.347	0.730
Dental cat: M1	−0.249	0.074	−3.378	0.001**	−0.021	0.047	−0.453	0.652
Dental cat: I's only	−0.389	0.085	−4.359	<0.001***	0.028	0.081	0.342	0.734
Dental cat: M2	−0.413	0.065	−6.407	<0.001***	−0.074	0.053	−1.412	0.163

adult testosterone variability in fact reflects a crucial element of the bonobo male reproductive strategy. In chimpanzees, aggression and dominance rank are effective strategies for obtaining conceptive mating opportunities [32,36]. Correspondingly, chimpanzee males show significant rank-dependent variation in testosterone production [42]. In our data set as well, it is likely that rank differences underlay the variation in adult male testosterone among chimpanzees, though we could not test the rank–testosterone correlation directly because the adults sampled were living in multiple social groups. In contrast, the reduced efficacy of aggression and competition for dominance in bonobo males may explain the reduced inter- and intra-individual variability in their testosterone levels [41,44], as well as a lesser sex difference in adult testosterone levels in bonobos relative to chimpanzees. Together with lesser variation over the course of development, bonobo males may optimize their immune function and overall life history strategy by elevating testosterone levels only when necessary during the months surrounding puberty and maintaining low testosterone levels otherwise. Future work investigating gonadotropin and adrenal androgen production in bonobos can determine whether these effects are specific to testosterone or instead reflect broader shifts in the features of bonobo pubertal maturation.

In addition to the minimal change in testosterone shown by bonobos during puberty, we also found no evidence for a decline in their testosterone levels between infancy and juvenility. Conversely, among chimpanzees, there was some signature of this neonatal decline in the dental category analysis (which provided the greatest resolution in grouping young individuals). Assuming that the chimpanzee pattern is the ancestral condition (in line with the patterns of infant testosterone production documented for multiple non-human primates), bonobos thus appear unusual in maintaining neonatal elevations of testosterone throughout infancy and juvenility. It is possible that because genital contacts are an important feature of bonobo social behavior even in infancy [94–98], testosterone levels remain high throughout infancy and juvenility to sustain high levels of libido. Alternatively, sexual contacts themselves might elevate testosterone levels in infant and juvenile bonobos, given the evidence from human males that sexual activity can increase testosterone levels and the finding that frequency of genito-genital rubbing correlates positively with androgen levels among adult bonobo females [99,100]. However, because we did not sample any bonobos younger than 1.5 years, we cannot say whether levels of testosterone among neonatal individuals would have been even higher than those measured here among infants and juveniles. Additional study of endocrine maturation and social behavior in neonatal bonobos is thus warranted.

Overall, our data suggest that differences in male reproductive strategies across species are associated with differences in the developmental patterns of testosterone production. Additional research on the ontogeny of testosterone production in closely-related species is essential to understand how slight variations in developmental trajectory can facilitate and constrain the reproductive strategies pursued by adults. Such inquiry will illuminate the role of hormones in shifting the maturation of a broad array of phenotypes, and will provide insight into the mechanisms by which evolution produces variation across species.

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