Behav Ecol Sociobiol (2009) 63:1539–1547
DOI 10.1007/s00265-009-0784-8

METHODS

Digit length ratio (2D/4D): comparing measurements from X-rays and photographs in field voles (*Microtus agrestis*)

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Received: 9 December 2008 / Revised: 8 May 2009 / Accepted: 11 May 2009 / Published online: 29 May 2009

Abstract The ratio of second-to-fourth digit length (2D/4D) has been suggested to be a useful adult age marker of intrauterine exposure to steroids because it should be sexually dimorphic and fixed already in utero. Numerous studies mainly on humans have supported this conclusion, but it is yet unclear how well this applies to other vertebrates. This information would be especially valuable to field biologists to whom measuring steroids in utero is often impossible. The non-human studies conducted so far have yielded inconsistent results, perhaps due to the variety of different methods employed in measuring 2D/4D. We examined the age and sex dependency and lateral asymmetry of 2D/4D in field voles (*Microtus agrestis*) and compared whether these effects differed between 2D/4D measurements taken from photographs and X-rays. Our results show that 2D/4D measurements from photos had a higher measurement error and gave consistently higher 2D/4D than those from X-rays. According to both measurement methods, the right paw showed higher 2D/4D values than the left paw (lateral asymmetry). Adult voles had a lower 2D/4D than juveniles when measured from X-rays, but not when measured from photographs. We found no evidence for a sex difference in 2D/4D using either of the measurement methods. Our findings thus suggest that X-rays, due to their greater accuracy, should be preferred over photographs for measuring digit ratios in rodents. Our results also indicate that in this species, 2D/4D is laterally asymmetric, but it may not be either fixed in utero or sexually dimorphic. In conclusion, 2D/4D appears to be a rather species- and method-specific measure and researchers should be careful when generalising its applicability to study early hormonal effects in vertebrates.

Keywords Phenotypic marker · Intrauterine · Steroids · Early development · Maternal effect

Introduction

The steroid environment that vertebrates encounter during their prenatal development has many important effects on their physiology, behaviour, phenotype and reproduction after birth (Metcalfe and Monaghan 2001; Groothuis et al. 2005; Foecking et al. 2008). Such effects are, however, very difficult, if not impossible to measure accurately. This holds particularly for mammals, as ethical and practical reasons make it hard for adulthood phenotype to be traced back to the steroid concentrations encountered prenatally. Manning et al. (1998) proposed a solution for this problem by suggesting that in mammals, steroid concentrations in utero could be estimated through the ratios of digit lengths in adulthood. This suggestion rests on the assumptions that digit ratios are fixed in utero and that digit lengths respond to prenatal levels of steroids. More specifically, oestrogen is believed to promote the growth of the index finger (2D) and testosterone the growth of the ring finger (4D) (Manning 2002). The ratio of second-to-fourth digit length (hereafter 2D/4D) is thus suggested to be a marker of prenatal exposure to steroids: The lower the 2D/4D, the higher testosterone, but the lower oestrogen levels during foetal

Communicated by A. Schulte-Hostedde

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life. The link between steroid levels in utero and digit ratios is thought to be the *Hox* genes, which are conserved in all vertebrates (Kondo et al. 1997). The *Hoxa* and *Hoxd* gene clusters are essential to the development of both limbs and genitals (Herault et al. 1997), and the expression of these genes is controlled by sex steroids (Buck et al. 2003).

Experimental evidence that prenatal steroids affect digit ratios is scarce at present. This is because most of the research on digit ratios has been conducted in humans where experimental approach has not been an option for ethical reasons. However, studies of congenital adrenal hyperplasia and 2D/4D strongly suggest that prenatal steroids are involved (Puts et al. 2008). In mammals, however, it has recently been shown that maternal testosterone enhancement during pregnancy lowered the 2D/4D of the pups born in rats (Talarovičová et al. 2009). In birds, manipulation of yolk steroids has also resulted in a response in digit ratios (Romano et al. 2005; Saino et al. 2007). These results provide support to Manning's hypothesis.

