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The development of sex differences in digital formula from infancy in the Fels Longitudinal Study

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Relative finger lengths, especially the second-to-fourth finger length ratio, have been proposed as useful markers for prenatal testosterone action. This claim partly depends on an association of relative finger lengths in adults with related sex differences in children and infants. This paper reports the results of a study using serial radiographs to test for both sex differences in the fingers of infants and children and for a relationship between sex differences in the children and infant finger and adult finger length ratios. This is the first study using long-term serial data to evaluate the validity of finger length ratios as markers. We found not only that sex differences in finger length ratios arise prior to puberty, but that sex differences in the fingers of children are highly correlated with adult finger length ratios. Our results strongly encourage the further use of finger length ratios as markers of perinatal testosterone action.

Keywords: second-to-fourth finger length ratio (2D:4D); digit ratios; sex dimorphism

1. INTRODUCTION

Peters *et al.* (2002) reviewed the long history of research into sex differences in the fingers, which has focused on measures contrasting the second and fourth digits. Manning *et al.* (1998) proposed that the sex difference in the second-to-fourth finger length ratio (2D:4D) reflects prenatal testosterone action (such that higher testosterone is associated with lower 2D:4Ds). Recent evidence has lent considerable support to their idea.

The strongest evidence for androgens playing a direct role in the development of digital formula is its association with congenital adrenal hyperplasia (CAH). CAH is a condition resulting in elevated androgen production, which is usually treated soon after birth. Prior to treatment, CAH often results in the masculinization of the external genitalia of newborn girls as well as other aspects of phenotype, including psychology (Hines 2004; Meyer-Bahlburg *et al.* 2004). Two studies have reported that both males and females with CAH have smaller, that is, more masculine, 2D:4Ds (Brown *et al.* 2002; Ökten *et al.* 2002). Although another study later failed to replicate this finding using 2D:4D measures obtained from left-hand radiographs, they did not employ a case–control design with age matching and their subjects ranged in age from 1 to 20 years old (Buck *et al.* 2003). The means obtained in that study were in the expected directions but not significant. In retrospect, their failure to replicate the earlier results can be understood partly in light of evidence that the 2D:4D increases during childhood (McIntyre *et al.* in press), potentially confounding comparisons between CAH-affected people and controls. Not having employed a case–control design might also have introduced other confounding factors, such as ethnic differences (Manning *et al.* 2000; McIntyre *et al.* in press).

As further evidence of a direct relationship between androgen action and digit ratio, other recent research has shown a relationship between a low 2D:4D and fewer CAH elements in the transactivational domain of the androgen receptor (Manning *et al.* 2003). The CAH elements encode a polyglutamine tract. The length of the tract is determined by the number of CAH elements in the allele, which is highly polymorphic in healthy people. The length of the tract has been inversely associated with both *in vitro* androgen receptor transactivation (Callewaert *et al.* 2003), and phenotypic masculinization of tissues (Ding *et al.* 2004). The association between this androgen receptor polymorphism and the 2D:4D suggests that not only do androgens directly influence the development of the digits, but they do so at least partly through the androgen receptor.

Sex steroids are known to play an important role in bone growth and skeletal maturation. Most steroid effects on growth plates in long bones have been shown to operate through oestrogen receptors alpha and beta (Cutler 1997; Kusec *et al.* 1998; Nilsson *et al.* 1999; Weise *et al.* 2001), with the effects of testosterone mediated through its local aromatization to estradiol (Öz *et al.* 2001). However, Abu *et al.* (1997) have reported that the androgen receptor is also expressed in the growth plates of long bones, though its direct physiological effects are unclear. Perhaps more relevant in the case of prenatal effects on bone growth is the finding by Ben-Hur *et al.* (1997) that both androgen and oestrogen receptors are expressed in foetal cartilaginous tissue, leaving open the possibility that androgens influence the development of the digital anlagen. Differences in the effect of androgens on the growth of different bones or digital rays could then be understood as resulting

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from differences in steroid sensitivity, enzyme activity or coactivational environments among tissues.

Garn *et al.* (1974) long ago noted sex differences in the foetal development of the fingers, with males having more advanced onset of ossification for a given crown–rump length (an unexpected pattern given the general female advancement at other ages and in other respects). In the following year, Garn *et al.* (1975) also showed that phalangeal length ratios typical of adulthood are attained early in gestation, a period of high testosterone production in males (Forest 1990). If, as an alternative to direct effects on particular bones, androgens are envisioned as having a global effect on digital development, such as advancing ossification, then sex differences could arise from global effects in one period affecting bones that develop at different rates. More research is needed to establish the physiological-developmental pathways mediating sex and relative digit lengths.

