

Patterns of Second-to-Fourth Digit Length Ratios (2D:4D) in Two Species of Frogs and Two Species of Lizards at La Selva, Costa Rica

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ABSTRACT

It is now well documented that androgen and estrogen signaling during early development cause a sexual dimorphism in second-to-fourth digit length ratio (2D:4D). It is also well documented that males of mammalian species have a smaller 2D:4D than females. Although there are discrepancies among 2D:4D studies in birds, the consensus is that birds exhibit the opposite pattern with males having a larger 2D:4D than females. The literature currently lacks substantial information regarding the phylogenetic pattern of this trait in amphibians and reptiles. In this study, we examined 2D:4D in two species of frogs (*Oophaga pumilio* and *Craugastor bransfordii*) and two species of lizards (*Anolis humilis* and *Anolis limifrons*) to determine the existence and the pattern of the sexual dimorphism. Male *O. pumilio* and *C. bransfordii* displayed larger 2D:4D than females in at least one of their two forelimbs. Male *A. humilis* had larger 2D:4D than females in both hindlimbs, but smaller 2D:4D than females in both forelimbs. Male *A. limifrons* may also have smaller 2D:4D than females in the right forelimb. Finally, digit ratios were sometimes positively related to body length, suggesting allometric growth. Overall, our results support the existence of the 2D:4D sexual dimorphism in amphibians and lizards and add to the knowledge of 2D:4D trait patterning among tetrapods. Anat Rec, 295:597–603, 2012. © 2012 Wiley Periodicals, Inc.

Key words: digit ratio (2D:4D); testosterone; androgens; sexual dimorphism; amphibia; reptilian

INTRODUCTION

Within the last decade, there has been a steady growth in the number of studies on the sexually dimorphic digit length ratios (Voracek and Loibl, 2009). Researchers have repeatedly found sexual dimorphism in the second-to-fourth digit length ratio (2D:4D; e.g. Brown et al., 2002; Putz et al., 2004; Forstmeier et al., 2010). Sexually dimorphic traits are affected by prenatal androgen and estrogen concentrations, as well as their respective receptors, which alter gene expression (Zheng and Cohn, 2011; also see references in Forstmeier et al., 2010). It was previously thought that differences in *Hox* gene expression cause the 2D:4D sexual dimorphism (Manning et al., 2003; reviewed by Forstmeier et al., 2010). However, more recently, Zheng and Cohn (2011)

reported that androgen and estrogen signaling during a critical period in development acts on skeletogenic genes, chondrogenic genes, and cell proliferation to influence 2D:4D sexual dimorphism in mammals. Sexual dimorphism of 2D:4D is evident in humans as early as

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the 14th week of gestation and is afterward stable through adulthood (Malas et al., 2006; Galis et al., 2010; but see Trivers et al., 2006). The second-to-fourth digit length ratio is also considered an indicator trait, because it correlates with numerous traits, such as sexual orientation (Grimbos et al., 2010), susceptibility to disease (Manning and Bundred, 2000; Devine et al., 2010; Gooding et al., 2010), sports performance (Bennett et al., 2010; Voracek et al., 2010), cognitive drive (Wakabayashi and Nakazawa, 2010), probability of developing eating disorders (Smith et al., 2010), and visual-spatial memory and numerical skills (Bull et al., 2010), among other characteristics and conditions (Voracek and Loibl, 2009) that are also likely influenced by prenatal hormones.

Thus far, 2D:4D has been studied extensively in both mammals and birds. In humans, mice, and rats, 2D:4D is sexually dimorphic (Brown et al., 2002; Lutchmaya et al., 2004; Talarovicová et al., 2009). The relationship between 2D:4D and sex seems to be well conserved among mammals such that male digit ratios are smaller than those of females (laboratory mice, *Mus musculus*, Brown et al., 2002; humans, *Homo sapiens*, Manning et al., 2002; baboons, *Papio hamadryas*, McFadden and Bracht, 2002; laboratory rats, *Rattus norvegicus*, McMechan et al., 2004; Anthropoids, Nelson and Shultz, 2010; but see field vole, *Microtus agrestis*, Lilley et al., 2009). Contrary to what has been found in other mammals, male Guinea Baboons (*Papio papio*) have larger digit ratios than females (Roney et al., 2004). This reversal or multiple origin of the trait could have been caused by genes on the sex chromosomes, changes in the relationship between digit ratios and the mechanism of sexual differentiation, or changes in the sexually dimorphic neurotransmitters, hormones, or hormone receptors (Roney et al., 2004; Chang et al., 2006).