In humans, 2D/4D is sexually dimorphic: Men, on average, have lower 2D/4D than women (Manning 2002; Saino et al. 2006). In other mammals, similar sexual dimorphism in 2D/4D has been found in laboratory mice (*Mus musculus*; Brown et al. 2002a; Manning et al. 2003) and wood mice (*Apodemus sylvaticus*; Leoni et al. 2005). Sexual dimorphism has also been reported in at least two species of birds, the zebra finch (*Taeniopygia guttata*; Burley and Foster 2004; but see Forstmeier 2005), house sparrows (*Passer domesticus*; Navarro et al. 2007) and ring-necked pheasants (*Phasianicus colchicus*; Romano et al. 2005; Saino et al. 2007). Moreover, in reptiles, sexual dimorphism of digit ratios has been reported in at least three species (Chang et al. 2006; Rubolini et al. 2006; Chang 2008; but see Lombardo and Thorpe 2008). The emerging pattern from these studies is that amphibians, reptiles and birds tend to have similarly sexually dimorphic 2D/4D such that males have higher 2D/4D than females, but mammals are sexually dimorphic such that males have lower 2D/4D than females (Lombardo et al. 2008; but see Burley and Foster 2004; Roney et al. 2004).

2D/4D has also been found to be laterally asymmetric in humans, especially in men; the right hand appears to have a lower ratio than the left hand (Manning 2002). Manning (1998) suggests that this is because the right side is more sensitive to androgens. Yet, the fundamental reason for this lateral asymmetry and the physiological factors behind it remain unclear. So far, lateral asymmetry in 2D/4D ratios in other species has been reported for mice (Brown et al. 2002a) and lizards only (Rubolini et al. 2006).

Digit ratios have traditionally been measured from either photocopy machine prints (Brown et al. 2002b; McFadden et al. 2005; McIntyre et al 2007; Martin et al. 2008), photos (Bailey et al. 2005; Hurd et al. 2008; Manno 2008), scans (Weis et al. 2007; Park et al. 2008) or by measurement of digits with a Vernier calliper by proxy (Dreiss et al. 2008) or directly (Manning 2002; Csathó et al. 2003; Manning et al. 2004). Because of ethical and logistic reasons, it has been rare to use X-ray scans to measure exact bone lengths (but see Manning et al. 2000; Leoni et al. 2008). This is unfortunate because most of the variation in digit ratios should arise from the differences in the length of phalanges, as bone growth is more sensitive to prenatal steroids than soft tissue growth (McIntyre 2006). Furthermore, when examining a mammal paw or a human hand, it is obvious that the proximal phalange of a digit extends far more towards the centre of the palm than where the soft tissue of the digits joins the tissue of the palm. This may introduce errors in digit ratio measurements taken from photos only. However, Manning (2002) suggests that there is a high enough correlation between 2D/4D measurements taken from photos and X-ray scans (r=0.45), indicating that measurements from photos would suffice, at least in humans. The same result (r=0.45) between radiographs and photos was obtained by Leoni et al. (2008) with hooded crows (*Corvus corone*). Manning et al. (2005) also pointed out that photocopy and direct measurements should not be mixed, especially when considering the measures of asymmetry. They further suggested a soft tissue effect in humans (from finger fat pads) that could cause the differences in photocopy measurements compared with direct assessments.

In order to seek the proper measurement technique for the digit ratios, there is thus an urgent need to examine the similarity of soft tissue and X-ray scan (measuring exact bone length) measurements in more detail.

Our aim was to examine whether the prevailing presumptions for using 2D/4D of the front paws as a marker of early developmental exposure to steroids in utero apply to field voles (*Microtus agrestis*) whose parents were caught from the wild. Manning (2002) has suggested that rodents are promising models for digit ratio research because they are amenable to experiments that cannot be conducted on humans. However, rather little is currently known about digit ratios in other than laboratory strain rodents (but see Leoni et al. 2005). This could be a problem when one is interested in the evolutionary and ecological significance of traits that the digit ratios are thought to measure. This is because domesticated and semi-domesticated species or strains might have undergone artificial selection whereby results based on them may not be applicable to wild populations. We tested the hypotheses that (1) there is sexual dimorphism in 2D/4D, (2) there is no difference in 2D/4D between adults and juveniles because 2D/4D should be fixed in utero and (3) 2D/4D differs between the left and right paws (lateral asymmetry). Because there currently seems to be no general consensus on which method should be preferred for measuring digit ratios, we examine these issues by contrasting two different methods of measurement, photos and X-ray scans. Therefore, our analyses should also shed light on whether it is the length of the bones or the amount of soft tissue that may explain the observed 2D/4D.