However, whatever the mediating physiological pathway anatomical sex differences in young children are most likely to result from perinatal androgen action, rather than, for example, from sex-linked genetic interactions or from the effects of other steroids, such as oestrogens. While the sex-determining region Y (RSY) certainly determines gonadal sex and, along with many other genes, regulates differentiation of internal reproductive organs, neither SRY nor other regions of the Y chromosome have been found to influence secondary sex differences in other tissues. Although X-linked genes often have different effects in males and females, such as the many well-known X-linked recessive conditions, X-linkage *per se* has not been found to play an important role in secondary sex differences. The process of X-inactivation seems to limit the potential role of X-linked genes in determining sex through, for example, reliable differences in gene product dosage between the sexes (though it fails to ameliorate the susceptibility of XY tissues to recessive allelic variants). Genes located on the X chromosome would normally be expressed in both males and females unless regulated by other sex-different factors, that is, hormones. Rather than genetic differences, sex steroids secreted from the differentiated gonads play the pivotal role in secondary sex differentiation in vertebrates. Furthermore, as discussed earlier, sex differences arising prior to puberty develop largely under the influence of testosterone alone, which is secreted by testes but not by the still-quiescent ovaries. If oestrogen was to play a role, it would likely be to promote masculinization and not feminization, as has been shown in other mammals. This pattern is attributable to the higher perinatal exposure of males, rather than of females, to oestrogens, due to the peripheral aromatization of testosterone. However, this pathway for oestrogen action is restricted by the action of alpha-fetoprotein in binding and inactivating oestrogens, probably to prevent foetal masculinization arising from placental oestrogens.

That said, in humans, the evidence thus far suggests that oestrogens normally play no role in masculinization prior to puberty, perhaps partly because of the action of alpha-fetoprotein which binds and inactivates oestrogens in foetal circulation (in contrast to the potentially substantial maculinizing effects of synthetic oestrogens, especially diethylstilbestrol, which are not bound by alpha-fetoprotein). This claim is most strongly evinced by the combined observations that (i) XY males with complete oestrogen insensitivity or aromatase deficiency do not present with signs of hypomasculinity and (ii) XY females with complete androgen insensitivity, who were nevertheless exposed to male-typical or even further elevated levels of oestrogens, while lacking female internal reproductive organs, have thus far not been observed as being masculine in other respects, including psychologically (Wilson 2001). Therefore, clear demonstration that sex differences in digital formula arise prior to puberty provides evidence for the involvement of perinatal androgens.

This paper, therefore, focuses on the most important question about digit ratio validity, namely, 'To what extent do digit ratios or other measures from the fingers approximate sex differences arising prior to puberty?' Identifying the age at which sex differences in digit ratios arise only partly answers this question. It is important to understand the developmental processes producing variance in adult digit ratios and, specifically, in sex differences. An association with childhood sex differences that have disappeared by adulthood would augment the utility of digit ratios as a marker of childhood or prenatal sex differences. A strong association between digit ratios and important growth processes which are not different between the sexes would warn us to interpret digit ratios carefully and to expect many spurious results.

To answer these questions, we have measured serial hand–wrist radiographs taken from subjects between birth and 18-years-old as part of the Fels Longitudinal Study (Roche 1992). This collection of radiographs allows for a complete description of the serial development of sex differences in the fingers and to test the relationship between sex differences arising early in development with sex differences observed in more mature fingers.

2. METHODS

The Fels Longitudinal Study began in 1929 as a study of the growth and development of children. Participants in the study have been randomly ascertained from the greater Dayton, Ohio, area; that is, they were not chosen on the basis of having any particular condition or risk factor. As a result, the Fels Longitudinal Study is a study of normative growth and development in a non-clinical population. During infancy, children in the study are seen at 1, 3, 6, 9 and 12 months, and then every 6 months thereafter until 18 years old. Hand–wrist radiographs (of the left hand only) have been collected since the beginning of the study for the purpose of determining skeletal maturity. The Fels Longitudinal Study, therefore, provides a unique opportunity to examine serial changes in digit lengths and their sex differences during childhood.

The criteria for the selection of subjects for this study were (i) having at least one measurable radiograph from the first year of life or (ii) having radiographs taken at or within two years of ages 1, 5, 9, 13, and 17 years. Analyses were conducted on the two, largely overlapping groups of radiographs corresponding to the two selection criteria, hereafter infant and serial. All available and measurable infant radiographs were measured (varying from one to five for each subject; median of three radiographs each). Exactly five serial radiographs, one corresponding to each target age, were measured from subjects included in the serial group. The infant sample contains 399 radiographs from 124 subjects

Table 1. First four principal components' discriminant functions and canonical correlations with nine length measures from infants, with correlations to geometric mean size, digit ratios and discriminant function scores below.
(2D refers to the second (index) finger, 3D to the third (middle) finger and 4D to the fourth (ring) finger.)