In birds, results are inconsistent. When the sexual dimorphism is reported, males have larger 2D:4D than females (zebra finches, *Taenopygia gutata*, Burley and Foster, 2004; house sparrows, *Passer domesticus*, Navarro et al., 2007; ring-necked pheasants, *Phasianus colchicus*, Saino et al., 2007; barn swallow, *Hirundo rustica*, Dreiss et al., 2008; hooded crows, *Corvus corone*, Leoni et al., 2008). However, other studies on the same and other bird species have reported no existence of the sexual dimorphism (zebra finch, *Taenopygia gutata*, Forstmeier, 2005; ring-necked pheasants, *Phasianus colchis*, Romano et al., 2005; collared flycatchers, *Ficedula albicollis*, Garamszegi et al., 2007). Lombardo et al. (2008) also did not detect differences in 2D:4D when analyzing digit ratios separately for four species of birds from three orders (Passeriformes, house sparrow, *Passer domesticus*; tree swallow, *Tachycineta bicolor*; Psittaciformes, budgerigar, *Melopsittacus undulates*; Galliformes, chicken, *Gallus domesticus*), but when the data from those species were pooled, males had larger 2D:4D and 2D:3D than females.

There have also been inconsistencies in studies of 2D:4D in lizards, but the patterning of the trait appears to be species-specific (Chang et al., 2006; Rubolini et al., 2006). Chang et al. (2006) found that male green anoles (*Anolis carolinensis*) had larger 2D:4D than females in the right hindlimb, and males had a smaller 2D:4D than females in the left forelimb. However, Lombardo and Thorpe (2008) did not find evidence to support sexual dimorphism of the trait in the same species. In another

study, male common wall lizards (*Podacris muralis*) had larger 2D:4D than females in both forelimbs, and tree skinks (*Mabuya planifrons*) were sexually monomorphic in relation to 2D:4D, but males had smaller 2D:3D ratios than females (Rubolini et al., 2006). Interestingly, male *P. muralis* are larger than females, and male *M. planifrons* are smaller than females, suggesting that the relative adult body size of a species may be related to the factors that organize the 2D:4D sexual dimorphism during development. Tobler et al. (2011) also reported males having smaller 3D:4D than females on the hindlimbs of painted dragon lizards, *Ctenophorus pictus*. Braña (2008) compared the length of the fourth digit on the right hindlimb of *Lacerta vivipara* hatchlings that were incubated with either same sex or mixed sex trios of eggs. The hatchlings of both sexes that were incubated with male eggs had longer fourth digits than the hatchlings incubated with female eggs, suggesting that hormonal leakage from male eggs was sufficient to influence digit patterning in developing lizards. To our knowledge, no other reports exist on 2D:4D in lizards, which leaves us with an unclear pattern of the trait in this group (reviewed in Voracek and Loibl, 2009).

In addition, little information is available regarding 2D:4D in amphibians. One study on strawberry poison frogs (*Oophaga pumilio*) found that males had larger 2D:4D than females in both hindlimbs (Chang, 2008), suggesting that patterning between the sexes is similar to birds. Overall, our knowledge of this sexually dimorphic trait among tetrapods, other than birds and mammals, is poorly developed and only includes one amphibian and a few lizard species. In this study, we measured the second and fourth digits of all four limbs of two species of frogs (*Oophaga pumilio* and *Craugastor bransfordii*) and two species of lizards (*Anolis humilis* and *Anolis limifrons*) to provide more information on the phylogenetic pattern of 2D:4D. Further understanding of the trait's pattern in amphibians will also provide insight to the possible evolutionary trajectories of the trait and why differences may exist in trait patterning among tetrapods.

MATERIALS AND METHODS

Procedure

Oophaga pumilio, *Craugastor bransfordii*, *Anolis humilis*, and *Anolis limifrons* individuals were caught at La Selva Biological Station in Sarapiquí, Costa Rica between June 2009 and March 2010. We chose these species because they are common, easy to collect, and the sexes are easily distinguished. Male *O. pumilio* have a darkened throat patch whereas females do not (Donnelly, 1989). Male *C. bransfordii*, typically, have a smaller body size than females and a tympanum that is approximately the same size as their eye, while females' tympanums are smaller than their eye (Guyer and Donnelly, 2005). Male *A. humilis* and *A. limifrons* can be distinguished from females by the presence of a significantly larger and more colorful dewlap.

