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Study of Sexual Dimorphism in Second-to-Fourth Digit Length Ratio (2D: 4D) in the Green-Bellied Lizard (*Darevskia cholorogaster*) from Iran

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ABSTRACT

Sexual dimorphism in digit ration is related to genetic differences between species in response to prenatal exposure to hormones. In this study, the development of tetrapod digits, preliminary the ratio between the length of digit II and IV (2D: 4D) was investigated in 34 samples of *Darevskia cholorogaster* in the North of Iran. The results showed no significant differences between sexes in any digit length. The digit ratio 2D: 4D was different between males and females in which the females had greater 2D: 4D in forelimb than the males. However, this difference was not significant. Sexual dimorphism in 2D: 4D, 3D: 4D and 2D: 3D ratios for the male and female showed no correlation within sex. Our results supported a genetic pattern seen in humans and most mammals, inconsistent with basic genetic pattern in reptiles. We concluded that the digits ratios observed in *D. cholorogaster* could be referred to microhabitat use by this species on different surfaces, besides basic genetic pattern and other ecological effects as foraging and mating.

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Introduction

Study of shape and size of the body structure in vertebrates indicates that steroid hormones such as testosterone effects on adult morphology, physiology and behavior (Collaer and Hines *et al*., 1995; Gil *et al*., 2008; Uller *et al.,* 2008). Sexual dimorphism in digits length ratio, particularly in the second-to-fourth (2D: 4D) is one of the most morphological features that is determined during embryonic development by the concentration of prenatal steroid hormones (Manning *et al*., 1998; Manning *et al*., 2002; McIntyre *et al*., 2005; Trivers *et al*., 2006). The results of this prenatal exposure of hormone caused a difference between genders of the vertebrates including humans. In synapsid mammals, females generally show larger values of 2D: 4D than males (Manning *et al*., 2002; Brown *et al*., 2002; Mcmechan *et al*., 2004; Leoni *et al*., 2005). Such difference in digit ratio of males have also been reported in other digits (such as 2D: 3D, 2D: 5D, and 3D: 4D: Leoni *et al*., 2005; Manning *et al*., 2003). In contrast, the diapsid taxa such as birds and reptiles showed larger digit ratio in males than females (Burley and Foster *et al*., 2004; Chang *et al*., 2006). However, contradictory results have been observed in the wall lizards (*Podarcis muralis*) (Rubolini *et al*., 2006), and green anole lizards (*Anolis carolinensis*) (Chang *et al*., 2006). Moreover, no sexual dimorphism in 2D: 4D was observed in tree skinks (Rubolini *et al*., 2006).

The development of the digits, limbs, and urogenital systems of the vertebrates are controlled by homeobox genes (Peichel *et al*., 1997) grouped in four gene clusters (HoxA - HoxD) (Lemons *et al*., 2006). The expressions of these genes are influenced by steroids hormones (Block *et al.,* 2000). Because of the conserved nature of these genes in animal taxa (Krumlauf *et al*., 1994), it can be expected sexual dimorphism of 2D: 4D follow in same rule in close groups of taxa. It was supposed if the urogenital system and the limbs have a primitive prenatal link, the pentadactyl terrestrial tetrapods will also have sexually dimorphic 2D: 4D ratios (Manning *et al*., 2002). However, despite generality of this hypothesis, a study on mammals, birds, and reptiles revealed inconsistent results in different species and populations of nonhuman tetrapods (Lombardo and Thorpe *et al*, 2008). Thus it indicates, the Hox genes do not consistently affect by steroid hormones (Lombardo and Thorpe *et al.*, 2008). Nevertheless, the study on digit ratio can reveal the difference of exploring of prenatal steroids hormones on different species and the population of animals. In addition, according to some theoretical context, the digit ratio 2D: 4D may have evolved in parallel with ecological diversity in some clades of Squamata. Thus the identifying the sign of the difference in digit ratio for each species and population could reflect multiple developmental changes related to sex differences in sex steroid hormone secretion during ontogeny (Damme *et al*., 2015).

The green-bellied lizard (*Darevskia cholorogaster* Boulenger *et al*., 1908) inhabits in temperate forest species (including Hyrcanian mixed deciduous forest) in a wide variety of biotopes from tree trunks and walls, in heaps of brushwood, or among the grass of the forest floor (Tuniyev *et al*., 2009). According to the theoretical model, the digit ratio 2D: 4D may have evolved in relation to the ecological diversity of some clades of Squamata (Gomes and Kohlsdorf *et al*., 2011). These ideas were examined in the green-bellied lizard, a species of lacertid lizards in Northern parts of Iran that distributed in southern and southeastern coasts of the Caspian Sea in Iran and Azerbaijan along the Elburz Mountains to northeastern regions in Kopet Dagh Mountains.