$n=124$	C-1 (size) (0.84 of variance)	C-2 (proximodistal) (0.09 of variance)	C-3 (medial phal.) (0.03 of variance)	C-4 (lateromedial) (0.02 of variance)	Sex-discriminant (0.17 of variance)
2D proximal	0.9386	0.2849	−0.1466	0.0260	−0.1194
2D medial	0.9253	0.0909	0.3233	0.0537	−0.3616
2D distal	0.8482	−0.4009	−0.0538	0.3301	−0.5953
3D proximal	0.9436	0.2737	−0.1655	0.0025	−0.1068
3D medial	0.9628	0.1144	0.1819	−0.0177	−0.4101
3D distal	0.8680	−0.4304	−0.0569	−0.0653	−0.5939
4D proximal	0.9373	0.2797	−0.1800	−0.0096	−0.1000
4D medial	0.9611	0.1168	0.1360	−0.0789	−0.4025
4D distal	0.8460	−0.4500	−0.0562	−0.2338	−0.7091
	Pearson's r (p)				
geo. mean	0.9998 (<0.0001)	0.6072 (<0.0001)	−0.3528 (<0.0001)	0.0409 (0.6524)	−0.3295 (<0.0001)
2D:4D	0.0834 (0.3580)	0.0674 (0.4577)	0.2130 (0.0174)	0.8264 (<0.0001)	0.0864 (0.3409)
3D:4D	−0.0575 (0.5270)	0.0190 (0.8343)	0.1490 (0.0987)	0.4986 (<0.0001)	0.1885 (0.0358)
discriminant	−0.3191 (0.0003)	0.4489 (<0.0001)	−0.4190 (<0.0001)	0.2400 (0.0071)	—

(56 females and 68 males). The serial sample contains 555 radiographs from 111 subjects (52 females and 59 males). Of these, 107 subjects (49 female, 58 male) were included in both datasets. From these subjects, 99 radiographs were also included in both datasets, including all radiographs taken at exactly one year old.

In most cases, serial radiographs were taken within the target birth month. When target-age radiographs were unavailable or not measurable, the nearest available age was selected. When two radiographs at equally younger and older ages were available, the older age was selected.

Radiographs were digitized to a resolution of 0.85 μm using a desktop scanner with backlight. The method of measurement and software used follow a published protocol (McIntyre *et al.* in press), yielding nine segment lengths: a proximal, medial and distal segment from each of the second (index), third (middle) and fourth (ring) fingers.

The observer making the original measurements also remeasured all 111 subjects in the serial group at ages one and five years to assess repeatability. At age one, the reliabilities (α) of the segment lengths range between 0.961 and 0.988; were 0.988 for the second digit (2D), 0.982 for the third digit (3D), and 0.985 for the fourth digit (4D); and were 0.970 for 2D:4D and 0.947 for 3D:4D. By age five, reliabilities were higher for all measures: between 0.987 and 0.994 for all segment lengths, 0.998 for 2D, 3D and 4D, 0.985 for 2D:4D and 0.969 for 3D:4D. None of these values would render measures unreliable, though the measures from younger ages are universally less reliable.

The analytical methods employed in this study involved more than simply measuring digit ratios at different ages and noting when sex differences arise. We had two goals in selecting analytical methods. First, we wanted to understand the development of digit ratios well enough to identify possible sources of bias in interpretation, especially any strong association with important growth processes that are not sex dimorphic, and to quantify the effect of any sources of bias. Second, we wanted to quantify the amount of information that digit ratios contain about pre-pubertal sex differences, which is the information of interest to most researchers.

Three classes of statistical analysis were performed. First, principal components, common principal components and discriminant function (by sex) analyses were performed in both infants and at all serial ages to describe basic patterns of growth and sex differences in the nine digit segments. Second, group comparisons and repeated-measures ANOVA analyses were performed to compare digit ratios between males and females at all ages. Third, correlation and regression analyses were performed to assess the longitudinal effects of derived developmental measures at younger ages (including principal components and discriminant function scores) on measures at older ages, especially on digit ratios at age 17 years. Analyses included both comparison between measures at two ages, one younger and one older, and also repeated ANOVA measures to assess effects over multiple age groups. Many analyses were performed to exclude unlikely hypotheses and to assess the reliability of measures. Therefore, in this paper we present only the quantitative results most relevant to testing the hypothesized association between sex differences in childhood with adult (age 17) digit ratios and summarize other results.