We caught frogs and lizards during the day by hand and by aquarium net along trails. We visited each location once to avoid recapturing individuals. After capture, we brought individuals back to the laboratory and measured their snout-to-vent length (SVL) with regular calipers. Then, one at a time, we extended each limb

onto the underside of a Petri dish to which a ruler had been taped. We separated their digits manually and flattened them using a microscope slide. Then, we photographed each foot repeatedly until a clearly focused and illuminated photograph was obtained. We measured the length of the second and fourth digits from photos zoomed in at 150% using the line tool in the image analysis software Image J (NIH). Captured individuals with missing digits or toe pads were not included in the study. G.V.D. conducted all measurements to better test the results of Chang (2008), where digits were measured by J.L.S. (formerly J.L.C.). The second and fourth digits of each limb were measured once. Five pairs of digits from each species were randomly chosen and re-measured to test the reliability of the original measurements. All resamples were within ± 0.04 mm of the original measurement. Resamples were not included in the analysis. We captured and measured 36 male and 48 female *O. pumilio*, 32 male and 40 female *C. bransfordii*, 46 male and 42 female *A. humilis*, and 40 male and 37 female *A. limifrons*. We released all captured individuals at their original area of capture.

Statistical Analysis

We used R (R Development Core Team, 2011) for all statistical analyses. We used a Shapiro Wilk's test to test for normality of SVL and 2D:4D of each limb, and we used Levene's test to test for homogeneity of variances (all $P > 0.05$). Data that were not normally distributed were analyzed for outliers using Grubb's test and log or square root transformed if necessary. Data were again tested for normality (all $P > 0.05$) and homogeneity of variances (all $P > 0.05$). We used parametric tests accordingly.

The aim of this study was to compare 2D:4D of each limb between the sexes of each species. We used multiple analysis of covariance (indicating sex as the experimental variable, 2D:4D as the response variable, and SVL as the covariate; Kratochvil and Flegr, 2009; Tobler et al., 2011). We also calculated effect sizes (Cohen, 1992), as have other digit ratio studies (Bailey et al., 2004; Rubolini et al., 2006; Lombardo and Thorpe, 2008), using the standard consensus that $d = 0.2$ is small, $d = 0.5$ is medium, and $d = 0.8$ is large. Following Chang et al. (2006) and Lombardo and Thorpe (2008), we used paired t -tests to compare left and right side 2D:4D within a sex for each species to test for directional asymmetry.

RESULTS

Male *O. pumilio* had significantly larger 2D:4D than females in both of the forelimbs (front left: $F_{1,79} = 19.508$, $P < 0.001$; front right: $F_{1,80} = 9.978$, $P = 0.002$), but digit ratios in the hindlimbs were not significantly different (Table 1). Effect sizes were medium to large for both forelimbs ($d > 0.65$), but small for both hindlimbs ($d < 0.2$, Table 2). Male *C. bransfordii* had significantly smaller SVL ($t_{69} = 1.714$, $P = 0.045$) and larger 2D:4D in the left forelimb than females ($F_{1,64} = 5.494$, $P = 0.022$). However, no sexual dimorphism was detected in the other three limbs. A medium effect size was observed in the left forelimb for *C. bransfordii* ($d = 0.618$), but effect sizes in all other limbs were small ($d < 0.35$; Table 2). Male *A. humilis* had a significantly smaller SVL than

females ($t_{84} = 8.327$, $P < 0.001$). Males *A. humilis* had smaller 2D:4D than females in both forelimbs (front left: $F_{1,78} = 7.940$, $P = 0.006$; front right: $F_{1,79} = 4.506$, $P = 0.036$), and males had significantly larger 2D:4D than females in both hindlimbs (back left: $F_{1,82} = 7.335$, $P = 0.008$; back right: $F_{1,77} = 9.915$, $P = 0.002$). All effect sizes were approximately medium sized for *A. humilis* ($0.4 < d < 0.75$, Table 2). Male *A. limifrons* were significantly smaller than females ($t_{76} = 3.544$, $P < 0.001$). Male *A. limifrons* showed a non-significant trend toward having a smaller 2D:4D than females in the right forelimb ($F_{1,70} = 2.925$, $P = 0.091$), but no sexual dimorphism was detected in any other limb. The effect size for the front right was approximately medium sized ($d = 0.412$), but was small for the other three limbs of *A. limifrons* ($d < 0.3$, Table 2).

