

Sexual Dimorphism in Digit Length Ratios in Marsh Frog, *Pelophylax ridibundus* (Ranidae) from Iran

Fariba Rajabi¹ Hossein Javanbakht^{1*}

¹Department of biology, Faculty of Science, University of Guilan, Rasht, Iran

*Corresponding Author

E-mail: h.javanbakht@gmail.com

Received: December 25, 2018

Accepted: April 26, 2019

Abstract

Sexual dimorphism in digit length ratios (preliminary 2D: 4D) is a morphological feature that affected by prenatal androgenic hormone during development. This dimorphism is controlled by the expression of home box genes which are highly conserved among vertebrate taxa. It is predicted that in most mammals, females have larger digit ratios than males and in diapsid species, males have larger digit ratios than females. However, this trend has not been studied sufficiently in lower taxa. In this study we examined this hypothesis in 50 specimen of marsh frog in north of Iran. Digit length and snout-ventral length (SVL) of frogs were measured by vernier caliper. The data analyzed by SPSS software version 18. The results showed unlike more basal vertebrate taxa, in marsh frog females had larger 2D: 4D than males. The digit length of D1 and D3 between males and females were significantly difference ($P < 0.05$). We did not observe any correlation between SVL and digit ratios. Nevertheless there were positive correlation between SVL and sex. We conclude that the evolution of morphological digits in frogs, deserve more attention, because it may be controlled by the association between prenatal exposure and environmental effects.

Keywords: Hox gene, prenatal development, vertebrate, limb

INTRODUCTION

In human societies, man are tended to have smaller digit ratios (primarily 2D: 4D) than women [1]. It has been speculated that prenatal exposure to sex hormones may be causally linked to adult morphology, physiology and behavior [2-4] which may result from the influence of maternally Hox genes and the endogenous production of sex steroids (testosterone and estradiol). This event occurs as a precursor of the urogenital system during embryonic development [5]. Thus urogenital systems and digit development are controlled by same genes. Under this hypothesis, study of digits ratio can be used a potential indicator to investigation of individual's prenatal hormone environment and past developmental pathways. These trends should also be visible in other tetrapod vertebrates because Hox genes are almost conserved in different vertebrate taxa [6]. It is very well documented that digit length ratios in several animal taxa are sexually dimorphic [7] Mammals [8].

Under this hypothesis in recent years, a growing number of studies have investigated digit ratios and their correlates in non-human vertebrates, especially in mammals and birds. These results suggest that female larger sexual dimorphism in 2D: 4D ratio seems to be the rule in mammal's taxa [8-10]. The only exception has been reported in Guinea baboons, in which 2D: 4D in females is less than in males [11]. Study on other taxa revealed the trait is vary among species [7,12-13] among populations [14-15] even between fore and hind limb in on species [13]. Study of sexual dimorphism in bird and reptiles indicated that digit ratios generally in males is larger than females (In birds- Zebra Finches [16] Ring-Necked Pheasants [17] lizards- Wall lizards [7] and Green anole lizards [14]. Nevertheless, males and females can exhibit different direction of the dimorphism among species and populations [7,12,13-15]. Moreover, Rubolini et al. [7]

reported no sexual dimorphism in 2D: 4D in three species of Skinks lizards.

Thus, investigation of lower vertebrate taxa could provide valuable data to connection between relative digit lengths and of Steroid hormones. Recently, a number of studies focused on relation of digit ratio with morphological and physiological [18] and ecological characters [12] in lizard species. In amphibian species, Chang [19] studied digit length ratios in the strawberry poison dart frog from Costa Rica. However study on frog has not received enough attention in the literature and to our knowledge such a survey has not yet been documented on frogs in Middle East and Iran. This is also important, because steroid hormones might be altered by the steroid-like metabolites of environmental contaminants by both pesticides and herbicides [17]. This contaminant can lead to abnormal gonadal development, feminization, and limb deformities in frogs [20]. Frogs are a common group of vertebrate in many localities that have received considerably less attention in morphological digit ratios than other vertebrates, what's making this group suitable to study the significance of digit ratios.

