Hormones and Behavior 66 (2014) 525–533

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Age-related changes in urinary testosterone levels suggest differences in puberty onset and divergent life history strategies in bonobos and chimpanzees

V. Behringer [a,*], T. Deschner [a], C. Deimel [b], J.M.G. Stevens [c], G. Hohmann [a]

[a] Max Planck Institute for Evolutionary Anthropology, Department of Primatology, Deutscher Platz 6, 04103 Leipzig, Germany
[b] Department of Anthropology, Indiana University Bloomington, 701 E Kirkwood Ave Bloomington, IN 47405, USA
[c] Centre for Research and Conservation, Royal Zoological Society of Antwerp, K. Astridplein 26, B 2018, Antwerp, Belgium

ARTICLE INFO

Article history:
Received 13 June 2014
Revised 19 July 2014
Accepted 23 July 2014
Available online 30 July 2014

Keywords:
Development
Steroid
Testosterone
Chimpanzee
Bonobo
LC–MS
Dispersal
Aggression
Maturation
Puberty

ABSTRACT

Research on age-related changes in morphology, social behavior, and cognition suggests that the development of bonobos (*Pan paniscus*) is delayed in comparison to chimpanzees (*Pan troglodytes*). However, there is also evidence for earlier reproductive maturation in bonobos. Since developmental changes such as reproductive maturation are induced by a number of endocrine processes, changes in hormone levels are indicators of different developmental stages. Age-related changes in testosterone excretion are an indirect marker for the onset of puberty in human and non-human primates. In this study we investigated patterns of urinary testosterone levels in male and female bonobos and chimpanzees to determine the onset of puberty. In contrast to other studies, we found that both species experience age-related changes in urinary testosterone levels. Older individuals of both sexes had significantly higher urinary testosterone levels than younger individuals, indicating that bonobos and chimpanzees experience juvenile pause. The males of both species showed a similar pattern of age-related changes in urinary testosterone levels, with a sharp increase in levels around the age of eight years. This suggests that species-differences in aggression and male mate competition evolved independently of developmental changes in testosterone levels. Females showed a similar pattern of age-related urinary testosterone increase. However, in female bonobos the onset was about three years earlier than in female chimpanzees. The earlier rise of urinary testosterone levels in female bonobos is in line with reports of their younger age of dispersal, and suggests that female bonobos experience puberty at a younger age than female chimpanzees.

Introduction

The slow transition from postnatal stages into adulthood distinguishes primate life histories from those of other mammals (e.g., Harvey and Clutton-Brock, 1985; Kappeler and Pereira, 2003; Schultz, 1963). In humans, postnatal development is even more prolonged and is differentiated into stages such as childhood and adolescence that are considered to be absent in non-human primates (Bock and Sellen, 2002; Bogin and Smith, 1996; Bogin, 2009; 1997; Dean et al., 2001). Changes in the timing of developmental events in one species relative to its ancestor are referred to as "heterochrony" (Smith, 2001). Bonobos (*Pan paniscus*) and chimpanzees (*Pan troglodytes*) are humans' closest living relatives and the three species share general life history parameters such as the slow development of motor skills, late onset of reproduction, and extended periods of socialization (Finch and Stanford, 2004; Kaplan et al., 2000). Previous studies investigating developmental trajectories of bonobos and chimpanzees have focused on dental development and endocranial growth (Boughner and Dean, 2008; Durrleman et al., 2012; Lieberman et al., 2007; Mitteroecker et al., 2005; Shea, 1983), the emergence of spatial memory and, the development of positional behavior (Brakke and Savage-Rumbaugh, 1991; Rosati and Hare, 2012; Wobber et al., 2010a, 2010b). Based on this work, there is a consensus that compared to chimpanzees, bonobos are characterized by the developmentally delayed expression of morphological, cognitive, and behavioral traits. A major developmental marker in humans is the onset of puberty. Puberty is defined as a "*complex series of coordinated neuroendocrine changes leading to internal and external physical changes in primary and secondary sexual characteristics and eventual reproductive competence*" (Dorn and Biro, 2011). Although the behavioral and endocrinological changes during puberty are well investigated for both sexes in humans and male chimpanzees (e.g., Anestis, 2006; August et al., 1972; Ducharme et al., 1976; Mitamura et al., 2000; Seraphin et al., 2008), less is known

* Corresponding author. Fax: +49 341 3550 299.
E-mail address: verena_behringer@eva.mpg.de (V. Behringer).

http://dx.doi.org/10.1016/j.yhbeh.2014.07.011
0018-506X/

about what and when puberty occurs in female chimpanzees and bonobos (Copeland et al., 1985; Nadler et al., 1987; Sannen et al., 2004).