We also found directional asymmetry within sexes. Female *O. pumilio* had significantly larger 2D:4D on the right forelimb than on the left forelimb and larger 2D:4D on the left hindlimb than on the right hindlimb ($t_{47} = 1.715$, $P = 0.046$; $t_{45} = -2.535$, $P = 0.007$, respectively). Female *C. bransfordii* also had significantly larger digit ratios on their right forelimb than on their left forelimb ($t_{37} = 2.457$, $P = 0.009$). No other directional asymmetries were significantly different ($P > 0.05$).

In general, some SVL and SVL-by-sex covariates were significantly correlated with 2D:4D (Table 1). In *O. pumilio*, SVL was correlated with 2D:4D on the front left limb ($F_{1,79} = 7.558$, $P = 0.007$) such that as SVL increased, 2D:4D decreased. SVL was also correlated with 2D:4D in both hindlimbs of *C. bransfordii* (back left: $F_{1,59} = 8.345$, $P = 0.005$; back right: $F_{1,63} = 11.531$, $P = 0.001$) such that as SVL increased, 2D:4D increased. The right forelimb and hindlimb of *A. humilis* and the left hindlimb of *A. limifrons* also showed effects of SVL ($F_{1,79} = 6.315$, $P = 0.014$; $F_{1,77} = 4.032$, $P = 0.048$; $F_{1,67} = 6.922$, $P = 0.010$, respectively); in all three limbs, as SVL increased, 2D:4D decreased. We also found a SVL-by-sex interaction in the left forelimb of *C. bransfordii* ($F_{1,64} = 7.285$, $P = 0.008$); as male SVL increased, 2D:4D increased, and as female SVL increased, 2D:4D decreased.

DISCUSSION

In this study, we report on 2D:4D in amphibians and reptiles in order to advance our understanding of the phylogenetic patterning of 2D:4D in tetrapods. The species that we investigated expressed the sexual dimorphism (or a trend toward sexual dimorphism in the case of one lizard species). These results are consistent with previous amphibian and lizard 2D:4D studies (see Introduction; Chang, 2008 for review).

Effect sizes ranged from 0.074 to 0.932. Most species had medium to large effect sizes when the sexual dimorphism was detected (amphibians: $0.618 < d < 0.932$; lizards: $0.425 < d < 0.746$), which is larger than reports in humans (detected: $d = 0.35$; Hönekopp and Watson, 2010), laboratory mice (not detected: $d = 0.02$; Bailey et al., 2004), and *A. carolinensis* (not detected: $0.18 < d < 0.37$; Lombardo and Thorpe, 2008), but smaller than *P. muralis* (detected: $0.88 < d < 1.09$; Rubolini et al., 2006).

Male *O. pumilio* have significantly larger 2D:4D than females in both forelimbs, but neither of the hindlimbs

TABLE 1. Results of MANCOVA testing for effects of SVL, sex, and SVL-by-sex interaction on 2D:4D in *O. pumilio*, *C. bransfordii*, *A. limifrons*, and *A. humilis* from La Selva, Costa Rica^a