Marsh frog (*Pelophylax ridibundus*) belongs to the family of true frogs (Ranidae) is widespread species in Western Europe and ranges as far eastwards as eastern Kazakhstan. The frog inhabits a wide variety of flowing from shallow puddles and ponds to large lakes and rivers. It's distributed in north, center and west of Iran [21]. In this report, we explore sex-related variation in 2D, 3D, and 4D ratios of the left forelimbs and hind limbs in marsh frogs from two locality in north of Iran, we also compare digit ratio of the frogs of two population in different locality with more than 300 km distance in border of Caspian sea.

MATERIALS AND METHODS

The marsh frogs are common species of amphibians in Iran and is not considered as endangered or protected species. All stages of this research were complied with Iranian laws and in authorizing of Guilan University. The marsh frogs were collected by hand and net in two localities in Rasht (N 49°33', E 37°17') and Sari (N 52°0', E 36°35') from border of Caspian Sea. Sex was determined by the presence or absence of vocal sac in male frogs. The snout-ventral length (SVL) was measured as a measure of body size. The length of all digits of left fore and hind limbs as well as snout-ventral length was measured. We measured the length of digits from the proximal end of the proximal phalanx bone to the distal end of the distal phalanx bone using vernier caliper accurate to 0.1 mm. all measurements were taken by the author. Totally, 50 specimens including 30 males and 20 females were measured (Rasht: 9 male and 11 female; Sari 21 male and 9 female). Only left-side digits were used in the comparative analyses. No animal was sacrificed specifically for the purposes of this study. Mean and standard deviation of digit ratios were calculated for each specimen separately for males and females.

We examined the data for normality by Kolmogorov-Smirnov test (all were $P > 0.05$). We compare male and female digit ratios and SVL by using t-tests analysis. We used Levene's test for equality of variances. To test significance of sexually dimorphic characters Independent Sample test (2-tailed) at the significance level of 0.05 as well as Principal Component Analysis (Ratio Statistics) were employed. We used MANOVA to determine the effect of SVL, sex, and SVL by sex interaction on the 2D: 4D of all digits. SPSS software version 22 was used for running the statistical analyses.

RESULTS

All examined specimen were adult. SVL ranged from 50.14–116.50 mm (77.05 ± 15.65 mm, mean \pm SD). Generally, female marsh frogs had larger SVL than males (mean female: 78.32 ± 18.71 , mean male: 76.21 ± 13.51 , $f=1$, $P=0.644$, Figure 1). Moreover, the specimen from Sari had larger SVL from Rasht (mean male: 63.43 ± 11.1 and mean female: 70.77 ± 15.29 in Rasht and mean male: 81.68 ± 10.11 and mean female: 87.55 ± 19.11 in Sari), (Figure 1). Sexual dimorphism in digit ratios was not significantly different among two localities ($P > 0.05$). Levene's test for equality of variances did not reveal differences between the sexes in any digit ratio ($P > 0.05$). Comparing of digits length between sexes in left fore and hind limb showed that female overall exhibited larger digit length than males (Table 1). However, according to pairwise analysis of differences between digit ratios D3 and D1 were significantly different between sex for both sides ($P < 0.05$). Sexual dimorphism in 2D: 4D, 3D: 4D and 2D: 3D digit length ratios for male and female are tightly correlated within sex with r values ranging from 0.176 to 0.393. Sexual dimorphism of digit length ratios for male and female are listed in Table 1. Variance in 2D: 4D in the fore and hind limb is not explained by SVL (ANOVAS: $F=0.005$, $P=0.946$, $F=1.331$, $P=0.254$). But SVL is affected by the sex and females were larger than males (ANOVA: $F=0.216$, $P=0.644$). However, variance in 2D: 4D, 3D: 4D and 2D: 3D in the hind limb is not explained by sex, SVL, or SVL by sex interaction ($P > 0.05$). Relationships between SVL and sex with digit ratios and their interaction are shown in Table 2.

