Sexual Dimorphism in Digit Length Ratios in Two Lizard Species

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ABSTRACT

Sexual dimorphism in digit length ratios has been reported for humans, a few other mammals, and two bird species. This dimorphism is thought to arise via an interaction between the prenatal exposure of the embryo to sex hormones and the Hox genes, which are highly conserved among vertebrates and control the development of both the appendices, including fingers and toes, and the urogenital system. In this study, we report on sexual dimorphism in 2D:3D, 2D:4D, and 3D:4D contralateral ratios of the forelimbs in two species of oviparous lizards, the common wall lizard (*Podarcis muralis*) and the tree skink (*Mabuya planifrons*), as measured on museum specimens. We found that male *P. muralis* had a larger 2D:4D ratio on both sides and larger 2D:3D ratio on the left side than females, whereas in *M. planifrons*, males had lower 2D:3D ratios than females on the left side. The two species show opposite patterns of sexual dimorphism in body size, males being larger than females in *P. muralis*, and the reverse in *M. planifrons*, suggesting that interspecific variation of sex differences in digit ratios could be associated with sex-specific growth trajectories. There was a limited evidence for directional asymmetry in digit ratios. Therefore, our findings provide the first evidence that digit ratios are sexually dimorphic in any reptile species and are consistent with the idea that the genetic link between limb development and the urogenital system had been established with the evolution of the earliest terrestrial tetrapods. Importantly, many lizard species with genetic sex determination, including the ones we studied, are oviparous and may represent valuable animal models for experimental tests of the association between prenatal exposure to androgens or estrogens and digit ratios. Anat Rec Part A 288A:491–497, 2006.

Key words: 2D:4D; lizards; Reptilia; sex steroids; Squamata; vertebrates

Sexual dimorphism in digit length ratios, particularly in the second-to-fourth (2D:4D) digit length of the hand, has long been described in humans, where males show lower 2D:4D ratios than females (i.e., a shorter index compared to the ring digit) (Manning, 2002; see also review by Peters et al., 2002). Such a dimorphism is established early during ontogeny and persists at later ages (Manning, 2002; McIntyre et al., 2005; Trivers et al., 2006). Sex differences in digit ratios were reported also for other pairs of forelimb digits (e.g., 2D:3D, 2D:5D, and 3D:4D) (McFadden and Shubel, 2002; Manning et al., 2003), males generally showing lower values than females (for hindlimb digit ratios, see also McFadden and Shubel, 2002). In addition, directional asymmetry in 2D:4D has

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been reported in some studies of men and women, the right hand showing lower ratios than the left hand, and directional asymmetry being larger in men (Williams et al., 2000; reviewed in Manning, 2002). Males have also been reported to have lower ratios among 2D, 3D, and 4D in wild and laboratory mice (Brown et al., 2002a; Manning et al., 2003; Leoni et al., 2005; but see Bailey et al., 2005), whereas variable patterns of sex differences in digit or metapodial ratios have been observed in nonhuman primates, males showing generally lower values than females in baboons (*Papio hamadryas*), but higher values in Guinea baboons (*Papio papio*), gorillas (*Gorilla gorilla*), and chimpanzees (*Pan troglodytes*), although the sign of the sex difference may vary depending on the specific digit ratio under scrutiny (McFadden and Bracht, 2003, 2005; Roney et al., 2004).