Materials and methods

General methods and study species

The study was conducted in autumn 2005 on first-generation laboratory-born field voles, whose parents were trapped from the wild in southwestern Finland during the spring 2005, and on their offspring from their first litter. Before pairing, the voles were housed in same-sex pairs in standard mouse cages (Tecniplast, Italy), maintained on a 16-h light/8-h dark–light cycle at approximately 20°C and fed with rat/mouse breeding diet pellets (Altromin GmbH, Germany), oats and potatoes ad libitum. Dry peat and hay were used as bedding.

We formed 28 random breeding pairs of adult voles with no formation of sibling pairs (see Helle et al. 2008 for details). The voles were paired for 14 days to ensure copulation/fertilisation, after which the males were removed. All pups born (n=129) were sexed visually under a microscope immediately after their birth on the basis of anogenital distance. This procedure usually took less than a minute per litter to conduct, during which the pups were kept warm and showed no signs of distress. All litters (mean litter size±SD=4.2±1.8) were monitored daily and dead pups (n=18, 14% of all pups born) recovered for gender verification by necropsy. No experimental procedures that could have caused pup mortality were practised. At the age of weaning (21 days; Innes and Millar 1994), all pups and their parents were euthanised by CO_2 gas, after which their front paws were removed from the individuals for 2D/4D measurements. At this stage, the sex of all pups was verified by necropsy. The study was approved by the Lab-Animal Care and Use Committee of the University of Turku (license number 1534/05).

The methodology of 2D/4D measurements

We measured 2D/4D from both digital and X-ray photographs to see whether our results were conditional on the measurement method used. Front paws were used for measurements, as most of the publications so far have measured 2D/4D of the front limbs as opposed to 2M/4M metapodial ratios (but see McFadden et al. 2005; Brown et al. 2002a). The paws were surgically removed from the voles above the wrists after their death [juveniles at 21 days and parents at mean (±SD) age of 125.5 (±28) days] and glued on to separate labelled hard plastic sheets. As with Bailey et al. (2005), we found this method more precise than pressing the paws down with a glass slide. We photographed the paws with a digital camera (Nikon Coolpix 5000) through a microscope with 10× magnification. After this, the paws were X-rayed with a high-resolution X-ray machine at the Turku University Hospital. The X-ray scans were transferred to digital media during this procedure.

The digit lengths from both microscope photographs and X-ray scans were measured with Image-J software (http://rsb.info.nih.gov/ij/). The digits from the X-rays were measured one phalanx at a time, with three phalanges per digit (Fig. 1a). The phalanges were then summed to