Table 1. Summary of sex differences in left fore and hind limb in marsh frog. 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}
fore							
2D:3D	.7392	.7944	.077	1	-1.214	.231	1
2D:4D	.9347	1.1032	2.322	1	-2.960	.005	1
3D:4D	1.2895	1.3832	.939	1	-1.783	.081	1
hind							
2D:3D	.7308	.7581	1.410	1	-.629	.532	1
2D:4D	.4998	.4815	.082	1	.563	.576	1
3D:4D	.7010	.6431	.521	1	1.237	.222	1

Table 2. The effect of sex, snout-vent length (SVL), and their interaction on the digit ratios 2D: 4D, 3D: 4D and 2D: 3D of the fore and hind limb.

fore	2D : 4D			3D: 4D			2D: 3D			
	Factor	df	F-ratio	P	df	F-ratio	P	df	F-ratio	P
Sex		1	7.008	0.011	1	2.693	0.108	1	0.860	0.359
SVL		1	0.05	0.946	1	0.269	0.606	1	0.081	0.777
SVL by sex		1	0.952	0.334	1	1.194	0.280	1	0.020	0.887
hind	2D: 4D			3D: 4D			2D: 3D			
Factor	df	F-ratio	P	df	F-ratio	P	df	F-ratio	P	
Sex	1	0.389	0.536	1	1.237	0.272	1	0.301	0.586	
SVL	1	0.827	0.368	1	0.201	0.656	1	0.017	0.898	
SVL by sex	1	0.189	0.666	1	0.334	0.566	1	0.942	0.337	

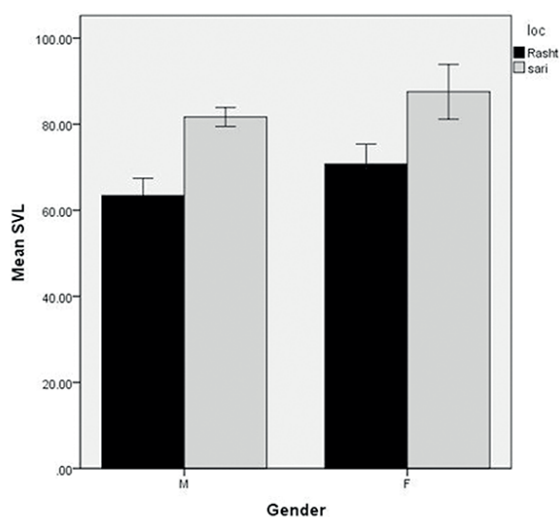


Figure 1. The snout-ventral length (SVL) in male and female of marsh frog captured in Rasht and Sari

DISCUSSION

The different pattern of sexual dimorphism in digit ratios such as 2D: 4D in vertebrate has been suggested in the existence of two non-mutually exclusive ways. First, the plasticity of sexual size dimorphism associated with the expression of prenatal steroid sex hormones during developmental program, shared by both sexes [22]. Second, variation in sex differences in digit ratios may affected by different amount of maternal steroids hormones or interspecific variation in sex-specific yolk androgen deposit in eggs that can lead to differential exposure of male and female embryos to hormones. It have repeatedly been observed in green anoles [23-25]. In fact, specie may vary in the effect of gonadal steroids hormones. The steroids hormones concentrations affect the hypothalamic GH-releasing and -inhibiting factors that associated with Hox gene expression, which coordinates autolimb development [1,26-27]. In addition, prenatal sexual dimorphism is thought may be affected by environmental pollutants. It has been reported some environmental contamination such as atrazine and dichloro-diphenyl-trichloroethane (DDT) have led to abnormal gonadal development, feminization, and limb deformities in amphibians [20]. Also 2D: 4D ratio may be altered, resulting steroid-like metabolites by the presence of contaminant [17].

In our study, we found that females had larger 2D: 4D on their fore limb than males of marsh frog. These results are consistent with Manning's [1] prediction that females have larger 2D: 4D than males. However, we found no statistical evidence of sexual dimorphism in 2D: 4D from hind limb. Inconsistent of our results, Chang [19] found that the digit ratio of the front fore limb in strawberry poison dart frog were not differ between the sexes. As well as males had a larger ratio of 2D: 4D in hind limb than females. Moreover, Direnzo and Stynoski [13] reported similar results in two species of frogs (*Oophaga pumilio* and *Craugastor bransfordii*) in Costa Rica. The most reason of lack of concordance between our results and those of Chang [19] and Direnzo and Stynoski [13], can be referred to different genus and species of frog that we examined. Different digit ratio 2D: 4D also can be associated with the use of different habitats where males and females exhibit different morphological patterns [12]. The