Endocrinological correlates of development

The transitions between developmental stages in human and non-human primates are linked to specific changes in endocrinological parameters that induce changes in morphology, physiology and behavior (Hochberg, 2012; Maggioncalda et al., 1999). In humans, extensive data on endocrinological correlates during development exist and hormones have also been used to trace developmental processes in non-human primates (e.g., Bercovitch and Ziegler, 2002; Wallen and Zehr, 2004). Thyroid hormones play a central role during development (Brown, 1997; Crockford, 2002). In a recent study we found that bonobo urinary thyroid hormone levels decreased several years later than in chimpanzees, which may relate to an expanded period of skeletal growth and cognitive development in bonobos (Behringer et al., 2014). While the specific consequences of this temporal asymmetry in the excretion of thyroid hormones between the species remain to be explored, multiple physiological functions are likely to be affected, including metabolic changes, behavioral responses to stress, and reproductive maturation (Crockford, 2006; Hadley, 1988). The developmental pattern of one hormone does not necessarily reflect the developmental pattern of other hormones and therefore cannot be interpreted as a general pattern differentiating the development of bonobos and chimpanzees. For example, changes in levels of urinary dehydroepiandrosterone-sulfate (DHEA-S), a hormonal indicator of adrenarche, occur at roughly the same age in both species (Behringer et al., 2012). These examples suggest that age-related changes in hormonal levels do not necessarily follow a uniform pattern within a species. Therefore, the temporal patterns of multiple hormones have to be analyzed to fully understand the developmental trajectories of species under study.

Testosterone during development in humans

Androgens, like testosterone, are another group of physiological markers that have been used to identify developmental stages in a large number of species (Harding, 1981; Hau, 2007; Staub and De Beer, 1997). Testosterone is the major sex steroid in males, but it is also secreted in females. Sexual maturation begins at the onset of puberty and is associated with an increased production of testosterone. Rising levels of circulating testosterone can thus be used as an indirect marker for reproductive maturation of testes and ovaries (Sizonenko and Paunier, 1975). Since specific developmental events such as rapid changes in muscle mass in adolescents and the onset of puberty are accompanied by changes in testosterone levels (Arslanian and Suprasongsin, 1997; Elmlinger et al., 2005; Gesquiere et al., 2005), this hormone can serve as a reference for these specific developmental processes. The majority of testosterone in adult males is secreted by the testes, whereas in females only 20 to 25% is released by the ovaries with the remaining balance resulting from the conversion of the adrenal gland-secreted, androstenedione and dehydroepiandrosterone (Crilly et al., 1981; Staub and De Beer, 1997).

In humans, postnatal testosterone concentrations change significantly across an individual's life span. During the first three months after birth, sex steroid levels are high in both sexes and decrease thereafter (Ducharme et al., 1976; Lalwani et al., 2003). Referred to as the 'juvenile pause', this phase lasts until 10–12 years of age (Byrd et al., 1998) and is characterized by low and stable levels of both gonadotropin and sex steroids, as the hypothalamic–pituitary–gonadal (HPG) axis is inhibited by the central nervous system (Styne, 1994). During the juvenile pause, low levels of androgens are mainly secreted by the adrenal cortex (Hobson et al., 1981; Sizonenko, 1978). Puberty starts with a reawakening of the HPG axis: the secretion of gonadotropin releasing hormone (GnRH) increases which results in a progressive rise of luteinizing hormone (LH) levels (Dorn and Biro, 2011; Kulin et al., 1976; Pinyerd and Zipf, 2005). These rising LH levels trigger the maturation of the testes (August et al., 1972; Winter and Faiman, 1972) and ovaries (Pinyerd and Zipf, 2005). During puberty, testosterone levels continue to increase progressively in both sexes before reaching adult levels between 14 and 15 years of age (August et al., 1972; Frasier et al., 1969; Winter and Faiman, 1972). Sexes differ in terms of the puberty-related increase in testosterone levels, with boys experiencing a more pronounced increase than girls (August et al., 1972; Frasier and Horton, 1966; Mitamura et al., 2000).

Testosterone during development in chimpanzees and bonobos

As in humans, the synthesis of androgens in non-human primates reflects the activity of cells in the testes and ovaries and testosterone levels gradually increase after the onset of puberty (Bribiescas, 2006). In captive chimpanzee males, testosterone levels start to rise around the age of seven in both sexes and increase steadily until ten years of age (Anestis, 2006; Copeland et al., 1985; Kondo et al., 2000; Marson et al., 1991; Martin et al., 1977; McCormack, 1971; Nadler et al., 1987). These developmental changes in testosterone excretion are accompanied by dramatic changes in somatic growth that facilitate physical performance, especially in males (Bribiescas, 2001). An increase in testosterone levels in adolescent female chimpanzees correlates with the onset of the cyclic changes of the sexual swellings, suggesting that increases in testosterone levels are also a useful marker for the onset of reproductive maturation in females (Pusey, 1990).