Limb development in vertebrates is coordinated by a subset of homeobox genes (HoxA and HoxD), which are also expressed in the genital bud, a precursor of the urogenital system during embryonic development (Kondo et al., 1997). Thus, digit development and genitalia are affected by the expression of the same set of genes. For example, in humans, the hand-foot-genital syndrome, which is characterized by synadactily of both fingers and toes, together with several urogenital anomalies, is caused by a mutation of Hox genes (Mortlock and Innis, 1997). Such evidence for a strong relationship between the development of limbs and gonads led Manning et al. (1998) to hypothesize that gonadal hormones may be causally linked to between- and within-sex variability in digit ratios, which may result from an interaction between the expression of Hox genes and the endogenous production of sex steroids (testosterone and estradiol) by the fetus in utero. In fact, there is direct evidence that endogenous sex steroids modulate Hox gene expression in the reproductive tract (e.g., Block et al., 2000), and, accordingly, the ratio of fetal testosterone to estradiol in the human amniotic fluid was found to be negatively related to 2D:4D ratio of infants (Lutchmaya et al., 2004; for studies relating to congenital adrenal hyperplasia and digit ratio, see also Brown et al., 2002b). Under this hypothesis, and considering the wide-ranging interactions of hormones with behavior- and fitness-related traits, a growing number of studies have investigated the relationships between sexual orientation, performance, health or other fitness traits, and digit ratios in both sexes, revealing that interindividual variation in digit ratios may predict human phenotypic, psychological or performance traits (for recent studies, see, e.g., Bailey and Hurd, 2005; Fink et al., 2005; Flegr et al., 2005; Kempel et al., 2005; Romano et al., 2006; Saino et al., 2006, and references therein; see also review in Manning, 2002; but see Putz et al., 2004). Interestingly, some studies reported stronger relationships for right- than for left-hand sides (e.g., Williams et al., 2000; Brown et al., 2002c; Rahman and Wilson, 2003), suggesting a directional asymmetry in the sensitivity of the limbs to hormonal exposure, which is consistent with the lower 2D:4D ratios in the right compared to the left sides observed in some studies of humans and mice (Williams et al., 2000; Brown et al., 2002a, 2002c). A recent study also indicates that inbred mouse strains differing in behavior show between-strain variation in digit ratios, suggesting that nonhuman models may also be utilized to investigate performance and fitness correlates of variation in digit ratio (Bailey et al., 2005).

Hox genes are evolutionary conserved across vertebrate classes (Krumlauf, 1994). This leads to the general prediction that prenatal androgen exposure should affect limb and digit development in vertebrate taxa other than mammals as well, resulting in inter- and intrasexual differences in relative digit lengths (Manning, 2002). To date, sex-related variation in digit ratios among nonmammalian vertebrates has been examined only in two bird species, the zebra finch (*Taenopygia guttata*), where males show a higher 2D:4D ratio in the right foot compared to females (data for the left foot were not provided) (Burley and Foster, 2004), and the pheasant (*Phasianus colchicus*), where no sexual dimorphism in all 2D, 3D, and 4D ratios for both feet was observed in one study (Romano et al., 2005), and where males had larger 2D:4D and 3D:4D ratios of the right foot in another (N. Saino et al., unpublished data). Furthermore, increase in prenatal testosterone exposure in eggs of the ring-necked pheasant resulted in a larger 2D:3D of the left foot in females (but not in males) (Romano et al., 2005), whereas an increase in prenatal estradiol feminized (i.e., lowered) the right 2D:4D ratio of males (N. Saino et al., unpublished data), therefore providing the first experimental evidence that prenatal sex steroid exposure is causally linked to variation in digit development.

Due to the ease of experimental manipulation of prenatal hormones independently of the maternal environment (Groothuis et al., 2005), cleidoic eggs of vertebrates, such as birds and reptiles, offer an ideal opportunity to provide direct tests of an association between prenatal androgen exposure and interindividual variation in digit ratios. However, the results of such experimental manipulations are more interpretable if a sexual dimorphism in digit ratios, which may be related to sex differences in sex steroid hormone secretion during ontogeny (Galli and Wassermann, 1973; Schumacher et al., 1988; Ottinger et al., 2001), or to sex differences in prenatal hormonal environment (e.g., Lovern and Wade, 2003), is observed.

In this report, we explore sex-related variation in 2D, 3D, and 4D ratios of the right and left forelimbs in two oviparous reptile species, the common wall lizard (*Podarcis muralis*) and the tree skink (*Mabuya planifrons*), based on measures obtained on whole digits of alcohol-preserved museum specimens. We also examine the correlation between contralateral digit ratios and analyze whether relative digit lengths show side-related patterns of variation in the two sexes. To our knowledge, this is the first study reporting on either inter- or intrasexual variation in digit ratios in any reptile species.