evolutionary changes in morphology of digit ratio associated with microhabitat and using of biomechanical implications for locomotion on different surfaces as well as effect of different ecological activity such as basking, foraging and mating [28-29]. Recently in a study on two Iguania species, Gomes and Kohlsdorf [12] found that males are tended to have larger digit ratios in 2D: 4D than female (inconsistence with diapsid species). They speculated ecological selection pressures are responsible for the differences in digit ratio in these species. The testosterone level is affected by these activities that can lead to change in Hox gene expression and morphological development [30]. Similar results were found in two lacertid lizards from several islands in the Adriatic Sea [18].

However, our results are consistent with humans and most mammals patterns that male tetrapods have a lower 2D: 4D than females [8,10]. In our study, digit ratios in hind limb were not statically difference in male and female. Study on lizard species suggested that the effects of prenatal steroid exposure on digit ratios in fore and hind limb may be different and a species with high finger digit ratios in fore limb not necessarily also have high toe digit ratios in hind limb [13,14]. This suggests that timing of forelimb development may have been shown heterochrony among the major vertebrate clades [31]. Our results were also consistent with those of some studies of 2D: 4D ratios in other reptiles (In *Menesida planifrons* [7] *Anolis humilis* and *Anolis limifrons* [13]). We did not observe any correlation between SVL with digit ratios. Similar results were obtained by Chang [19] in strawberry poison dart frog. In contrast, Gomes and Kohlsdorf [12] reported in Iguania lizard, SVL significantly correlated with front and hind limb, both in males and females that may be related to microhabitat and ecological activities. However, information about related between digit ratios and ecological requirements is scant.

In conclusion, this study provides evidence that digit ratio 2D: 4D in marsh frog associated with sex which is restricted to fore limbs. This dimorphism was important because our results were inconsistent with those reported in other studies on frogs [13,19]. This experiment showed study of digit ratio in frogs should be more considered. Future study on frogs should be focused on interaction of environmental pollutant and morphological development.

REFERENCES

- [1] Manning, JT. 2002. Digit ratio. New Brunswick, NJ: Rutgers University Press.
- [2] Collaer ML, Hines M. 1995. Human behavioral sex-differences: a role for gonadal hormones during early development. *Psychological bulletin*. 118: 55-107.
- [3] Gil, D. 2008. Hormones in avian eggs: physiology, ecology and behavior. *Advances in the Study of Behavior*. 38: 37-398.
- [4] Uller, T. 2008. Developmental plasticity and the evolution of parental effects. *Trends Ecology and Evolution*. 23: 432-438.
- [5] Kondo T, Zakany J, Innis JW, Duboule D. 1997. Of fingers, toes and penises. *Nature*. 390: 29.
- [6] Krumlauf, R. 1994. Hox genes in vertebrate development. *Cell*. 78: 191-201.
- [7] Rubolini D, Pupin F, Sacchi R, Gentili A, Zuffi MAL, Galeotti P, Saino N. 2006. Sexual dimorphism in digit length ratios in two lizard species. *Anatomical Record*. 288:

491-497.

[8] McFadden D, and Bracht MS. 2005. Sex differences in the relative lengths of metacarpals and metatarsals in gorillas and chimpanzees. *Hormones and Behavior*. 47: 99-111.

[9] Brown WM, Hines M, Fane BA, and Breedlove SM. 2002. Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Hormone and Metabolic Research*. 42: 380-386.

[10] Nelson E, Shultz S. 2010. Finger length ratios (2D:4D) in anthropoids implicate reduced prenatal androgens in social bonding. *American Journal of Physical Anthropology*. 141: 395-405.

[11] Roney JR, Whitham JC, Leoni M, Bellem A, Wielebnowski N, Maestriperi D. 2004. Relative digit lengths and testosterone levels in Guinea baboons. *Hormones and Behavior*. 45: 285-290.