Compared to chimpanzees, much less is known about age-related patterns of testosterone levels in bonobos. Sannen et al. (2004) found that one immature female bonobo had lower urinary testosterone metabolite levels than adult females, and an eight year old male bonobos had lower testosterone metabolite levels comparable to those in adult males. However, due to small sample sizes of young individuals no clear overall developmental pattern for urinary testosterone metabolite levels could be discerned. Another research group reported that salivary testosterone levels did not change significantly with age in bonobos in either sex (Wobber et al., 2013). Compared to chimpanzees, infant bonobos had higher salivary testosterone levels and therefore showed a smaller increase with puberty (Wobber et al., 2013), which would suggest that unlike humans and chimpanzees, bonobos do not experience a juvenile pause.

Life history patterns in bonobos and chimpanzees

Males

Bonobos and chimpanzees differ in terms of early development, timing of maturation, and the expression of adult behaviors (Furuichi et al., 2012; Lieberman et al., 2007; Parish, 1996; Wrangham, 1993). Adolescent male chimpanzees engage in aggressive dominance interactions which are considered a prerequisite to dominate female group members (Anestis, 2006) and to establish their position within the male hierarchy (Muller et al., 2009). These age-related changes in social behavior correlate with the enlargement of muscle tissue and visible growth of the testes (Copeland et al., 1985; Kraemer et al., 1982; Nadler et al., 1987). In male bonobos, reproductive maturation appears to be largely independent of aggressive dominance behaviors against females (Hashimoto, 1997) and dominance interactions among males are moderate when compared with chimpanzees (Kano, 1992). While dominance relations among males show a hierarchical structure (Surbeck et al., 2011), maturing males integrate into the cohort of resident females by maintaining strong bonds with their mothers and invest in differentiated relations with adult females (Hohmann et al., 1999; Surbeck et al., 2012). Physiological and behavioral tradeoffs represent long- and short-term strategies within male life histories and the ability to regulate energy allocation between reproductive and survivorship functions during various life stages, and according to social circumstances, therefore determines the progression of male

senescence (Bribiescas, 2006; 2001; Bribiescas et al., 2012; Muehlenbein and Flinn, 2011).

Females

As in males, key components of female life history strategies affect the onset of reproduction (Hochberg, 2012). Captive female bonobos give birth to their first infant at a younger age than chimpanzees (Parish, 1996), but other data suggest that females start their reproductive carriers at a similar age (de Lathouwers and Van Elsacker, 2005; Emery Thompson, 2013; Kuroda, 1989). While the onset of reproduction remains a matter of debate, other sources hint at species-specific differences in female life history strategies. While females of both species transfer from their natal communities, bonobos disperse considerably earlier than chimpanzees (7–9 years versus 11–13 years of age, Emery Thompson, 2013; Furuichi, 1989). Migration is costly as it exposes individuals to novel environments, predation and often to aggression from residents; therefore species-differences in the timing of transfers are usually explained by its costs. The dispersal of female chimpanzees is associated with high energetic costs and elevated levels of social stress (Kahlenberg et al., 2008). Corresponding information from bonobos is scarce, but the available evidence suggests that the costs of transfer are relatively low: aggression from resident females against immigrants is rare or absent (Idani, 1991), residents of both sexes seem to be attracted to unfamiliar females (Furuichi, 2011), and resident females play an active role in the integration process of newcomers (Idani, 1991).

The information outlined above indicates species-specific differences in life history patterns in both sexes. Large body size and physical power seem to be more important for male chimpanzees than male bonobos and therefore, the former may grow faster or invest longer into somatic growth than the latter. Because of the different costs associated with female dispersal, we assume an earlier onset of reproduction in bonobos. Using changes in urinary testosterone levels as a marker for the onset of puberty we tested the following predictions:

1. If testosterone secretion is similar in bonobos and chimpanzees to humans, with adrenal glands being the only source of testosterone production during the juvenile pause, we predict younger individuals of both sexes to have lower levels of urinary testosterone than adults.
2. If testosterone secretion during puberty and adulthood is similar to that found in humans, we predict a sexually dimorphic pattern in testosterone secretion with males showing a more pronounced urinary testosterone level increase with the onset of puberty than females in chimpanzees and bonobos.

3a. If puberty follows the general pattern of heterochrony observed in both species, with bonobos being delayed relative to chimpanzees, we predict that chimpanzee age-related increases in urinary testosterone levels will precede that of bonobos in both sexes.
Alternatively, if heterochrony is not a generalized hormonal pattern in the two species we would predict:

3b. If the rise in testosterone levels in males reflects species-specific patterns of male mate competition and that more intense mate competition among male chimpanzees requires a longer investment into somatic growth and accumulation of muscle mass, we predict that urinary testosterone levels will increase later in male chimpanzees relative to male bonobos.

3c. If the rise in testosterone in females corresponds with an earlier onset of puberty and a younger age at dispersal in bonobos, we predict an earlier increase in female bonobo urinary testosterone levels in comparison to female chimpanzees.