3. RESULTS

(a) *Infant sex differences in the digits*

Principal components analysis was performed on the nine digit segment lengths measured from all 399 radiographs obtained from 124 infant participants. Table 1 (upper part) shows the canonical correlations of the first four principal components with the measured segments. The first component (C-1) reflects overall size or segment length, loading all segment lengths strongly and in the same direction, and accounts for over 80% of the total variance. The second component (C-2) contrasts proximal segments with distal segments, hence the proximodistal component, and accounts for about 9% of the total variance. The third component (C-3) contrasts the medial component with the proximal component primarily, but also with the distal component (albeit weakly, hence the medial phalange component) and accounts for almost 3% of the total variance. The fourth component (C-4) contrasts segments on the second digit (especially the distal segment) with segments on the fourth digit (again, especially the distal segment; hence the lateromedial

Table 2. First four principal components' discriminant functions and canonical correlations with nine length measures from children at age five, with correlations to geometric mean size, digit ratios and discriminant function scores below. (2D refers to the second (index) finger, 3D to the third (middle) finger and 4D to the fourth (ring) finger.)

$n=111$	C-1 (size) (0.75 of variance)	C-2 (proximodistal) (0.12 of variance)	C-3 (medial phal.) (0.06 of variance)	C-4 (lateromedial) (0.03 of variance)	Sex-discriminant (0.19 of variance)
2D proximal	0.8988	0.3320	−0.1512	0.1529	0.0393
2D medial	0.8581	0.0168	0.4289	0.2165	−0.1774
2D distal	0.7882	−0.5326	−0.0171	0.1861	−0.2107
3D proximal	0.8868	0.3756	−0.2081	0.0494	0.1425
3D medial	0.9207	0.0777	0.3185	−0.0826	−0.1926
3D distal	0.8543	−0.4448	−0.1498	−0.0288	−0.3267
4D proximal	0.8699	0.3871	−0.2451	−0.0067	−0.0047
4D medial	0.9045	0.1259	0.2013	−0.3208	−0.2346
4D distal	0.8287	−0.4444	−0.2030	−0.1412	−0.4934
	Pearson's r (p)				
Geo. mean	0.9979 (<0.0001)	0.0629 (0.5125)	−0.0064 (0.9473)	0.0098 (0.9189)	−0.2045 (0.0311)
2D:4D, age 5	0.0904 (0.3464)	−0.2286 (0.0156)	0.4292 (<0.0001)	0.8263 (<0.0001)	0.2592 (0.0058)
3D:4D, age 5	0.0449 (0.6404)	−0.0657 (0.4941)	0.2783 (0.0030)	0.5033 (<0.0001)	0.4743 (<0.0001)
2D:4D, age 17	0.0385 (0.6890)	−0.0107 (0.9113)	0.3641 (<0.0001)	0.5896 (<0.0001)	0.1695 (0.0754)
3D:4D, age 17	−0.0012 (0.9902)	0.0079 (0.9349)	0.2870 (0.0021)	0.3739 (<0.0001)	0.3656 (<0.0001)
discriminant	−0.1323 (0.0554)	0.4482 (<0.0001)	−0.1287 (0.1787)	0.4443 (<0.0001)	—

component) and accounts for almost 2% of the total variance.

Likewise, a sex-discriminating function was estimated from all 399 radiographs using the LINDA program (Cavalcanti 2001). Positive loading indicates that greater relative segment length characterizes girls. That all canonical correlations of discriminant function scores with segment lengths are negative indicates that boys have longer fingers, especially in the distal segments and especially in the fourth distal segment. In correlational analyses (table 1, lower part), each subject was assigned a single score for each component, the sex-discriminant and digit ratios, by averaging the scores from all of that subject's radiographs. This approach treats differences in scores from infant radiographs as arising from error rather than from real fluctuations or developmental changes, which is probably not entirely warranted but allows for maximum use of the data available. Selecting single radiographs from each subject using a target age or restricting inclusion of radiographs to a specified age (which, for all ages, substantially reduces samples size) both yield similar results in all analyses.

Sex differences in infant hands are notable. Given that infant boys are longer and heavier, it is unsurprising that male infants are partly characterized by having longer finger bones. Of the roughly 17% of variance in sex-discriminant scores accounted for by sex (16.9% of variance in radiograph scores, 17.6% in subject average scores), approximately 3% consists of sex differences in overall size (whether taken as the size component scores or geometric mean size). Most of the remaining sex differences in the fingers are associated with sex differences in the second (proximodistal) component, with boys having relatively longer distal segments. The relationship between the sex-discriminant and the fourth (lateromedial) factor is weaker, explaining why the relationships between sex discriminant scores and digit ratios are weak and why neither 2D:4Ds nor 3D:4Ds are sex-different in this sample (female 2D:4D 0.0030 greater, $p=0.5277$; female 3D:4D 0.0038 greater, $p=0.2831$).