MATERIALS AND METHODS

Specimens and Digit Measurement

Specimens were obtained from 75% alcohol-preserved collections of the Museo Zoologico "La Specola" (University of Florence, Florence, Italy). From the whole set of lizard specimen series available, we selected the two species of different families that had the largest sample size of individuals from a single site of collection, in order to avoid potential biases due to interpopulation variation in sexual dimorphism. The selected species were *Podarcis muralis* Laurenti, 1768 (Squamata: Lacertidae), a common lizard of the Mediterranean region (Corti and Lo Cascio, 2002), and *Mabuya planifrons* Peters, 1878 (Squamata: Scincidae), a widespread African scincid (Spawls et al., 2001). Both species are oviparous, with genetic sex

TABLE 1. Summary of sex differences in left and right digit length ratios in *P. muralis* and *M. planifrons*. *F*-tests refer to the Levene's test for the equality of variances, whereas the *t*-tests refer to sex differences. Effect sizes (Cohen's *d*) are calculated for each of these two tests (d_{var} and d_{mean}, respectively)

Measure	Males	Females	*F*	d_{var}	*t*	*P*	d_{mean}
P. muralis[a]							
Right							
2D:3D	0.6932 (0.0096)	0.6711 (0.0087)	0.17	0.13	1.71	0.096	0.57
2D:4D	0.6388 (0.0102)	0.6054 (0.0074)	1.15	0.35	2.64	0.012*	0.88
3D:4D	0.9216 (0.0077)	0.9030 (0.0086)	0.14	0.12	1.61	0.116	0.53
Left							
2D:3D	0.7058 (0.0099)	0.6701 (0.0089)	0.88	0.31	2.69	0.011*	0.89
2D:4D	0.6352 (0.0101)	0.5983 (0.0058)	7.06*	0.88	3.18[b]	0.004*	1.06
3D:4D	0.8998 (0.0059)	0.8940 (0.0070)	0.31	0.18	0.64	0.527	0.21
M. planifrons[c]							
Right							
2D:3D	0.7313 (0.0046)	0.7482 (0.0058)	0.04	0.07	−2.30	0.030	0.89
2D:4D	0.7126 (0.0074)	0.7250 (0.0077)	0.28	0.20	−1.12	0.274	0.43
3D:4D	0.9741 (0.0061)	0.9690 (0.0062)	1.30	0.44	0.56	0.578	0.21
Left							
2D:3D	0.7280 (0.0049)	0.7494 (0.0056)	0.24	0.19	−2.83	0.009*	1.09
2D:4D	0.7126 (0.0076)	0.7206 (0.0061)	2.07	0.56	−0.75	0.459	0.29
3D:4D	0.9785 (0.0060)	0.9615 (0.0047)	0.78	0.34	2.02	0.054	0.78

Mean (SE) digit ratio values are reported (see Results and below for sample sizes of males and females). Significant differences after standard Bonferroni correction of the *t*-test *P*-values ($\alpha = 0.10/6 = 0.017$; see Methods) are marked with an asterisk.
[a]: measured specimens: males ($N = 18$), 8535, 8544, 8547, 8548, 8552, 8555, 8560, 8563, 8571, 8572, 8581, 8590, 8596, 8600, 8603, 8605, 8607, 8612; females ($N = 18$), 8545, 8546, 8551, 8566, 8567, 8570, 8575, 8577, 8580, 8583, 8587, 8591, 8593, 8594, 8598, 8599, 8601, 8610.
[b]: *t*-test for unequal variances.
[c]: measured specimens: males ($N = 17$), 1601, 1603, 1606, 1608, 1609, 1643, 1644, 1699, 1701, 1713, 1715, 1716, 1731, 1762, 1763, 1765, 1770; females ($N = 11$), 1605, 1641, 1698, 1714, 1718, 1720, 1729, 1760, 1761, 1766, 1768.