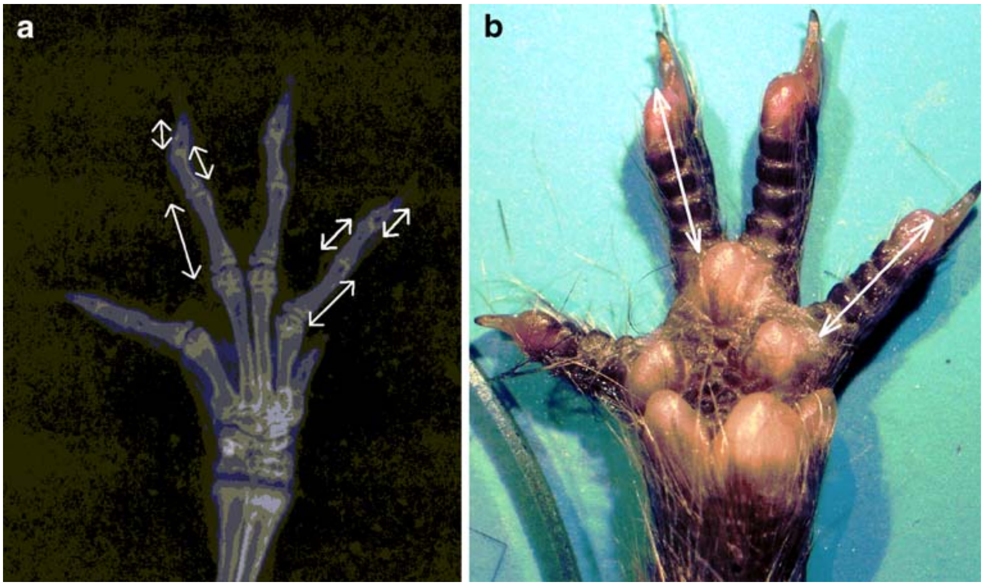

Fig. 1 Examples of X-ray and microscope photographs used in the study to measure 2D/4D. The pictures are of the same paw. The *arrows* indicate the measurement locations. The phalanges in the X-rays were measured individually, whereas in the photographs, the digit was measured as a whole

calculate the length of the digits. The microscope photographs were measured from a mid-point of the flexure crease proximal of the palm to the tip of the digit (see Fig. 1b). The digit measurements from microscope photographs were calibrated with a millimetre grid placed in every picture, whereas the digit measurements from X-rays were calibrated with a precisely measured lead ball (diameter 4,742 mm) which was placed in every X-ray scan. Means (±SD) of 2D/4D by laterality, sex and age are shown in Table 1.

Statistical analyses

In order to compare the magnitude of measurement error related to measuring 2D/4D with these two methods, we included all adult paws (n=66) and a comparable and randomly chosen sample of the juvenile data in the analysis. These photos and X-ray scans were measured twice, independently of each other in random sequence. All measurements were made by T. Lilley. A one-way analysis of variance was then performed to obtain the between- and within-group mean squares, which were used to estimate the measurement error of 2D/4D for each method (Lessells and Boag 1987). We were unable to estimate true repeatability of the measurements because after glueing the paws to the plastic sheets for X-raying, it was not possible to detach the paws anymore without damaging them. However, we are confident that, for example, X-raying the digits a second time would not affect the length of the bones measured, since this method is robust to the positioning of the paws. Moreover, in order to know whether the measurements give similar information about the relative differences among individuals, we also examined whether there was a correlation between the 2D/4D measurements taken from photos and X-rays using Pearson's correlation coefficients (2D/4D measurements from photos and X-rays were normally distributed, Kolmogorov–Smirnov test, all p>0.1).

Using general linear mixed models (Littell et al. 2006), we examined whether sex, age or lateral asymmetry explains variation in 2D/4D. It became soon clear that it made no sense to apply a full-factorial model including both X-ray and photograph data in the same analysis, since there was a difference in the average 2D/4D between the measurement methods (Table 1) and no correlation between the 2D/4Ds measured with the two methods (see below). This means that any estimates from models including both data would have yielded estimates that lie somewhere in between the two measurements, thus not corresponding to either of the actual 2D/4D measurement. Such estimates could not be used for comparisons with other data, and it is unlikely that they would have biological interpretation either due to the lack of correlation between the two measurements. We therefore analysed data separately for both methods. However, before doing that, we tested the main effect of the method and its interactions with the other factors included by combining both data. This was done to obtain formal statistical tests for the differences in 2D/4D between X-rays and photographs with respect to age and sex dependency and lateral asymmetry.

Because the data included two measurements (2D/4D from both the left and right paws) per animal, individual identity was included as a random factor in all the models, and hence, Kenward–Roger method was used to estimate the denominator degrees of freedom of fixed effects (Littell et al. 2006). In the case of X-ray data, the variance component of individual identity was negative. Since the variance component cannot be negative by definition, we modelled this covariance as an intra-class correlation using repeated measures approach with compound symmetry covariance structure (Littell et al. 2006). This approach properly controls for type I error rate and resumes the power of the analysis (Littell et al. 2006). Furthermore, we included all interactions between fixed factors in our initial models. Model reduction by backward elimination of non-significant interactions and main terms using two-tailed F tests with a p value of 0.05 as an elimination criterion was applied to obtain final models. The normality of the residuals of the final models was confirmed with Kolmogorov–Smirnov test (in all cases, p>0.15).