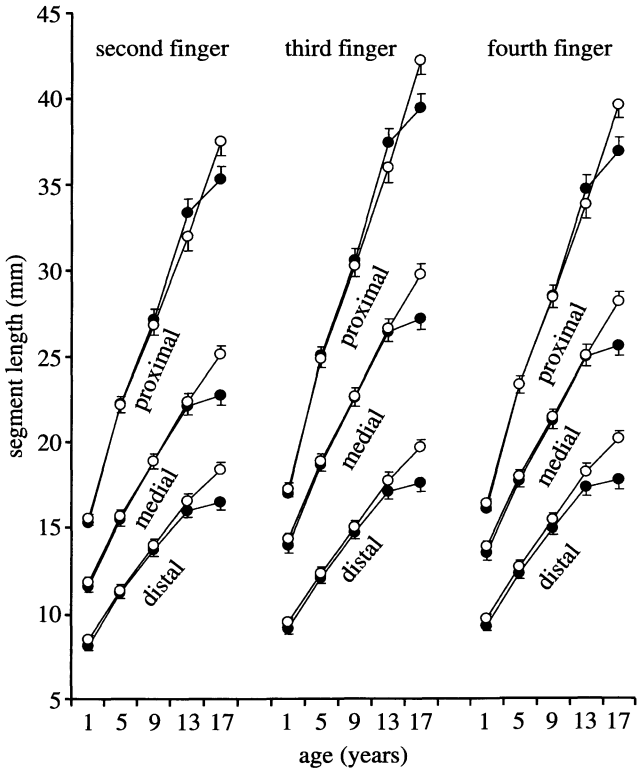

Figure 1. Growth in lengths of segments from second (index), third (middle) and fourth (ring) digits. Mean lengths (±1 s.d.). Closed circles are for females, open circles are for males.

(b) *Serial development of sex differences in the digits through to age 17*

Figure 1 shows the growth in length of the measured digit segments, according to sex, from age 1 to 17 years. The fourth distal segment is significantly longer in males at each age. Overall size differences in the fingers are only present at ages 1 and 17, with males having longer fingers at both ages. Proximal segments are significantly longer in females only at age 13, perhaps owing to acceleration in ossification accompanying pubertal maturation, though their fingers do not become significantly longer overall.

Table 3. Correlations among sex-discriminant scores estimated at different ages and with digit ratios at age 17 years old.

	infant[a]	age 1	age 5	age 9	age 13	age 17	2D:4D (age 17) r (p)	3D:4D (age 17) r (p)
infant[a]	—	0.7982	0.4342	0.4557	0.4992	0.6010	0.1329 (0.1727)	0.1219 (0.2115)
age 1	0.7982	—	0.4466	0.3935	0.4247	0.4991	0.1609 (0.0916)	0.1700 (0.0745)
age 5	0.4342	0.4466	—	0.6970	0.6132	0.4266	0.1695 (0.0754)	0.3656 (<0.0001)
age 9	0.4557	0.3935	0.6970	—	0.7351	0.5618	0.4691 (<0.0001)	0.4798 (<0.0001)
age 13	0.4992	0.4247	0.6132	0.7351	—	0.7324	0.2940 (0.0016)	0.3725 (<0.0001)
age 17	0.6010	0.4991	0.4266	0.5618	0.7324	—	0.2510 (0.0077)	0.2854 (0.0023)

[a] For all correlations $n=111$, except for correlations with 'infant' scores, in which cases $n=107$.