determination (Ciofi and Swingland, 1997; Corti and Lo Cascio, 2002), but show different patterns of sexual dimorphism in size, because males of *P. muralis* are larger than females (Corti and Lo Cascio, 2002), whereas *M. planifrons* shows the reversed pattern (see Results). *P. muralis* specimens were collected at a single site of the Venice Lagoon (Italy; 45°25′N–12°19′E) in May 1967. *M. planifrons* specimens were from Dinsor (Somalia; 2°28′N–42°58′E), and were collected during July 1962. All measured individuals were sexually mature adults, and sexes were determined by gonadal inspection, as specimens were opened short after collection to allow the alcohol to penetrate into the internal organs and cavities in order to obtain a long-lasting preservation. All individuals whose sex could not be reliably determined were discarded. Although the validity of museum collections for the study of digit ratios may be questioned (McFadden and Bracht, 2005), because specimens may not represent a random sample of the species under scrutiny, it should be emphasized that the specimens measured in this study were collected in order to study phenotypic variation and not for aesthetic or exhibition purposes. We therefore believe that they should be regarded as a representative sample of the original population with respect to the analysis of sex differences in digit ratios. In addition, the use of well-preserved museum specimens had the advantage that no animal was sacrificed specifically for the purposes of this study.

Since limbs (and especially digits) of alcohol-preserved specimens are relatively less flexible than those of freshly dead or live lizards, are very thin and delicate, and could easily break when manipulated, in order to preserve as much as possible the integrity of the specimens, we decided to concentrate our attention on the measurements of the lengths of 2D, 3D, and 4D, which are the longest digits of the forelimbs and were the easiest to measure on preliminary trials on a few freshly dead wall lizards. Only individuals with intact forelimbs and 2D, 3D, and 4D on both sides were measured. Hindlimbs were disregarded also because of their peculiar shape, with outer digits being very elongated, thin, and often already broken, damaged, or difficult to stretch correctly in order to obtain reliable measurements without the risk of permanently altering specimens. Overall, specimens measured for both species are listed in footnotes to Table 1. Digits were measured by gently stretching the forepaw on a rigid entomological plastic sheet. The base of the forepaw was fixed to the side of the plastic sheet by using a thin tape line, and digits to be measured were carefully stretched; in order to keep them as straight as possible, they were inserted into a microhematocrit capillary glass tube to approximately half the finger. Tape was used to fix capillary tubes. The upper side of forepaws was then scanned under an Epson Perfection 2480 scanner at 4,800 dpi and imported into the Adobe Photoshop image processing software. Digit lengths were measured in pixels on scanned images, from a proximal landmark of the finger to the distal fleshy tip, excluding toenails. In both species, the proximal landmark was identified as the unequivocal landmark closest to the basal joint of the finger (Fig. 1). In *P. muralis*, this was identified as the second dorsal scale on each digit. In *M. planifrons*, where the basal joints are hidden by imbricated dorsal scales, we identified the basal landmark as the middle point of a line perpendicular to the digit axis and tangential to the imbricated scale covering the metacarpals (Fig. 1). Images of the right forepaw