Table 1 Means (±SD) and sample sizes of 2D/4D measurements by age, sex and measurement method (X-ray or photo)

		X-ray	Number	Photo	Number
Juvenile	Male right	0.964±0.047	45	1.034±0.071	47
	Male left	0.939±0.042	47	0.995±0.058	46
	Female right	0.960±0.045	60	1.016±0.059	60
	Female left	0.946±0.059	60	1.002±0.042	58
Adult	Male right	0.922±0.033	8	1.026±0.080	9
	Male left	0.920±0.030	7	1.011±0.063	7
	Female right	0.928±0.033	25	1.023±0.035	25
	Female left	0.936±0.026	16	1.018±0.051	15

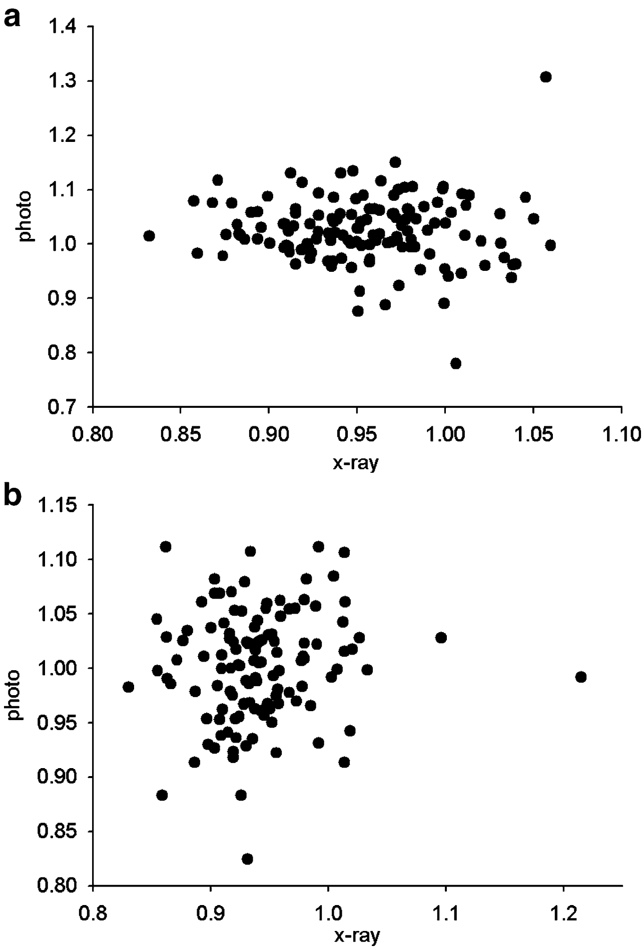

Fig. 2 The correlation between 2D/4D measures taken from photo and X-ray in the right (**a**) and left (**b**) paw

Results

The measurement error of 2D/4D measurements from photos and X-rays

The 2D/4D measured from X-rays showed high accuracy of the measurements, i.e. low measurement error (juveniles $n=66$, $r=0.88$, $F=16.48$, $p<0.0001$; adults $n=66$, $r=0.90$, $F=19.24$, $p<0.0001$). Instead, the 2D/4D measured from photos showed much higher measurement error and the two measurements from photos were not statistically repeatable (juveniles $n=66$, $r=0.50$, $F=3.04$, $p=0.08$; adults $n=66$, $r=0.52$, $F=3.18$, $p=0.08$).

Differences in 2D/4D measured from X-ray scans and photos

We found no association between the 2D/4D measurements from photographs and X-rays either in the right ($n=141$, $r=-0.032$, $p=0.75$) or left paw ($n=126$, $r=0.127$, $p=0.20$; Fig. 2a, b). The reduced model for the combined data furthermore showed a difference of means in 2D/4D measured from X-ray scans or photographs and that the effect of age on 2D/4D differed between measurement methods (method: $F_{1,399.5}=197.6$, $p<0.0001$, age: $F_{1,156.7}=2.81$, $p=0.096$, method × age: $F_{1,399.4}=9.35$, $p=0.002$, whilst controlling for laterality). In general, the measurements taken from photos showed significantly higher 2D/4D than the X-ray measurements in both left and right paws; 2D/4D values measured from photographs were on average 6% higher than those measured from X-ray scans (Table 1). The method by age interaction is examined in more detail below in separate models for the two measurement methods.