Materials and Methods

A total of 34 samples of green-bellied lizard including 24 males and 10 females were collected by hand and net from Rasht (N 49°33′ 18", E 37°17′ 18") in the North of Iran. The sexuality of adult specimens was determined by the presence or absence of hemipenis, in adult individuals. The snout-vent length (SVL), and the length of all digits of fore and hind limbs of each lizard was measured to the nearest 0.01 mm using analogue Vernier caliper. Only left-side digits were used in the comparative analyses The length of 2D, 3D and 4D were measured from the mid-point of the proximal end of the proximal phalanx bone to the mid-point of the distal end of the distal phalanx bone. All variables were screened for normality and homogeneity of variance using the Kolmogorov-Smirnov normality test and Levene's test, respectively (Chang *et al*., 2006). The T-test was used to examine the effects of sex on variables. All statistical analyses were performed in SPSS version 19.00 (SPSS Inc. 2011). General linear model MANOVAs were used to determine the effects of sex and SVL on 2D: 4D, 3D: 4D and 2D: 3D ratios of the left limbs.

Results

All lizards examined in this study were adult. There was no statistically significant difference in SVL between males and females samples (mean female: 57 ± 5.05, mean male: 53.4± 9.7, F= 1.7, *P*=0.29, Fig. 1).

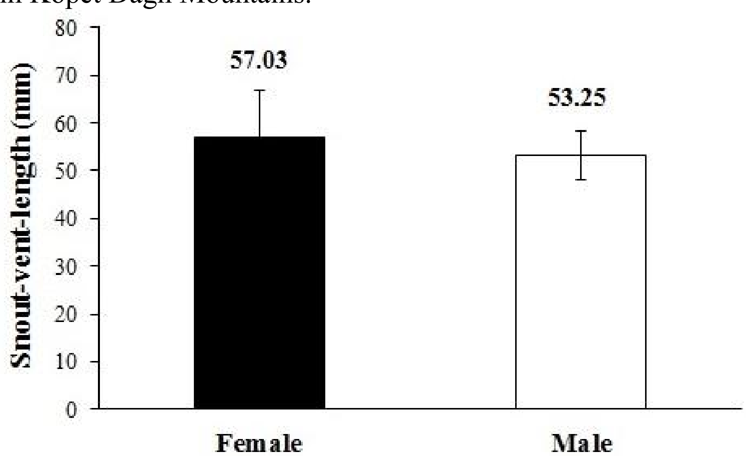

Fig.1. The snout-ventral length (SVL± SE) in male and female of *D. cholorogaster*

The SVL ranged from 32.3- 83.8 mm for males and 46.9- 66.1 mm for females. No significant differences were found between the sexes in any digit length (T-test, *P*> 0.05). The digits length ratio were compared between sexes in left fore and hind limbs (2D: 4D, 3D: 4D and 2D: 3D). The results indicated females had larger 2D: 4D digits length ratios than males in both fore and hind limbs, but these differences were not significant (T-test, *P*> 0.05). Results for all digit length ratios are shown in table 1.

Variance in 2D: 4D in the fore and hind limbs is not explained by sex (MANOVAs: fore limb (F= 0.39, *P*=0.53); hind limb, F=0.54, *P*= 0.46) as well as variance in 2D: 4D in the forelimb is not explained by SVL. However, 2D: 4D in females were larger than males (ANOVA: F=16.19, *P*=0.02). The ratio of 2D: 4D in the hind limb was affected by the SVL. Relationships between SVL and sex with digit ratios (Table 2) indicated that no relationship was seen between SVL and sex (Pearson correlation, *P*= 0.29). Sexual dimorphism in 2D: 4D, 3D: 4D and 2D: 3D digit length ratios for male and female were not correlated within sex, and the r values calculated ranging from -0.18 to 0.38.

Table 1. Summary of sex differences in left fore and hind limb in *D. cholorogaster*

Measure*	**Males**	**Females**	**F**	**T**	***P***	**d_{mean}**
Fore limb						
2D: 3D	0.74	0.71	0.76	1.05	0.38	0.03
2D: 4D	0.66	0.68	2.34	0.62	0.53	0.02
3D:4D	0.88	0.95	16.21	-2.35	0.025	-0.07
Hind limb						
2D: 3D	0.61	0.56	4.58	1.25	0.21	0.04
2D:4D	0.45	0.42	2.94	0.73	0.46	0.26
3D: 4D	0.73	0.74	0.35	-0.16	0.86	-0.005

* *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).