Materials and methods

We measured urinary testosterone levels in 251 urine samples of 48 male and 64 female captive bonobos and in 42 male and 97 female captive chimpanzees (Table A.1). Bonobo ages ranged from less than one year to 55 years of age, and in chimpanzees from one year to 56 years of age. Animals of both species were housed in social groups in European and American zoos, and were in good health at the time of sample collection. The apes were fed a mix of fruits and vegetables several times per day and had ad libitum access to fresh water. The protocol for urine sample collection was approved by the authorities of each zoo and supported by the coordinators of the EAZA Western Chimpanzee EEP and Robust Chimpanzee ESB (Frands Carlsen and Tom de Jongh) as well as by the EEP bonobo coordinators (Jeroen Stevens and Zjef Pereboom).

Urine samples were collected throughout the day (between 07:00 and 18:00). After collection, the samples were stored at −20 °C and transported frozen to the Max Planck Institute for Evolutionary Anthropology in Leipzig (MPI-EVA), Germany. At the MPI-EVA samples were stored at −20 °C before being analyzed. More detailed information on sample collection is available in Behringer et al. (2014). Urine samples were analyzed for testosterone with LC–MS/MS (Liquid Chromatography–Tandem Mass Spectrometry) (Hauser et al., 2011; Murtagh et al., 2013).

Urinary testosterone extraction and analyses

The extraction of testosterone from urine was done following the extraction protocol described in Hauser et al. (2008a, 2008b), with the following modifications: we used 100 μl and 900 μl phosphate buffer mixed with 50 μl of internal standard (Hauser et al., 2008a) and 40 μl β-glucuronidase. Because a considerable proportion of testosterone in urine is conjugated, the correlation of urinary and serum testosterone in chimpanzees increases after solvolysis and hydrolysis, therefore we additionally hydrolyzed and solvolyzed the samples (Hauser et al., 2011; 2008a; Surbeck et al., 2012). We used an internal standard described in Hauser et al. (2008a), which was added to each sample as a quality control and sample extraction was repeated if the internal standard recovery was lower than 50% for a given sample.

Urinary hormone levels must be adjusted for variable water content among spot urine samples, which depends on an individual's hydration status and time since the last urination. In primate studies, this is typically achieved by correcting with the creatinine concentration of the sample. Since however, creatinine is a breakdown product of muscle tissue, higher muscle mass leads to higher urinary creatinine concentrations (Emery Thompson et al., 2012). Therefore, in studies comparing urinary steroid levels of individuals with high variability in sex and age, a correction of hormone levels via urinary creatinine concentration is inadequate. Instead, specific gravity (SG) should be used as an alternative for creatinine correction for urinary steroids in these studies (Miller et al., 2004). Therefore, to compensate for variation in urine concentration, we measured SG with a digital handheld refractometer (TEC, Ober-Ramstadt, Germany) and calculated urinary testosterone corrected for SG (Miller et al., 2004). The SG population average for chimpanzees was 1.0043 and 1.0047 for bonobos. In urine samples in which SG was measurable, but the urinary testosterone was not quantifiable, testosterone levels were set to 0.01, the lowest value detectable by the LC–MS/MS. This was done to avoid an overestimation of the results for young individuals, as otherwise we would have had to exclude five chimpanzee and three bonobo females, as well as two bonobo males younger than ten years of age.

Statistical analyses

We used a general linear mixed model (GLMM, Baayen, 2008) to explore differences of the influence of age on urinary testosterone levels in both sexes of bonobos and chimpanzees. The model was run in R (R Core Team, 2013) using the function lmer provided in the package lme4 (Bates et al., 2013). It was fit with a Gaussian error distribution,

identity link function, and using maximum likelihood (argument 'REML' set to 'FALSE').

To investigate the influence of the predictor variables species, sex, age, and sampling time (predictors with fixed effects) on urinary testosterone levels (log-transformed response variable), we used a GLMM and included these four main effects as well as the three-way interaction between species, sex and age (and also all the two-way interactions between them). The interactions were included because we expected that the age dependent changes of urinary testosterone levels would differ between the two species and between the sexes of each species, and that the degree of sex differences is species-specific. We also included location (zoos) as a random effect, to control for a possible influence of relevant animal husbandry conditions of zoos (e.g., diet and group size). Furthermore, we included random slopes of age within zoo to keep type I error rates at the nominal level of 5% (Barr et al., 2013; Schielzeth and Forstmeier, 2009). Female reproductive status or ovarian cycle phase was not included in the model as information on precise reproductive state was not available for all females. However, although an inclusion of female reproductive state into the model might have reduced the unexplained variation in adult female urinary testosterone levels, it would not have changed the results on the overall developmental pattern of urinary testosterone levels, which was the aim of this study. Chronological age of each individual was square root transformed. To achieve comparable estimates, the transformed age and time of sample collection (to control for diurnal variation) were z-transformed to a mean of zero and a standard deviation of one (Schielzeth, 2010). The required normal distribution and homogeneity of residuals were verified by visual inspections of a histogram and a q-q plot of the residuals, and by plotting residuals against fitted values. Neither of them indicated deviations from these assumptions. Model stability was tested by excluding zoos one by one which did not indicate any obvious influence of this random effect. To establish the significance of species, sex, years of age (and all their interactions) as a whole (Forstmeier and Schielzeth, 2011) we used a likelihood ratio test (Dobson and Barnett, 2008; R function anova) to compare the full model with a null model excluding the predictor variables and the interactions, but retaining day time, the random effect of zoo as well as the random slopes component.