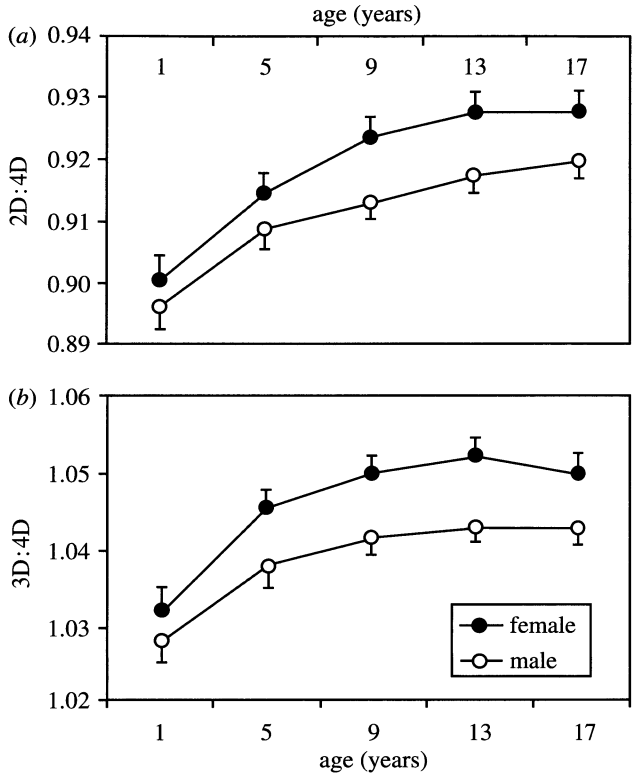

Figure 2. Serial changes and sex differences in the ratios of (*a*) the second finger to the fourth finger (2D:4D) and of (*b*) the third finger to the fourth finger (3D:4D). Mean ratios (±1 s.e.m.).

Table 2 shows canonical correlations with measured segments of the first four principal components and the sex-discriminating function at age five. The first four principal components at age five can be interpreted similarly to the components obtained from infants, and to components described in a different sample of children (McIntyre *et al.* in press). These components arise at all ages (in the same order of importance) and are serially correlated. Common principal components analyses with Flury's method reveal that covariance matrices at different ages, and between the sexes, are not equal (owing to differences in mean lengths and variances) but are proportional and share all principal components.

Table 3 (left part) shows that, like the principal components, sex-discriminant scores are highly intercorrelated at all ages (all $p<0.0001$). The correlation between age-one and age-17 discriminant scores is inflated by their respective associations with overall size (which is also correlated at all ages). Removing the shared association with overall size at age one weakens the relationship (partial $r=0.3869$, $p<0.0001$). As in infants, the digit ratios themselves do not differ by sex at age one (see figure 2). However, by age five, females have higher 3D:4Ds (by 0.0076, $p=0.0238$) but not 2D:4Ds (by 0.0058, $p=0.2127$). By age nine, females have higher 3D:4Ds (by 0.0085, $p=0.0095$) and 2D:4Ds (by 0.0107, $p=0.0143$). Sex differences persist, except the difference in 2D:4Ds becomes marginally non-significant in this sample by age 17 (females 0.0082 greater, $p=0.0536$). Both ratios grow with age, especially in early childhood. In a two-way repeated-measures ANOVA, 3D:4Ds vary both by sex and by age (for sex $F=5.83$, $p=0.0174$; for age $F=64.04$, $p<0.0001$), whereas 2D:4Ds only vary significantly by age (for sex $F=3.74$, $p=0.0558$; for age $F=70.25$, $p<0.0001$), with no significant sex-by-age interaction in either case.

(c) *The validity of digit ratios and/or other measures of digital formula at age 17*

The central use of serial data for the problem of validating digit ratios as markers of androgen action is not to identify sex differences, nor even to describe when they arise (both of which have been done before) but, returning to our original question, to ask: 'Are digit ratios measured in adults useful for approximating the sex-differentiating processes of the prenatal or perinatal period?' Serial analysis of digit ratios partly answers this question by establishing the high reliability of digit ratios as trait descriptors, even in growing children and despite serial changes in the ratios (2D:4D $\alpha=0.88$, 3D:4D $\alpha=0.89$). Serial analysis also confirms that, while sex differences in digit ratios probably do not arise before birth, they certainly arise before, and are little affected by, puberty (Manning *et al.* 1998; McIntyre *et al.* in press).

Likewise, the reliability of sex-discriminant scores ($\alpha=0.79$) suggests that adult fingers can be used to assess sex-typicality as a trait that arises early in childhood. However, the question can be further addressed in this sample by testing the relationship of digit ratios at 17 years old with a continuous measure of sex differences at younger ages, particularly in infancy and early childhood. Put another way, each radiograph is assigned a sex-discriminant score, which can be posed as describing the extent of exposure to sex-differentiating factors (perhaps testosterone) prior to the given age. Are these sex-differentiating factors well approximated by digit ratios in mature hands?

The right part of table 3 shows the correlation between sex-discriminant scores at each age and digit ratios at 17 years old. Sex differences appearing by age five describe more than 13% of the variation in age-17 3D:4Ds and sex

differences at age nine explain more than 20% of the variation. Moreover, the sex-discriminating function obtained at age nine fully explains the binary sex differences observed at age 17 in both 2D:4Ds (full regression $r^2=0.22$, $F=15.24$, $p<0.0001$; sex dummy partial $t=0.07$, $p=0.9404$; age-nine discriminant partial $t=5.08$, $p<0.0001$) and 3D:4D (full regression $r^2=0.23$, $F=16.17$, $p<0.0001$; sex dummy partial $t=-0.19$, $p=0.8500$; age-nine discriminant partial $t=5.12$, $p<0.0001$). In general, the relationship between sex differences in 2D:4Ds is weaker than those of 3D:4Ds, just as left-hand 2D:4Ds are less sex different than left-hand 3D:4Ds (McIntyre *et al.* in press).