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

Fore limb	**2D:4D**			**2D:3D**			**3D:4D**		
Factor	df	f-ratio	*p*	df	f-ratio	*p*	df	f-ratio	*p*
Sex	1	0.39	0.53	1	1.1	0.3	1	5.58	0.25
SVL	32	1.85	0.53	32	0.34	0.9	32	2.1	0.5
Hind limb	**2D:4D**			**2D:3D**			**3D:4D**		
Factor	df	f-ratio	*P*	df	f-ratio	*p*	Df	f-ratio	*p*
Sex	1	0.54	0.46	1	1.57	0.21	1	0.028	0.86
SVL	32	1.619	0.02	32	5.47	0.32	32	4.8	0.34

Discussion

Sexual dimorphism in digit ratio has been reported in the various tetrapod groups (Damme *et al*., 2015; Burley and Foster *et al.,* 2004; Leoni *et al*., 2005). The reason for the observed pattern is still unclear. It is speculated that different level of maternal steroids hormones deposited in the eggs will result in differential exposure of male and female embryos to the hormones (Rubolini *et al*., 2006; Lombardo and Thorpe *et al*., 2008). Variations in amount of testosterone deposited in egg have been reported from green anoles (Lovern and Wade *et al*., 2001; Lovern and Wade *et al*., 2003) and birds (Whittingham and Schwabl *et al*., 2002), resulted in digits ratios (Lombardo and Thorpe *et al*., 2008). In the other hand, it has also been suggested that sexual dimorphism in digit ratio may be affected by growth hormones secretion in different ways in different taxa (Rubolini *et al*., 2006). Direnzo and Stynoski (2012) have suggested digit ratio in male and female may be determined by the chromosomal sex-determination system and the heterogametic sex may have smaller digit ratio (males in mammals and females in birds). The above idea may corroborate in anole lizards (Damme *et al*., 2015). Some authors have pointed out that the ecological selection pressures role, for example, the locomotor performance may be responsible for the differences in digit ratio (Gomes and Kohlsdorf *et al*., 2011; Damme *et al*., 2015).

In the present study, there were no significant differences in digit length observed between males and females. However, the digit ratio 2D: 4D was different between sexes. Males and females had greater value in 2D: 4D ratio in fore and hind limbs, respectively. Rubolini *et al*. (2006) and Damme *et al*. (2015) reported that in two species of lizards (*Podarcis muralis* and *Mabuya planifrons*), males had a larger 2D: 4D ratio than females on the left sides, that it was a basic pattern in diapsid species. However, they found inconsistent results in different digits as well as fore and hind limbs. They concluded that according to Badyaev (Badyaev *et al*., 2002), sex-specific growth patterns is dependent on the expression of sex and age-specific modifiers and are controlled by high sex specificity in growth hormone (GH) secretion and sensitivity. In this context, GH release and synthesis is regulated by the hypothalamic GH-releasing and -inhibiting factors that they are influenced by gonadal steroids. Although, their relative importance is species-specific (Manning *et al*., 2002). Our results are also inconsistent with the genetic sex hypothesis pattern seen in humans and several other tetrapods that heterogametic sex in vertebrate animals has smaller digit ratio than homogametic (Direnzo and Stynoski *et al*., 2012). Because it was known that female in lacertid lizards are heterogametic (Rovatsos *et al*., 2016).

It is possible that digit length is also influenced by performance selective pressure in a specific ecological setting that is at least slightly distinct among sexes (Gomes and Kohlsdorf *et al*., 2011). It was seen some species of lizards are sexually dimorphic in microhabitat usage. For example, species of Iguania females of arboreal species in the basking time and diameter of used perches are differ from males (Gomes and Kohlsdorf *et al*., 2011). Also, some species of Tropidurinae lizards seem sexually dimorphic for ecological patterns (Vansluys *et al*., 1992). The larger ratio of 2D: 4D have seen in males hind limb in our study can be referred to earlier development of hind limb than forelimb and in consequence heterochrony in timing and concentration of steroids hormones (Gomes and Kohlsdorf *et al*., 2011). In accordance with our results, Direnzo and Stynoski (Direnzo and Stynoski *et al*., 2012) found in *Anolis humilis* males had smaller 2D: 4D than females in the forelimb, but larger 2D: 4D in the hind limb. Similar results in 2D: 4D digit ratio was found in green anolis lizard (Chang *et al*., 2006).

In the present study, no correlation was found between digits ratios with sex in fore and hind limbs as well as no correlation between digit ratios with SVL in the fore limb. But 2D: 4D in the hind limb was explained by SVL. Study on strawberry poison dart frog (*Oophaga pumilio*) by Chang (2008) indicated that variation in 2D: 4D was not explained by sex, SVL or SVL by sex in fore and hind limbs. But he found a positive correlation between 2D: 4D and sex in the hind limb. Nerveless, our results are inconsistent with Gomes and Kohlsdorf (Gomes and Kohlsdorf *et al*., 2011) who found that correlations between SVL and the digits ratios in 25 lizard species of 3 families of Iguania (Iguanidae, Polychrotidae, and Tropiduridae) were significant both in males and in females. However, they found significant associations between both digits ratios and the sexual dimorphism with microhabitat usage. Therefore, concur with two previous studies, the correlation between 3D: 4D can be referred to microhabitat use by this species and biomechanical implications for locomotion on different surfaces, besides other ecological effects as basking, foraging, and mating.