To describe the pattern of age-related changes in urinary testosterone levels in more detail, we fitted a sigmoidal model, separately for each species and sex. We parameterized the dependency of testosterone on age using a formula describing a sigmoid curve (Jensen et al., 2009). The model was fitted using the R function nls. The data were bootstrapped 1000 times to obtain parameter coefficients. This allowed producing confidence intervals for the curve estimated by the sigmoidal model.

Results

The comparison of the full model with the null model revealed significance ($\chi^2 = 192.72$, df = 7, $P < 0.001$). The three way interaction of species, sex and age was not significant (estimate = −0.059, SE = 0.182, z-value = −0.321; P = 0.721) and was thus excluded from the model. In the resulting model, two of the three two-way interactions revealed significance (Table 1). The interaction between age and species was not significant, demonstrating that the urinary testosterone pattern is comparable between species, with low levels in younger individuals and increased urinary testosterone levels in older individuals, thereby following the general mammalian pattern (Fig. 1). The two significant two-way interactions showed that urinary testosterone levels in females and males depended on both species and age (Table 1): The urinary testosterone levels increase earlier in female bonobos than in males, but later in female chimpanzees when compared to males.

Table 1
Results of the general linear mixed model (GLMM) obtained by analyzing urinary testosterone levels from chimpanzees and bonobos of both sexes, with species, age (in years) and sex in interactions and time of sample collection as fixed effects, and zoo as random effect (SE = standard error, bold numbers indicate significance, * refers to an interaction term).

Term	Estimate	SE	DF	χ^2	P-value
Intercept	1.3978	0.108			
Time of sample collection	−0.045	0.047	1	0.851	0.356
Age * sex	0.546	0.121	1	30.044	<**0.001**
Age * species	0.053	0.106	1	0.123	0.725
Sex * species	0.434	0.175	1	5.852	**0.016**

The sigmoid model in male bonobos and in chimpanzees of both sexes showed an increase in urinary testosterone levels with increasing age, with an onset around eight to nine years of age (Fig. 1). In female bonobos the sigmoid model implies a less clear increase in urinary testosterone levels with age, but this is a result of the small sample size for individuals under five years of age. The confidence intervals clearly demonstrate an increase of urinary testosterone levels at around five years (Fig. 1). In comparison to females, the increase in urinary testosterone levels in males was more pronounced. In adult females of both species urinary testosterone levels returned to lower levels after the puberty increase with levels ranging between <1–11 ng/ml corr SG in bonobos and <1–15 ng/ml corr SG in chimpanzees (Table 2). In adult males of both species, urinary testosterone levels stayed high after the onset of puberty. While variation in male bonobos was similar to that of females, male chimpanzees showed the highest variability in urinary testosterone levels (Fig. 1, Table 2).

Discussion

In accordance with data on a large body of literature on other mammalian species, the results of our study show that both sexes in bonobos and chimpanzees experience similar age-related changes of urinary testosterone levels, with older individuals having significantly higher levels than younger individuals. This indicates that bonobos and chimpanzees experience a juvenile pause during their early development. In both species, urinary testosterone levels of males increased sharply around the age of eight years. Male bonobos were not delayed in terms of the age-related increase in urinary testosterone levels in comparison to male chimpanzees. In contrast, urinary testosterone levels increased at a younger age in female bonobos than in female chimpanzees.

The pace of development of captive animals probably differs from those living under natural conditions. But given the similarity in husbandry conditions for bonobos and chimpanzees across zoos, the differences in age-related urinary testosterone levels can be considered to reflect true differences in life history traits that are relatively independent of their captive living conditions. Comparative studies indicate considerable intraspecific robustness of life history profiles which suggests inter-generational consistency of timing and ordering of developmental stages (Oyama, 2000). For example, the effect of the captive environment is similar across the sexes and species. An additional advantage of using captive populations is that the exact age is known for most individuals (Leigh, 1992). Moreover, the captive setting offers the advantage to interpret differences in developmental timing without the confounding effects of seasonality in resource abundance and related inter-individual variation of energy supply that potentially influence development and growth in the wild. Furthermore, the detection of age-related changes in urinary testosterone levels requires a large sample size. Therefore, urine samples for this study were collected from captive animals, because a large sample size is not yet available from the wild.