Factor	Parameter estimate	SE	df	F	P
<i>O. pumilio</i>					
Front left			3,79 ^b	9.636 ^b	<0.001 ^{b,*}
Sex	-0.348	0.300	1,79	19.508	<0.001*
SVL	-0.029	0.009	1,79	7.588	0.007*
SVL × sex	0.021	0.015	1,79	1.809	0.182
Front right			3,80 ^b	4.552 ^b	0.005 ^{b,*}
Sex	0.526	0.355	1,80	9.978	0.002*
SVL	-0.002	0.011	1,80	1.854	0.177
SVL × sex	-0.024	0.018	1,80	1.823	0.180
Back left			3,79 ^b	0.811 ^b	0.491 ^b
Sex	-0.058	0.134	1,79	0.115	0.734
SVL	0.003	0.004	1,79	2.139	0.147
SVL × sex	0.002	0.007	1,79	0.179	0.672
Back right			3,78 ^b	1.356 ^b	0.262 ^b
Sex	0.072	0.125	1,78	0.756	0.387
SVL	0.006	0.004	1,78	3.030	0.085
SVL × sex	-0.003	0.006	1,78	0.280	0.598
<i>C. bransfordii</i>					
Front left			3,64 ^b	4.311 ^b	0.007 ^{b,*}
Sex	-0.483	0.212	1,64	5.494	0.022*
SVL	-0.006	0.006	1,64	0.154	0.695
SVL × sex	0.033	0.012	1,64	7.285	0.008*
Front right			3,66 ^b	0.112 ^b	0.952 ^b
Sex	0.019	0.161	1,66	0.146	0.702
SVL	-0.001	0.004	1,66	0.153	0.697
SVL × sex	-0.001	0.009	1,66	0.038	0.844
Back left			3,59 ^b	3.240 ^b	0.028 ^{b,*}
Sex	0.048	0.047	1,59	0.743	0.392
SVL	0.003	0.001	1,59	8.345	0.005*
SVL × sex	-0.002	0.002	1,59	0.630	0.430
Back right			3,63 ^b	5.357 ^b	0.002 ^{b,*}
Sex	-0.122	0.062	1,63	0.658	0.419
SVL	0.003	0.001	1,63	11.531	0.001*
SVL × sex	0.007	0.003	1,63	3.879	0.053
<i>A. humilis</i>					
Front left			3,78 ^b	2.976 ^b	0.036 ^{b,*}
Sex	0.005	0.273	1,78	7.940	0.006*
SVL	-0.002	0.006	1,78	0.893	0.347
SVL × sex	-0.002	0.008	1,78	0.092	0.761
Front right			3,79 ^b	4.525 ^b	0.005 ^{b,*}
Sex	0.168	0.125	1,79	4.506	0.036*
SVL	-0.001	0.002	1,79	6.315	0.014*
SVL × sex	-0.006	0.003	1,79	2.752	0.101
Back left			3,82 ^b	3.373 ^b	0.022 ^{b,*}
Sex	0.192	0.102	1,82	7.335	0.008*
SVL	0.003	0.002	1,82	0.007	0.931
SVL × sex	-0.005	0.003	1,82	2.775	0.099
Back right			3,77 ^b	5.618 ^b	0.001 ^{b,*}
Sex	0.160	0.087	1,77	9.915	0.002*
SVL	<-0.001	0.002	1,77	4.032	0.048*
SVL × sex	-0.004	0.002	1,77	2.907	0.092
<i>A. limifrons</i>					
Front left			3,73 ^b	0.820 ^b	0.486 ^b
Sex	0.076	0.115	1,73	1.486	0.226
SVL	0.001	0.002	1,73	0.324	0.570
SVL × sex	-0.002	0.003	1,73	0.651	0.422
Front right			3,70 ^b	1.392 ^b	0.252 ^b
Sex	0.056	0.102	1,70	2.925	0.091
SVL	<0.001	0.002	1,70	0.690	0.408
SVL × sex	-0.002	0.002	1,70	0.558	0.457
Back left			3,67 ^b	3.218 ^b	0.028 ^{b,*}
Sex	-0.097	0.072	1,67	0.424	0.517
SVL	<-0.001	0.001	1,67	6.922	0.010*
SVL × sex	0.003	0.001	1,67	2.309	0.133
Back right			3,67 ^b	0.496 ^b	0.686 ^b
Sex	0.033	0.066	1,67	0.041	0.838
SVL	<0.001	0.001	1,67	1.129	0.291
SVL × sex	0.001	0.001	1,67	0.316	0.575

^aParameter estimates, standard error, degrees of freedom, *F*-value, and *P*-value for each limb of each species are provided.

^bIndicates overall model values for limb of that species.

*A significant difference (*P* < 0.05).