However, sex differences in infants and at age one, as described by the discriminant function, are not significantly correlated with age-17 digit ratios in this sample, just as digit ratios at those ages are not yet sex different. The relationships between age-one sex differences and digit ratios are slightly confounded by the presence of sex differences in overall size at age one (which are related to the sex-discriminating function, as discussed above, but which are unrelated to digit ratios). Controlling for this confounding effect, whether by multivariate control or by estimating a new sex-discriminating function using scaled length measures (segment lengths divided by geometric mean length), only slightly increases the significance of the relationship.

4. CONCLUSION

These results confirm many previous findings about digit ratios, namely: digit ratios are sex different by as early as five years old (Manning *et al.* 1998; Manning *et al.* 2004; McIntyre *et al.* in press), lateromedial digit ratios like 2D:4Ds increase with age in children (McIntyre *et al.* in press), similar principal components of digit segment length, along with similar sex-discriminating functions, have been repeatedly obtained in different samples and at different ages (McIntyre *et al.* in press) and, finally, 3D:4Ds may be a better measure for approximating childhood sex differences, even if it is less sex dimorphic in adults (McIntyre *et al.* in press).

Beyond further substantiating previous claims about digit ratios, this study also extends our understanding of adult digit ratios by relating them to early childhood growth processes, including the development of sex differences which include, but are not limited to, sex differences in the digit ratios. Our most striking finding was that digit ratios in the most mature hands reflect childhood sex differences in the fingers, expressed as a continuous variable, much more strongly than might be expected on the basis of the small group sex differences observed among adults. While binary sex (namely, being male or female) accounts for less than 5% of the variance in mature digit ratios, patterns of sex difference in childhood might account for as much as 20% of the variance in mature digit ratios. The high serial reliability both of digital formula measures (including digit ratios and principal component scores) and of sex-discriminant scores contributes to the reported pattern.

It is important to note that these measures were taken from radiographs of the left hand. Most research employing digit ratios involves measures taken on the skin surface, often from the right hand. Therefore, direct, quantitative comparisons are problematic, even if most conclusions can be applied generally.

Differences in method might explain why we found adult 3D:4Ds to be a more valid descriptor of childhood sex differences. Using measures taken on the skin surface, both right and left hand 2D:4Ds are more sexually dimorphic than 3D:4Ds. However, digit ratios other than 2D:4Ds have not been widely reported for children. Therefore, contrary findings using radiographs from children might at least argue for the continued investigation of digit ratios other than 2D:4Ds. In particular, the size of adult sex differences ought not to be taken as primary evidence for the validity of 2D:4Ds. Rather, the associations between digit ratios and relevant developmental variables (such as independent proxies of pre-pubertal androgen production or pre-pubertal sex differences) are crucial.

Our results support the proposal that pre-pubertal sex differentiation, which is largely guided by testosterone in the perinatal period, determines sex differences in adult digit ratios. The results also help to explain why the correlations between 2D:4Ds and investigational variables are so much greater than the sex differences in either.

REFERENCES

Abu, E. O., Horner, A., Kusec, V., Triffitt, J. T. & Compston, J. E. 1997 The localization of androgen receptors in human bone. *J. Clin. Endocrinol. Metab.* **82**, 3493–3497.

Ben-Hur, H., Thole, H. H., Mashiah, A., Insler, V., Berman, V., Shezen, E., Elias, D., Zuckerman, A. & Ornoy, A. 1997 Estrogen, progesterone and testosterone receptors in human fetal cartilaginous tissue: immunohistochemical studies. *Calcif. Tissue Int.* **60**, 520–526.

Brown, W. M., Hines, M., Fane, B. A. & Breedlove, S. M. 2002 Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Horm. Behav.* **42**, 380–386.

Buck, J. J., Williams, R. M., Hughes, I. A. & Acerini, C. L. 2003 In-utero androgen exposure and 2nd to 4th digit length ratio—comparisons between healthy controls and females with classical congenital adrenal hyperplasia. *Hum. Reprod.* **18**, 976–979.

Callewaert, L., Cristiaens, V., Haelens, A., Verrijdt, G., Verhoeven, G. & Claessens, F. 2003 Implications of a polyglutamine tract in the function of the human androgen receptor. *Biochem. Biophys. Res. Commun.* **306**, 46–52.