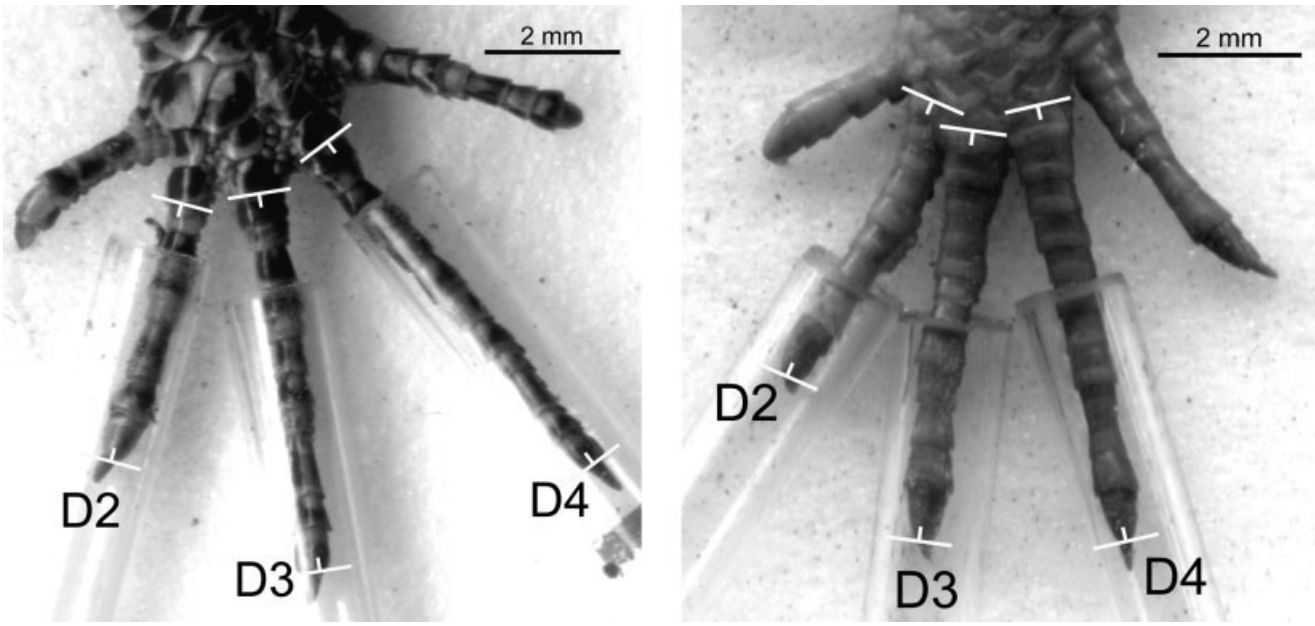

Fig. 1. Picture of a right *P. muralis* forelimb (left, translated along the vertical axis; specimen 8577) and a left *M. planifrons* forelimb (right; specimen 1768) illustrating the proximal and distal landmarks used to measure digit lengths. See Materials and Methods for details of landmark identification and footnotes to Table 1 for the complete list of measured specimens.

were reflected on their vertical axis and then measured in order to avoid any bias during image processing (Bailey et al., 2005). Repeatability of digit ratios (2D:3D, 2D:4D, and 3D:4D), as measured on a repeated preparation of 10 randomly selected *P. muralis* (5 males, 5 females) and 10 randomly selected *M. planifrons* (4 males, 6 females), was high (mean of both sides: *P. muralis*, 0.94 ± 0.02 SE; *M. planifrons*, 0.87 ± 0.02 SE; the lowest value for both species was 0.82, and all values always associated with $F_{9,10} > 10.90$; $P < 0.0001$). For all specimens, we recorded the snout-vent length (SVL) as a measure of body size.

We measured all 2D, 3D, and 4D on both sides in 18 male and 18 female specimens of *P. muralis*, and in 17 male and 11 female specimens of *M. planifrons*. Therefore, we will refer to these sample sizes in all comparisons.

Statistical Procedures

The statistical analysis of digit length ratios involves two main intrinsic problems. First, several ratios are calculated for each individual, and each ratio is likely to be informative in itself (Manning et al., 2003); however, this obviously leads to an increased likelihood of obtaining statistically significant results by chance, i.e., rejecting the null hypothesis when it is true. This is often controlled for by introducing corrections to P values (Nakagawa, 2004). Second, the different ratios cannot be considered as fully independent measures, because the length of each digit is included in more than one ratio. To date, there is no standard way to present and analyze these data, although most researchers emphasized the importance of providing extensive descriptive statistics, and several relied on effect sizes for the interpretation of results (e.g., McFadden and Shubel, 2002; McFadden and Bracht, 2003, 2005). To be consistent with previous literature on this topic, we therefore followed this approach.

Since commonly used corrections for multiple tests may lead to dramatic reductions of statistical power (Chandler, 1995; Nakagawa, 2004), and considering the exploratory purpose of this study, we performed a standard Bonferroni correction but set the experiment-wise error rate at 10%, according to earlier suggestions (e.g., Chandler, 1995), in order to limit an excessive loss of power in individual tests. We report uncorrected P values, and those that were significant after correction are marked with an asterisk. All digit ratios for both species were normally distributed (Kolmogorov-Smirnov test, all P values > 0.4), so parametric statistics (t-tests for unpaired and paired samples, Pearson's correlation coefficients) were used throughout. In addition, we report the size of the effect (Cohen's d, absolute values) for within- and between-sex comparisons of digit ratios, as performed in other recent descriptive studies concerning sexual dimorphism of digit ratios (McFadden and Shubel, 2002; McFadden and Bracht, 2003, 2005). In the absence of reference values for this specific field, effect sizes d of 0.8 are considered as large, those of 0.5 medium, and 0.2 small (Cohen, 1992; Rosenthal et al., 2000). Means are reported together with their associated standard errors.