TABLE 2. SVL (mm) and 2D:4D in *O. pumilio*, *C. bransfordii*, *A. limifrons*, and *A. humilis* from La Selva, Costa Rica^a

Measure	Male	<i>n</i>	Female	<i>n</i>	<i>d</i>
<i>O. pumilio</i>					
SVL	19.292 ± 0.852	36	19.086 ± 0.908	48	0.234
Front left	1.095 ± 0.072	35	1.032 ± 0.065 ^b	48	0.932*
Front right	1.100 ± 0.075	36	1.050 ± 0.070	48	0.697*
Back left	0.321 ± 0.027	36	0.323 ± 0.027 ^b	47	0.074
Back right	0.317 ± 0.024	35	0.312 ± 0.026	47	0.199
<i>C. bransfordii</i>					
SVL	16.665 ± 2.356	31	17.954 ± 3.675	39	0.411*
Front left	1.102 ± 0.158	30	1.014 ± 0.131 ^b	38	0.618*
Front right	1.059 ± 0.097	30	1.072 ± 0.121	40	0.117
Back left	0.398 ± 0.037	25	0.386 ± 0.037	38	0.327
Back right	0.391 ± 0.039	31	0.387 ± 0.044	36	0.096
<i>A. humilis</i>					
SVL	30.788 ± 2.878	44	35.119 ± 2.515	41	1.608*
Front left	0.571 ± 0.065	41	0.614 ± 0.050	41	0.746*
Front right	0.571 ± 0.078	42	0.602 ± 0.068	41	0.425*
Back left	0.306 ± 0.045	44	0.281 ± 0.033	42	0.632*
Back right	0.307 ± 0.037	40	0.288 ± 0.030	41	0.565*
<i>A. limifrons</i>					
SVL	33.530 ± 5.094	40	36.905 ± 2.866	37	0.816*
Front left	0.476 ± 0.044	40	0.489 ± 0.050	37	0.278
Front right	0.483 ± 0.042	40	0.500 ± 0.041	34	0.412
Back left	0.249 ± 0.031	39	0.244 ± 0.031	32	0.162
Back right	0.247 ± 0.028	38	0.245 ± 0.026	33	0.074

^aMeans ± SD of SVL and 2D:4D of each sex for each species is indicated. Sample size (*n*) per sex follows the mean. Effect size (Cohen's *d*) also calculated.

^bA significant difference between left and right side 2D:4D within a sex ($P < 0.05$).

*A significant difference between sexes ($P < 0.05$).

expressed the sexual dimorphism. In contrast, Chang (2008; now author JLS) reported that male *O. pumilio* from the same wild population have larger 2D:4D in both of the hindlimbs, and no sexual dimorphism was found in either of the forelimbs. These opposing findings might be attributed to the difference in methods. In the current study, we used software analysis of digital photos to measure digits. While this technique offers very precise measurements, one obvious source of error is the degree to which toes are straightened and flattened prior to being photographed, which is less consistent in the long-toed rear feet than in the short-toed front feet. In contrast, Chang (2008) used hand-held calipers, which lack precision in small digits, such as those of the front feet. It is extremely important to accurately measure digits and keep methods consistent when using small-sized living animals because measurement error can mask the presence of traits. The disparity between these two studies serves as a cautionary tale for choice of methods in future digit ratio studies.

Other 2D:4D studies that have examined the same species twice have also found inconsistencies as to which limb expresses the sexual dimorphism. In laboratory mice, Brown et al. (2002) found the 2D:4D sexual dimorphism in the right hindlimb, Manning et al. (2003) found it in the left hindlimb, and Bailey et al. (2004) did not find the sexual dimorphism. Chang et al. (2006) reported 2D:4D in *Anolis carolinensis* for two populations and found the sexual dimorphism in the right hindlimb in both populations but only found it in the left forelimb in one population, while Lombardo and Thorpe (2008) did not find the sexual dimorphism in this species. Possible reasons for the observed variations could

be differences in populations, observers, effective sample sizes, ages, and methods (Bailey et al., 2004; Chang et al., 2006; Trivers et al., 2006). Results may also differ because of the use of living as opposed to museum-preserved individuals, and differences in interspecific and intrapopulation variability in digit ratios (also see Burley and Foster, 2004; Romano et al., 2005; Rubolini et al., 2006).