Age-related changes in urinary testosterone levels in male chimpanzees and bonobos

Based on the data from our study, bonobo and chimpanzee males experience a steep rise in urinary testosterone levels around the age of

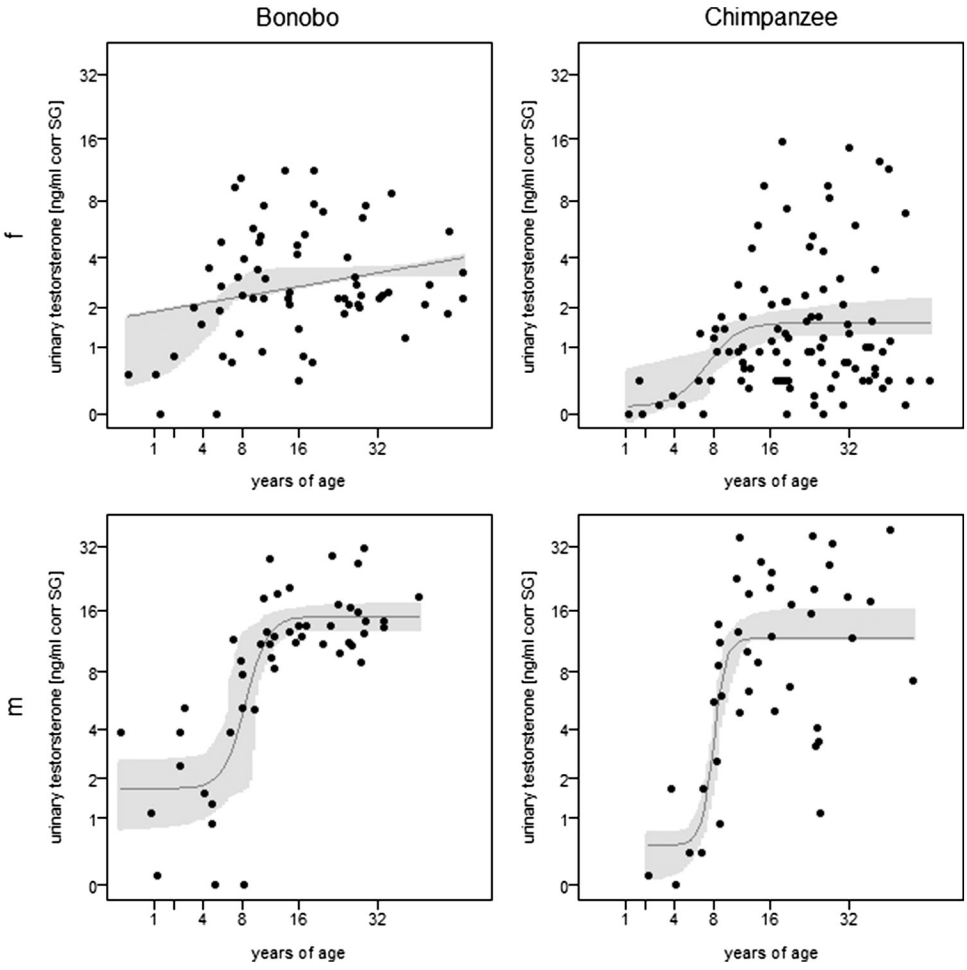

Fig. 1. Measures of urinary testosterone levels corrected for specific gravity (corr SG) obtained from bonobos (left) and chimpanzees (right) (top: females (f); bottom: males (m)) in relation to chronological age (in years). Each filled circle represents one individual. Lines represent the fitted sigmoidal model for the data set. Shaded areas represent bootstrapped 95% confidence intervals for expected urinary testosterone levels. The x- and y-axes are displayed on a log scale. Sample sizes: total N = 251, $N_{female\ bonobos} = 64$, $N_{male\ bonobos} = 48$; $N_{female\ chimpanzees} = 97$, $N_{male\ chimpanzees} = 42$.

eight years. This is in line with previous work on chimpanzees (Kondo et al., 2000; Martin et al., 1977; Smail et al., 1982; Young et al., 1993) and matches results of an earlier study in bonobos (Sannen et al. 2004). In contrast, a recent study found no significant changes in salivary testosterone levels with age in bonobos (Wobber et al., 2013). This negative result could be due to methodological issues with the cotton rolls used for collecting the saliva (Dabbs Jr, 1991; Kutsukake et al., 2009). A detailed study on the trouble with salivary testosterone found that samples collected with cotton rolls (Salivette® device) had testosterone values almost threefold higher in comparison to untreated samples when measured with an immuno-assay (Granger et al., 2004). These findings were confirmed for chimpanzee salivary testosterone measures (Kutsukake et al., 2009). It was proposed that the artificially higher testosterone measurements in samples collected with cotton rolls might be caused by the presence of an unknown substance in cotton, maybe plant hormones, which possibly cross-reacts with the assay's antibody or that the interference is due to non-specific binding (Dabbs Jr, 1991; Granger et al., 1999; Shirtcliff et al., 2001). Since this effect causes unsystematic errors in testosterone measurements of saliva samples collected with cotton rolls, this method should be avoided if one wants to measure testosterone and several other steroids (Shirtcliff et al., 2001). In general, problems with erroneous measurements of hormones due to the cross reactivity of the assay antibody can be avoided by measuring hormones with an LC–MS as exemplified in this study (Murtagh et al., 2013).