RESULTS

In *P. muralis*, males had larger SVL than females (61.85 ± 1.22 mm vs. 57.88 ± 0.94 mm; $t_{34} = 2.58$; $P = 0.014$). The Levene's test for the homogeneity of variances did not reveal differences between the sexes in any digit ratio for both sides, with the exception of left 2D:4D (Table 1). Out of the six between-sex comparisons, males had larger right 2D:4D and left 2D:3D and 2D:4D ratios than females (Table 1). Effect sizes for significant differences were always > 0.89. The correlation coefficients between contralateral digit ratios were always positive in both

TABLE 2. Pearson correlation coefficients between contralateral digit ratios of male and female *P. muralis* and *M. planifrons*

	Males		Females	
Measure	*r*	*P*	*r*	*P*
P. muralis				
2D:3D	0.602	0.008*	0.687	0.002*
2D:4D	0.539	0.021*	0.824	< 0.001*
3D:4D	0.720	0.001*	0.475	0.046
M. planifrons				
2D:3D	0.751	0.001*	0.884	< 0.001*
2D:4D	0.937	< 0.001*	0.939	< 0.001*
3D:4D	0.862	< 0.001*	0.578	0.062

See Results and footnotes to Table 1 for sample sizes of males and females.
Significant differences after standard Bonferroni correction of *P*-values ($\alpha = 0.10/3 = 0.033$; see Methods) are marked with an asterisk.

sexes, with r values ranging from 0.48 to 0.82 (Table 2). The pairwise analysis of differences between contralateral digit ratios showed that values were similar for each side in both sexes (paired-samples *t*-tests, all *P* values > 0.12; absolute d < 0.30), with the exception of 3D:4D ratio in males, which was larger for the right compared to the left side (paired-samples *t*-test, $t_{17} = -3.05$; $P = 0.007$*; d = 0.74), suggesting the existence of a limited directional asymmetry in digit ratio values.

In the subsample of *M. planifrons* specimens where we measured digit ratios, male and female SVL was similar (80.53 ± 1.77 mm vs. 83.98 ± 2.15 mm; $t_{26} = -1.23$; $P = 0.23$), although females were slightly larger than males. However, across the whole series of known-sex specimens available, including those on which digits could not be measured (see Materials and Methods), females were significantly larger than males (80.32 ± 1.63 mm vs. 85.27 ± 1.76 mm; $t_{40} = -2.06$; $P = 0.046$). The Levene's test for the homogeneity of variances did not reveal differences between the sexes in any digit ratio for both sides (Table 1). Between-sex comparisons of digit ratios revealed that males had a smaller 2D:3D ratio on the left side (Table 1). The sex difference in the 2D:3D ratio for the other side was not significant after the Bonferroni correction, but was associated with a large effect size (d = 0.89), indicating that the strength of the difference was similar to the other ratios that showed significant sex differences (Table 1). Contralateral digit ratios were positively correlated in both sexes, with r values ranging from 0.58 to 0.94 (Table 2). The pairwise analysis of differences between contralateral digit ratios did not reveal any evidence of a clear directional asymmetry (paired-samples *t*-tests, all *P* values > 0.15; absolute *d* values always < 0.16).

DISCUSSION

In this study, we have examined sexual dimorphism in contralateral 2D, 3D, and 4D length ratios of the forelimbs for the first time in any reptile species. We found that, in two oviparous lizard species differing in the degree of sexual size dimorphism, digit length ratios showed variable patterns of sexual dimorphism, males having greater 2D:4D ratios (both sides) and 2D:3D ratios (left side) than females in *P. muralis*, a species where males are larger than females, and males showing a lower 2D:3D ratios on the left side than females in *M. planifrons*, a species where females are larger than males. Differently from several studies of humans and mice, but similarly to studies of birds (see Introduction), we found limited evidence for directional asymmetry in digit ratios.