Age and sex dependency and laterality of 2D/4D in relation to the measurement method used

When the analyses were conducted separately for the two measurement methods, adults showed a lower 2D/4D than juveniles in the X-ray scans data (Table 2 and Fig. 3), but not in the photograph data (Table 3 and Fig. 3). The measurements taken from both X-ray scans and photographs showed lateral asymmetry in 2D/4D, as the right paw showed higher 2D/4D compared to the left paw (Tables 2 and 3; Fig. 4). Finally, irrespective of the measurement method, no evidence for sex differences in 2D/4D were found (Tables 1, 2 and 3).

Table 2 Results of the final minimal model of the effects of sex, age and laterality on 2D/4D measured from X-ray scans

	Estimate	SE	ndf, ddf	*F*	*p*
Final mode effects					
Age	0.025	0.007	1,158	14.08	0.0002
Laterality	0.014	0.006	1,141.6	5.75	0.0178
Removed effects					
Sex	−0.003	0.006	1,137.6	0.25	0.6187
Laterality × Age	0.024	0.014	1,159.1	2.78	0.0972
Laterality × Sex	0.009	0.012	1,138.8	0.58	0.4459
Sex × Age	0.009	0.015	1,155.9	0.37	0.5419
Laterality × Sex × Age	0.000	0.032	1,156.4	0	0.9954

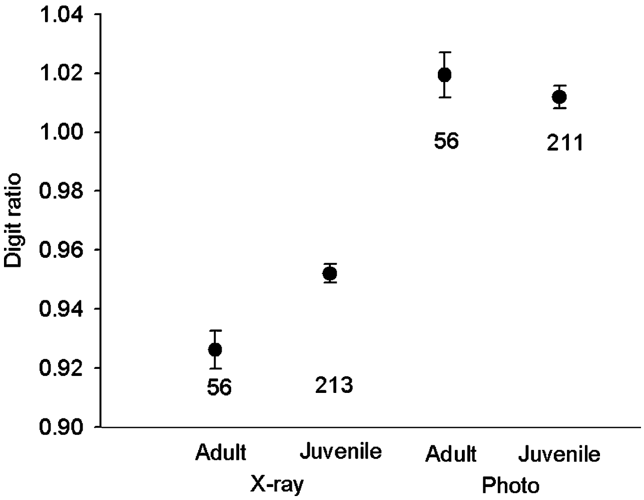

Fig. 3 Mean (±SEM) of 2D/4D in the right and left paws of juveniles and adults measured from X-rays and photographs. The X-ray measurements indicated a significant difference between juveniles and adults, whereas in the digital photographs, no difference between age groups was observed. *Numbers above the bars* represent the sample size

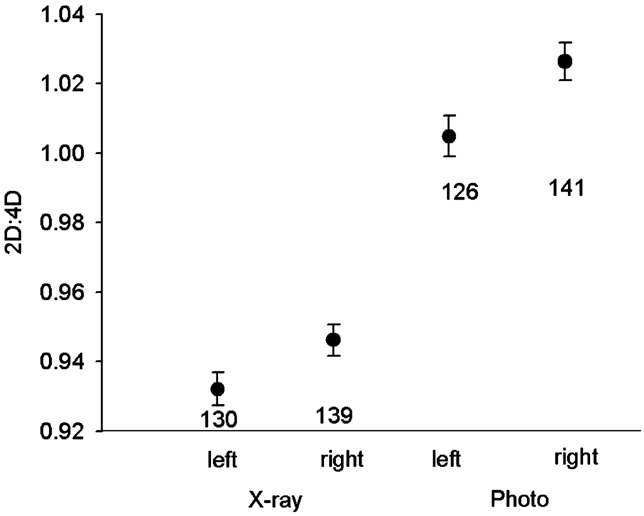

Fig. 4 Mean (±SEM) of 2D/4D measured from X-rays and digital photos in the right and left paws. There is a significant difference between the X-ray and digital photograph measurements, but also between left and right paw measurements in both measurements. *Numbers below the bars* represent the sample size

Discussion

Our results show that in field voles, 2D/4D is more accurately measured using X-ray scans than photographs. Measurements from photographs also gave higher 2D/4Ds than measurements from X-ray scans, and there was no significant correlation between these two methods in either of the paws. Moreover, we found no evidence for sexual dimorphism of 2D/4D in this species, but the measurements from X-ray scans showed a lower 2D/4D in adults compared to juveniles. In addition, irrespective of individual sex or age, 2D/4D was laterally asymmetrical using both measurement methods.