We also report other species that display the 2D:4D sexual dimorphism that has not yet been documented. Male *C. bransfordii* have larger 2D:4D than females in the left forelimb. Male *A. humilis* have larger 2D:4D than females in both of the hindlimbs and smaller 2D:4D than females in both of the forelimbs. In addition, male *A. limifrons* showed a trend toward smaller 2D:4D than females in the right forelimb. Our results are consistent with other studies of 2D:4D in lizards. Chang et al. (2006) reported larger 2D:4D in the right hindlimb of male *A. carolinensis*, and male smaller than female 2D:4D in the left forelimb (but see Lombardo and Thorpe, 2008). Rubolini et al. (2006) reported that male *P. muralis* had larger 2D:4D in both forelimbs and larger 2D:3D in the left forelimb than females. Rubolini et al. (2006) also did not find evidence to support the existence of the 2D:4D sexual dimorphism in *M. planifrons*, but males did have smaller 2D:3D than females on the left forelimb.

We also report evidence for directional asymmetry in 2D:4D (also see Rubolini et al., 2006). Female *O. pumilio* had significantly larger 2D:4D on the right forelimb than on the left and on the left hindlimb than on the right. Female *C. bransfordii* also had significantly larger 2D:4D on the right forelimb than on the left. Other

studies have also documented directional asymmetry in 2D:4D. For example, male rats and mice have larger 2D:4D on the right forelimb than on the left (McMechan et al., 2004; Leoni et al., 2005). In addition, a meta-analysis of 2D:4D studies on humans showed that the right hand displays a larger sex difference than the left hand (Hönekopp and Watson, 2010; also see Williams et al., 2000; Lutchmaya et al., 2004). Directional asymmetry has also been observed in studies of 2D:4D in birds (Burley and Foster, 2004; Romano et al., 2005; but see Forstmeier, 2005).

We also found some effects of SVL and an SVL-by-sex interaction. This suggests that dimorphism in 2D:4D is due at least in part to an allometric effect on body size. Blackburn (2009) reported a positive allometric correlation between SVL and the length of the sexually dimorphic third digit in anurans of the genus *Arthroleptis* and *Cardioglossa*. Rubolini et al. (2006) also reported SVL but did not report whether SVL or its interaction with sex had an effect on digit ratios in either *P. muralis* or *M. planifrons*. Chang (2008) included *O. pumilio* SVL in the analysis and did not find an effect of SVL or SVL-by-sex on 2D:4D in any of the limbs. Lombardo and Thorpe (2008) also included SVL as a covariate in their model, but did not indicate if SVL or SVL-by-sex were significant. Human 2D:4D increases slightly with age, which may suggest allometric growth (Trivers et al., 2006; Kratochvil and Flegr, 2009; but see Fink et al., 2005; Hönekopp and Watson, 2010; Manning, 2010). Tobler et al. (2011) also reported inflated digit ratios in *C. pictus* when SVL was not accounted for in the statistical model. However, it is clear that the sexual dimorphism is not solely due to an effect of size (Zheng and Cohn, 2011). If SVL does act allometrically on digit ratios, future studies should include SVL as a covariate.

Digit ratio patterns among lineages may differ due to differences in androgen and estrogen concentrations and receptor activity (Zheng and Cohn, 2011), or differences in chromosomal sex determination, which influences androgen and estrogen signaling (Chang et al., 2006; Lombardo et al., 2008). In birds, females are the heterogametic sex (ZW), whereas in mammals it is the male (XY). In amphibians, reptiles, and fish, either males or females can be heterogametic depending on the species (Hillis and Green, 1990; also reviewed by Stöck et al., 2011), and in amphibians, sex steroids signaled by environmental cues can induce sex changes during later life stages (Nakamura, 2008). It is unknown if male *O. pumilio* or *C. bransfordii* are the homo- or heterogametic sex. Heteromorphy of sex chromosomes is diverse in the genus *Anolis* and known for some species, such as *A. carolinensis* where males are the heterogametic sex (XY; Alfoldi et al., 2011) but is not known for *A. limifrons* or *A. humilis* (Organ and Janes, 2008; Janes et al., 2010). To address the chromosomal sex determination hypothesis, future studies should compare patterns of 2D:4D among sexes of frog or lizard species with known male homo- or heterogametic sex chromosomes.

This study establishes that 2D:4D in amphibian forelimbs and lizard hindlimbs is larger in males than in females and that 2D:4D in lizard forelimbs is smaller in males than in females, which is consistent with previous studies. We also report allometric growth of 2D:4D in amphibians and lizards, and conclude that this sexu-

ally dimorphic trait is evident in basal extant tetrapod taxa.

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