Against our prediction, males of both species showed similar profiles of age-related changes in urinary testosterone levels. Testosterone is one of the major sex steroids in males and changing levels have been linked to male mate competition, dominance status and aggression (Anestis, 2006; Bernstein et al., 1983; Hau, 2007; Wingfield et al., 1990). There are clear species-differences between chimpanzees and bonobos in terms of male aggression, male mate competition, and the expression of male dominance: Male chimpanzees are dominant over females and engage in intense aggression and coercive mating strategies. In male bonobos, aggression is less intense, coercive mating strategies are rare and sexes are co-dominant (Muller and Wrangham, 2004; Surbeck et al., 2011; Wrangham and Peterson, 1996). The results of this study suggest that these behavioral traits emerge independently of the developmental patterns of testosterone level changes in males. However, the fact that variation of urinary testosterone levels was higher in male chimpanzees than in male bonobos could be related to differences in species specific male rank acquisition. In addition, we cannot exclude that housing conditions such as single male versus multi-male groups affect aggression levels and thereby impact variation in urinary testosterone levels as well.

Age-related urinary testosterone levels in female chimpanzees and bonobos

Our results show that urinary testosterone levels start to increase much earlier in female bonobos than in female chimpanzees. This indicates an earlier onset of reproductive maturation in female bonobos

Table 2
Description of urinary testosterone levels (ng/ml corr SG) in male and female chimpanzees and bonobos.

			Age categories					
			0–5	6–10	11–15	16–20	20–30	<30
Male	Bonobo	Median	1.45	6.49	12.70	12.69	13.75	14.10
		Mean	2.09	6.69	15.19	12.54	16.30	15.30
		Std. dev.	1.74	3.90	6.12	1.20	7.32	2.89
		Std. error	0.55	1.38	1.94	0.60	1.96	1.67
		Min.	0.01	0.01	8.30	11.18	8.90	13.20
		Max.	5.2	11.52	27.9	13.60	31.30	18.60
		N	10	8	10	4	14	3
	Chimpanzee	Median	0.27	2.15	9.57	6.71	15.48	11.7
		Mean	0.57	4.39	12.39	9.87	15.79	13.52
		Std. dev.	0.79	4.68	11.34	8.88	13.58	13.13
		Std. error	0.40	1.35	3.27	2.96	4.53	4.96
		Min.	0.01	0.01	0.18	0.90	1.14	3.01
		Max.	1.72	13.79	35.12	24.08	35.69	38.27
		N	4	12	12	9	9	7
Female	Bonobo	Median	1.19	3.1	2.77	2.20	1.35	2.30
		Mean	1.68	3.81	3.95	3.69	2.26	2.76
		Std. dev.	1.61	3.11	3.29	3.89	2.36	2.46
		Std. error	0.51	0.86	1.04	1.30	0.68	0.74
		Min.	0.01	0.59	0.88	0.01	0.26	0.37
		Max.	4.90	10.30	11.3	11.3	7.52	8.70
		N	10	13	10	9	12	11
	Chimpanzee	Median	0.12	0.86	0.90	1.15	1.58	0.78
		Mean	0.14	0.94	2.18	2.23	2.41	2.55
		Std. dev.	0.16	0.52	2.62	3.80	6.62	3.99
		Std. error	0.06	0.16	0.68	0.92	0.57	0.75
		Min.	0.01	0.01	0.27	0.20	2.4	0.95
		Max.	0.43	1.72	9.46	15.48	9.46	14.62
		N	6	11	15	17	21	28

compared to female chimpanzees. The accuracy of assessing the timing of puberty is limited in females because urinary testosterone levels of adult females in both species overlap with those of young individuals. This variability in testosterone levels is caused by cyclic ovarian activity which causes testosterone and other androgen levels to change during the menstrual cycle, with testosterone rising for a few days in mid-cycle (McNatty et al., 1976; Sinha-Hikim et al., 1998).

Reproductive maturation is a key life history event that has strong implications for lifetime reproductive success, and therefore its timing is under strong selection (Promislow and Harvey, 1990; Stearns, 1992). The driving force for the observed variation in life histories within and between species is the tradeoff between energy allocations towards somatic growth versus those for reproduction (Charnov, 1991; Stearns, 1992). The optimal allocation of resources into growth or reproduction at different time points depends on a species' ecology and social system (Charnov and Berrigan, 2005; Kaplan et al., 2000; Kozłowski, 1992). One key element in an animal's life history is the transition from adolescence to adulthood as it requires not only the achievement of somatic and physiological maturation but also integration into a novel social environment. In both species females disperse from their natal groups and in most cases, age of dispersal occurs with the onset of reproduction (Emery Thompson, 2013; Furuichi, 1989).