Comparison between the two methods of 2D/4D measurement

Measurements from photographs showed a much higher measurement error compared to X-rays measurements. Difficulty in measuring the digits accurately from photographs, due to the nature of the formations on the anterior side of the vole paw (see Fig. 1), was the most likely reason for this difference. Most voles had a pronounced sole, a fatty nodule, at the base of the third and fourth digits that often extends to cover the crease where the fourth digit joins the palm of the paw. This is the point where the length of the digit is measured from, increasing the measurement error. The high measurement error and higher mean 2D/4D of the photographs may have also been due to a variable amount of soft tissue at the tip of the distal phalanx, since the soft tissue at the end of the distal phalanx forms a part of the sole on which the vole treads on. There is a possibility that the soft tissue at the end of the distal phalanx on the second digit plays a large part in the locomotion of the vole, i.e. bears a lot of pressure, and therefore, it is enlarged in comparison to the similar soft tissue at the end of the fourth digit. A similar phenomenon has been demonstrated, for example, in the left hand digits

Table 3 Results of the final minimal model of the effects of sex, age and laterality on 2D/4D measurements from photographs

	Estimate	SE	ndf, ddf	*F*	*p*
Final mode effects					
Laterality	0.022	0.007	1,139.3	10	0.002
Removed effects					
Age	−0.007	0.009	1,154.3	0.62	0.430
Sex	0.004	0.007	1,135	0.39	0.535
Laterality × Sex	0.023	0.014	1,136.8	2.77	0.098
Laterality × Age	0.014	0.017	1,152.1	0.62	0.432
Age × Sex	0.009	0.019	1,146.8	0.21	0.647
Laterality × Age × Sex	0.015	0.037	1,146.5	0.15	0.695

of guitarists and cellists compared to their right hands (Kloeppel 2000). Hence, although photocopies and photographs may work well for non-musician humans (but see Buck et al. 2003), the method may be very error-prone for other species in which the paw structure is different and soft tissue has a strong effect on the digit lengths measured. This issue should be given more consideration when applying the rationale found in humans to wild animals. Additionally, although measuring 2D/4D from digital images with software has been proven effective (Kemper and Schwerdtfeger 2009), it should also be noted that photocopies, scans and photographs of fingers convert three-dimensional structures to two-dimensional images with consequent loss of accuracy (Manning et al. 2007).

Currently, most studies on 2D/4D have been conducted by either measuring the digits from photocopies (Fink et al. 2004) or by the measurement of soft tissue with a calliper or a similar precise measuring device (Manning et al. 1998; Brown et al. 2002a). Manning (2002) proposed that at least in humans, there is no need for X-raying the digits because photocopies, ink prints and soft tissue measurements, in the authors opinion, correlate highly (r=0.45) with digit measurements from X-rays. A corresponding result was obtained by Leoni et al. (2008) in hooded crows (*C. corone*). However, if interpreted in terms of the coefficients of determination (R^2), these correlation coefficients show that the 2D/4D measured from X-rays explain as little as 20% of the variation in the 2D/4D measured from photographs only. We found no correlation between the photograph and X-ray measurements in the field voles studied. Furthermore, the photograph measurements showed a consistently higher 2D/4D than the X-ray measurements. There is thus clearly a need to take the different characteristics (e.g. repeatability and/or measurement error) of these two different methods into consideration, especially in non-human species with hard-to-interpret digits.