This study provides the first evidence that digit length ratios show a degree of sexual dimorphism in another class of vertebrates, after mammals and birds. Remarkably, the patterns of sexual dimorphism in digit ratios were different in the two species and involved partly different digit ratios. Therefore, interspecific differences in sexual dimorphism of digit length ratios could be difficult to predict among reptiles. Among the two other oviparous vertebrate species investigated so far (the pheasant and the zebra finch), there appears to be both interspecific and intrapopulation variability in digit ratios (Burley and Foster, 2004; Romano et al., 2005). In the zebra finch and in one pheasant subspecies (*P. c. colchicus*), males appear to have larger ratios than females (right 2D:4D ratios in zebra finch and right 2D:4D and 3D:4D ratios in *P. c. colchicus*) (Burley and Foster, 2004; N. Saino et al., unpublished data), although in a different captive pheasant breed there was no sexual dimorphism in any of the digit ratios of the two sides (Romano et al., 2005). Similarly, a marked interspecific variability in sexual dimorphism in digit ratios is observed among primate species (see Introduction).

The different pattern of variation in sex differences in digit ratios observed in two species showing opposite patterns of sexual size dimorphism may suggest the existence of a common underlying mechanism shaping both sexual dimorphism in body traits and digit ratios. In vertebrates, the ontogeny of sexual dimorphism depends on the expression of sex- and age-specific modifiers of a developmental program shared by both sexes (see review in Badyaev, 2002). In fact, sex-specific growth patterns are accomplished by a high sex specificity in growth hormone (GH) secretion and sensitivity (Badyaev, 2002). In this context, gonadal steroids play a major role, because the hypothalamic GH-releasing and -inhibiting factors, which regulate GH release and synthesis, are under the control of gonadal steroids. Both androgens and estrogens can stimulate the secretion of GH, but their relative importance can vary between species (see review in Badyaev, 2002). Therefore, we may speculate that sex-specific patterns of sex hormones secretion occurring during embryonic development, which are observed as early as at day 7–8 of incubation in embryos of avian species (Galli and Wassermann, 1973; Schumacher et al., 1988; Ottinger et al., 2001), may concomitantly affect the expression of limb development (hence digit ratios) and later GH secretory patterns in different ways in the two species, resulting in interspecific variation in the expression of sexual dimorphism in size and digit ratios.

Alternatively, the observed interspecific variation in sexual dimorphism in digit ratios may be independent of sexual dimorphism and may simply originate from interspecific variation in sex-specific yolk androgen or estrogen deposition by females, resulting in differential exposure to prenatal sex steroids in male and female embryos of the two species. For instance, sex-specific yolk testosterone levels have repeatedly been observed in one lizard species, the green anole (Lovern and Wade, 2001, 2003; Lovern et al., 2001).

Whatever the proximate mechanisms generating the observed patterns, our findings may have broader implications concerning the etiology of sexual dimorphism in digit ratios. In fact, Manning (2002) hypothesized that the genetic control of the formation of both the urogenital system and the extremities (fingers, toes, and penises) (Kondo et al., 1997) is a primitive association that may have originated with the transition from an aquatic to a terrestrial lifestyle, leading to the prediction that most terrestrial tetrapods with pentadactyl limbs will show sexually dimorphic digit ratios. The observation of sex differences in digit ratios among reptiles, representing the first true terrestrial tetrapods, is therefore consistent with this evolutionary hypothesis.

In conclusion, this study indicates that oviparous reptiles showing genetic sex determination, such as several lizard species (Ciofi and Swingland, 1997), could represent promising animal models to explore the link between prenatal sex steroid exposure and variation in digit ratios. Similarly to birds, where experimentally elevated egg androgen or estrogen levels differentially affected the digit ratio of females and males, respectively (Romano et al., 2005), the manipulation of egg hormones in these species could provide a further direct test of the link between prenatal hormonal influences and digit ratios, and thus of the usefulness of digit ratios as markers of prenatal exposure to sex hormones.

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