Cavalcanti, M. J. 2001 *LINDA—linear discriminant analysis and Hotteling's T-squared with randomization tests.* Rio de Janeiro: Setor de Paleovertebrados, Departamento de Geologia e Paleontologia, Museu Nacional/Universidade Federal do Rio de Janeiro.

Cutler Jr, G. B. 1997 The role of estrogen in bone growth and maturation during childhood and adolescence. *J. Steroid Biochem. Mol. Biol.* **61**, 141–144.

Ding, D., Xu, L., Menon, M., Reddy, G. P. V. & Barrack, E. R. 2004 Effect of a short CAG (glutamine) repeat on human androgen receptor function. *Prostate* **58**, 23–32.

Forest, M. G. 1990 Pituitary gonadotropin and sex steroid secretion during the first two years of life. In *Control of the onset of puberty* (ed. M. M. Grumbach, P. C. Sizonenko & M. L. Aubert). Baltimore: Williams & Wilkins.

Garn, S. M., Burdi, A. R. & Babler, W. J. 1974 Male advancement in prenatal hand development. *Am. J. Phys. Anthropol.* **41**, 353–360.

Garn, S. M., Burdi, A. R., Babler, W. J. & Stinson, S. 1975 Early prenatal attainment of adult metacarpal-phalangeal rankings and proportions. *Am. J. Phys. Anthropol.* **43**, 327–332.

Hines, M. 2004 Sex steroids and human behavior: prenatal androgen exposure and sex-typical play behavior in children. *Ann. NY Acad. Sci.* **1007**, 272–282.

Kusec, V., Virdi, A. S., Prince, R. & Triffitt, J. T. 1998 Localization of estrogen receptor-alpha in human and rabbit skeletal tissues. *J. Clin. Endocrinol. Metab.* **83**, 2431–2428.

Manning, J. T., Scutt, D., Wilson, J. & Lewis-Jones, D. I. 1998 The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Hum. Reprod.* **13**, 3000–3004.

Manning, J. T. *et al.* 2000 The 2nd:4th digit ratio, sexual dimorphism, population differences, and reproductive success: evidence for sexually antagonistic genes? *Ev. Hum. Behav.* **21**, 163–183.

Manning, J. T., Bundred, P. E., Newton, D. J. & Flanagan, B. F. 2003 The second to fourth digit ratio and variation in the androgen receptor gene. *Ev. Hum. Behav.* **24**, 399–405.

Manning, J. T., Stewart, A., Bundred, P. E. & Trivers, R. L. 2004 Sex and ethnic differences in 2nd to 4th digit ratio of children. *Early Hum. Dev.* **80**, 161–168.

McIntyre, M. H., Cohn, B. A., Ellison, P. T. In press. Sex dimorphism in the digital formulae of children. *Am. J. Phys. Anthropol.*

Meyer-Bahlburg, H. F. L., Dolezal, C., Baker, S. W., Carlson, A. D., Obeid, J. S. & New, M. I. 2004 Prenatal androgenization affects gender-related behavior but not gender identity in 5–12-year-old girls with congenital adrenal hyperplasia. *Arch. Sex Behav.* **33**, 97–104.

Nilsson, L. O., Boman, A., Sävendahl, L., Grigelioniene, G., Ohlsson, C., Ritzén, E. M. & Wroblewski, J. 1999 Demonstration of estrogen receptor-beta immunoreactivity in human growth plate cartilage. *J. Mol. Endocrinol.* **84**, 370–373.

Ökten, A., Kalyoncu, M. & Yaris, N. 2002 The ratio of second- and fourth digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Early Hum. Dev.* **70**, 47–54.

Öz, O. K., Millsaps, R., Welch, R., Birch, J. & Zerwekh, J. E. 2001 Expression of aromatase in the human growth plate. *J. Mol. Endocrinol.* **27**, 249–253.

Peters, M., Mackenzie, K. & Bryden, P. 2002 Finger length and distal finger extent patterns in humans. *Am. J. Phys. Anthropol.* **117**, 209–217.

Roche, A. F. 1992 *Growth, maturation, and body composition: The Fels Longitudinal Study 1929–1991. Cambridge Studies in Biological Anthropology.* Cambridge University Press: Cambridge.

Weise, M., De-Levi, S., Barnes, K. M., Gafni, R. I., Abad, V. & Baron, J. 2001 Effects of estrogen on growth plate senescence and epiphyseal fusion. *Proc. Natl. Acad. Sci.* **98**, 6871–6876.

Wilson, J. D. 2001 Androgens, androgen receptors, and male gender role behavior. *Horm. Behav.* **40**, 358–366.

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