Age- and sex-dependent differences and lateral asymmetry in 2D/4D

According to X-ray measurements, the 2D/4D of juveniles was significantly higher than the 2D/4D of adults. If not due to sampling bias caused by a smaller sample size amongst adult voles, this result suggests that in field voles, 2D/4D may continue to develop after birth and even after reaching independence. Therefore, our results contrast the original view, based mainly on human studies, that digit ratios are fixed already in utero, in humans, for example, as early as at week 14 (Manning 2002). Our results are, however, in line with the more recent studies that have suggested that this assumption may not fully hold even in humans, as 2D/4D may show subtle rise with age although not affecting the rank order of individuals by 2D/4D (McIntyre et al. 2005; Trivers et al 2006). Ali Malas et al. (2006) presented data suggesting that 2D/4D may not be established by week 14 of pregnancy or in utero in humans, but later in childhood. Moreover, Manning et al. (2004) also noticed that in humans, digit ratios may change during childhood, especially during periods of rapid growth. A similar result to our study was also found in mice where 2D/4D of the right paws of juvenile males were considerably lower than those of adult males (Brown et al. 2002a). Based on this study and on those mentioned above, one could speculate that in some mammals, digit ratios may not be fixed prenatally as widely believed. Nevertheless, more longitudinal data (in contrast to cross-sectional data used here and in most previous studies) with larger data are needed to determine whether 2D/4D or other digit ratios can be used as proxies of in utero steroid concentrations. For example, we cannot completely exclude the possibility of selective mortality between weaning and adulthood based on individual 2D/4D, biasing the 2D/4D between juveniles and adults.

One of the most fundamental predictions of the 2D/4D hypothesis is that since 2D/4D should be determined by sex steroids in utero, it should differ between the sexes. We, however, found no evidence for sexual dimorphism in 2D/4D in field voles, irrespective of age or the measurement method used. Sexual dimorphism has previously been observed in humans (Manning 2002; Saino et al. 2006), outbred laboratory mice (Brown et al. 2002a; Manning et al. 2003), Guinea baboons (Roney et al. 2004), wood mice (Leoni et al. 2005), zebra finches (Burley and Foster 2004; but see Forstmeier 2005) and house sparrows (*P. domesticus*; Navarro et al. 2007) and experimentally demonstrated by the manipulation of egg yolk testosterone in the ring-necked pheasant (*P. colchicus*; Romano et al. 2005; Saino et al. 2007). In reptiles, sexual dimorphism of digit ratios has been reported in three species (Chang et al. 2006; Rubolini et al. 2006; Chang 2008). However, Bailey et al. (2005) reported that in eight inbred laboratory mouse strains, there was no evidence of sexual dimorphism of 2D/4D. The same conclusion has been reached in the barn swallow (*Hirundo rustica*) by Dreiss et al. (2008) and anolis lizard (*Anolis carolinensis*) by Lombardo and Thorpe (2008). It may thus be that amphibians, reptiles and birds have sexually dimorphic 2D/4D such that males have higher 2D/4D than females, but in mammals, this sexual dimorphisms is reversed (Lombardo et al. 2008). The reasons for this between-species variation in sexual dimorphism in 2D/4D remain unknown. One potential explanation for variation in sexual dimorphism patterns amongst species may be that the developmental timing of sex differentiation varies between different groups of vertebrates. Alternatively, these inconsistent results may be due to variation in the measurement methods used. Since we used two different measurement methods and found no evidence for sexual dimorphism, our results suggest that these patterns may indeed vary amongst species.

Finally, we found significant lateral asymmetry in 2D/4D in field voles. The biological significance and the mechanism producing lateral asymmetry in 2D/4D are currently unknown, but it is exciting to consider that the existence of lateral asymmetry might indicate, for example, the degree of developmental stress experienced by the individual (Møller and Swaddle 1997). Further studies on other vertebrate species on one hand and on the sex-specific hormonal correlates of digit ratios on the other will be needed to explore this possibility.

To conclude, our results suggest that in field voles, 2D/4D may not be fixed or influenced by intrauterine sex steroids. This result did not depend on the measurement method (X-ray vs. photos) used. Therefore, 2D/4D appears to be a rather species- and method-specific measure and researchers should be careful when generalising its applicability to study early hormonal effects across vertebrates.

Acknowledgements We thank J. Paranko for help during the study and M. Lilley and anonymous reviewers for commenting on the manuscript. This study was funded by the Finnish Cultural Foundation (S.H. and T.Lilley) and the Academy of Finland (grant no. 106036 to O.H. and grant no. 209108 to T. Laaksonen).

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