[12] Gomes CM, Kohlsdorf T. 2011. Evolution of Sexual Dimorphism in the Digit Ratio 2D:4D - Relationships with Body Size and Microhabitat Use in Iguanian Lizards. *PLoS One*. 6(12): e28465.

[13] Direnzo GV, Stynoski JL. 2012. 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. *Anatomical Record*. 295: 597-603.

[14] Chang JL, Doughty S, Wade J, Lovern MB. 2006. Sexual dimorphism in the second to fourth digit length ratio in Green Anoles, *Anolis carolinensis* (Squamata: Polychrotidae) from the southeastern United States. *Canadian Journal of Zoology*. 84: 1489-1494.

[15] Lombardo MP, Thorpe PA. 2008. Digit Ratios in Green Anolis Lizard (*Anolis carolinensis*). *The Anatomical Record*. 291: 433-440.

[16] Burley NT, Foster VS. 2004. Digit ratio varies with sex, egg order, and strength of mate preference in zebra finches. *Proceedings of the Royal Society of London B*. 271: 239-244.

[17] Saino N, Rubolini D, Romano M, Boncoraglio G. 2007. Increased egg estradiol concentration feminizes digit ratios of male pheasants (*Phasianus colchicus*). *Naturwissenschaften*. 94: 207-212.

[18] Damme RV, Wijnrocx K, Boeye J, Huyghe K, Dongen SV. 2015. Digit ratios in two lacertid lizards: sexual dimorphism and morphological and physiological correlates. *Zoomorphology* 134: 565-575.

[19] Chang, JL. 2008. Sexual Dimorphism of the Second-to-Fourth Digit Length Ratio (2D: 4D) in the strawberry poison dart frog (*Oophaga pumilio*) in Costa Rica. *Journal of Herpetology*. 42(2): 414-416.

[20] Hayes TB, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A. 2002. Feminization of male frogs in the wild. *Nature*. 419: 895-896.

[21] Frost, DR. 2011. *Amphibian species of the world: An online reference*. Version 5.5, New York. Available at: <http://research.amnh.org/vz/herpetology/amphibia/AmMusNatHist>.

[22] Badyaev, AV. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends in Ecology and Evolution*. 17: 369-378.

[23] Lovern MB, McNabb FMA, Janssen TA. 2001. Developmental effects of testosterone on behavior in male and female green anoles (*Anolis carolinensis*). *Hormones and Behavior* 39: 131-143.

[24] Lovern MB, Wade J. 2001. Maternal plasma and egg yolk testosterone concentrations during embryonic

development in green anoles (*Anolis carolinensis*). *General and Comparative Endocrinology*. 124: 226-235.

[25] Tobler M, Healey M, Olsson M. 2011. Digit ratio, color polymorphism and egg testosterone in the Australian painted dragon. *PLOS One*. 6: e16225.

[26] Lutchmaya S, Baron-Cohen S, Raggatt P, Knickmeyer R, Manning JT. 2004. 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Human Development*. 77: 23-28.

[27] Malas MA, Dogan S, Evcil EH, Desdicioglu K. 2006. Fetal development of the hand, digits and digit ratio (2D:4D). *Early human development*. 82: 469-475.

[28] Spezzano LC, and Jayne B. 2004. The effects of surface diameter and incline on the hind limb kinematics of an arboreal lizard (*Anolis sagrei*). *Journal of Experimental Biology*. 207: 2115-2131.

[29] Toro E, Herrel A, Irschick D. 2004. The evolution of jumping performance in Caribbean Anolis lizards: solutions to biomechanical trade-offs. *American Naturalist*. 163: 844-856.

[30] Losos JB, Creer D, Glossip D, Goellner R, Hampton A, Roberts G, Haskell N, Taylor P, Ettling J. 2000. Evolutionary Implications of Phenotypic Plasticity in the Hindlimb of the Lizard *Anolis sagrei*. *Evolution*. 54: 301-305.

[31] Bininda-Emonds ORP., Jeffery JE, Sanchez-Villagra MR, Hanken J, Colbert M, Picau C, Selwood L, Cate CT, Raynaud A, Osabutey CK, Richardson MK. 2007. Forelimb-hindlimb developmental timing changes across tetrapod phylogeny. *BMC evolutionary biology*. 7: 182.