In chimpanzees, immigrants meet opposition from resident females and can be exposed to severe aggression (Boesch and Boesch-Achermann, 2000; Kahlenberg et al., 2008; Pusey, 1990). In some populations, reproductive success varies with the quality of core areas that each resident female occupies and it is mainly the selective range use, not access to food sources, that promotes competition among resident females (Williams et al., 2002). Accordingly, because individual resource holding potential determines access to high quality ranges, females may delay dispersal until their physical condition permits successful competition with residents. While this strategy improves the competitive potential of immigrants, it compromises the onset of reproduction. Occasionally young females give birth to their first infant before emigrating from their natal community (Williams et al., 2002). While this can be considered as circumstantial support for the second scenario, it may actually represent a compromise solution that combines delayed dispersal with an earlier onset of reproduction.

In contrast to chimpanzees, immigrant female bonobos are not highly aggressed by residents of either sex (Furuichi et al., 2012; Idani, 1991). New immigrants actively establish relationships with older resident females by engaging in frequent affiliative behaviors such as grooming and socio-sexual contacts, which eventually lead to stable positions within the community (Furuichi, 1989; Hohmann and Fruth, 2000; Idani, 1991). Furthermore, adult males are sexually attracted to immigrant females long before they have fertile cycles (Takahata et al., 1999). Given the insignificance of bodily condition in relation to dispersal, female bonobos are expected to disperse at a younger age than female chimpanzees. Reports from a number of field sites support this prediction. Adolescent female chimpanzees do leave their natal community at an older age than adolescent female bonobos (11–13 years versus 6–10 years of age, (Emery Thompson, 2013; Furuichi, 1989)).

Whether or not the inter-species differences in the timing of dispersal are causally related to the timing of urinary testosterone level increases remains to be explored in future studies in free ranging populations. However, the data obtained in our study suggest that female bonobos and chimpanzees differ in their timing of the onset of puberty. As both species show similar timings in their age at first birth, this means that they differ in the length of the period between onset of puberty to first conception. How this difference relates to the socialization of immigrants, social systems and other female life history parameters remains to be seen.

The timing of puberty in an evolutionary context

In human populations, there is a strong trend towards earlier puberty with improvements in diet and parasite loads (Karlberg, 2002) and a dissociation of biological and psychosocial maturation (Gluckman and Hanson, 2006). Interestingly, the results of our study show a comparable phenomenon with female bonobos experiencing puberty at a younger age than female chimpanzees. These differences in the onset of puberty could be linked to a higher abundance of food sources, modest fluctuations in food production, and a relaxation of resource competition in bonobos in comparison to chimpanzees (Wrangham, 1986). The onset of human puberty shows considerable plasticity and is considerably altered by environmental conditions (Stearns and Koella, 1986). As there is considerable variation in ecological conditions across the range inhabited by bonobos and chimpanzees (Doran et al., 2002) it can be surmised that the onset of puberty will have been subject to differing evolutionary pressures in the two species. The incorporation of data on life history parameters with ecological variation from multiple great ape populations will be essential for elucidating changes in the timing of puberty which is considered a hallmark of human life history evolution (Kaplan et al., 2000).

Conclusion

Urinary testosterone levels are useful markers to detect the onset of puberty in male and female chimpanzees and bonobos. Both species experience age-related changes in urinary testosterone levels, with older individuals of both sexes having significantly higher levels than younger individuals. The low urinary testosterone levels in younger individuals indicate that bonobos and chimpanzees experience a juvenile pause during their early development. In males of both species, urinary testosterone levels increased sharply around the age of eight years, and these age-related changes in urinary testosterone levels are more pronounced in males than in females. This suggests that behavioral traits differentiating the males of the two species emerged independently of the developmental patterns of urinary testosterone level changes. Female chimpanzees show a similar timing of age-related urinary testosterone

level increase as male chimpanzees and bonobos, but female bonobo urinary testosterone levels start to increase about three years earlier than in female chimpanzees. The earlier rise of urinary testosterone levels in female bonobos corresponds with an earlier time of first dispersal and suggests that female bonobos may reach puberty at a younger age than female chimpanzees.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2014.07.011.

Acknowledgments

Sincere thanks go to the directors and curators of the facilities in Aalborg, Amsterdam, Apenheul, Arnhem, Augsburg, Badoca Safari Park, Belfast, Berlin, Boras, Bremerhaven, Dudley West Midland, Cologne, Frankfurt, Fuengirola, Furuviksparken, Gdansk, Gelsenkirchen, Givskud, Halle, Heidelberg, Jerusalem, Kristiansand, Leipzig, Ljubljana, Madrid, Milwaukee, Muenster, Munich, Planckendael, Romagne, San Diego wild animal park, San Diego zoo, Stuttgart, Twycross, Verona, Veszprém, Warsaw, and Wuppertal. Special thanks go to the caregivers of the facilities for collecting urine samples of bonobos and chimpanzees. Thanks to the EEP coordinators Frands Carlsen, Tom de Jongh, and Zjef Pereboom for their support. The statistical help by Roger Mundry and Colleen R. Stephens is gratefully acknowledged. The authors are also grateful to Róisín Murtagh for sample measurement, to Barbara Fruth and Pamela Heidi Douglas for valuable comments on earlier drafts, and Mimi Arandjelovic for editing the manuscript. This project was funded by the Max Planck Society.

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