In conclusion, our results indicate that prenatal development of digit ratio not only affected by steroids hormone following a basic pattern but also differed dependent on species and ecological performance. Thus it would be valuable to investigate sexual dimorphism in different species and their correlation to performance and environmental factors.

References

Badyaev AV. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol Evol* 17: 369-378.

Block K, Kardana A, Igarashi P, Taylor HS. 2000. In utero diethylstilbestrol (DES) exposure alters Hox gene expression in the developing mullerian system. *FASEB J* 14: 1101-1108.

Brown WM, Finn CJ, Breedlove SM. 2002. Sexual dimorphism in digit-length ratios of laboratory mice. *Anat Rec* 267: 231-234.

Burley NT, Foster VS. 2004. Digit ratio varies with sex, egg order and strength of mate preference in zebra finches. *Proc Biol Sci* 271: 239-244.

Collaer ML, Hines M. 1995. Human behavioral sex-differences: a role for gonadal hormones during early development. *Psychol Bull* 118: 55-107.

Chang JL, Doughty SDS, Wade JWJ, Lovern M. 2006. Sexual dimorphism in the second-to-fourth digit length ratio in green anoles, *Anolis carolinensis* (Squamata: Polychrotidae), from the southeastern United States. *Can J Zool* 84: 1489-1494.

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. *J Herpetol* 42(2): 414-416.

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.

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. *Anat Rec* 295: 597-603.

Gil D. 2008. Hormones in avian eggs: physiology,ecology and behaviour. *Adv Study Behav* 38:37398.

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.

Krumlauf R. 1994. Hox genes in vertebrate development. *Cell* 78: 191- 201.

Lemons D, McGinnis W. 2006. Genomic evolution of Hox gene clusters. *Science* 313: 1918-1922.

Leoni B, Canova L, Saino N. 2005. Sexual dimorphism in metapodial and phalanges length ratios in the wood mouse. *Anat Rec A Discov Mol Cell Evol Bio* 286(A): 955-961.

Lombardo MP, Thorpe PA. 2008. Digit ratios in green anolis lizards (*Anolis carolinensis*). *Anat Rec* 291: 433-440.

Lovern MB, Wade J. 2001. Maternal plasma and egg yolk testosterone concentrations during embryonic development in green anoles (*Anolis carolinensis*). *Gen Comp Endocrinol* 124: 226-235.

Lovern MB, Wade J. 2003. Yolk testosterone varies with sex in eggs of the lizard, *Anolis carolinensis*. *J Exp Zool A Comp Exp Biol* 295(A): 206-210.

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

Manning JT. 2002. Digit ratio: New Brunswick**,** NJ: Rutgers University Press.

McIntyre MH, Ellison PT, Lieberman DE, Demerath E, Towne B. 2005. The development of sex differences in digital formula from infancy in the Fels Longitudinal study. *Proc Biol Sci* 272: 1473-1479.

Mcmechan AP, O'Leary-Moore SK, Morrison SD, Hannigan JH. 2004. Effects of prenatal alcohol exposure on forepaw digit length and digit ratios in rats. *Dev Psychobiol* 45: 251-258.

Manning JT, Callow M, Bundred PE. 2003. Finger and toes ratios in humans and mice: implications for the aetiology of deseases influenced by Hox genes. *Med Hypotheses* 60: 340-343.

Peichel CL, Prabhakaran B, Vogt TF. 1997. The mouse Ulnaless mutation deregulates posterior HoxD gene expression and alters appendicular patterning. *Development* 124: 3481-3492.

Roney JR, Whitham JC, Leoni M, Bellem A, Wielebnowski N, Maestripieri D. 2004.

Relative digit lengths and testosterone levels in Guinea baboons. *Horm Behav* 45: 285-290.

Rovatsos M, Vukic J, Kratochvil L. 2016. Mammalian X homolog acts as sex chromosome in lacertid lizards. *Heredity* (*Edinb*) 117(1): 8-13.

Rubolini D, Pupin F, Sacchi R, Gentilli A, Zuffi MAL, Galeotti P, Zuffi MA, Galeotti P, Saino N. 2006. Sexual dimorphism in digit length ratios in two lizard species. *Anat Rec A Discov Mol Cell Evol Bio* 288(A): 491-497.

Trivers R, Manning J, Jacobson A. 2006. A longitudinal study of digit ratio (2D: 4D) and other finger ratios in Jamaican children. *Horm Behav* 49:150-156.

Tuniyev B, Ananjeva N, Agasyan A, Orlov N, Tuniyev S, Anderson S. 2009. *Darevskia chlorogaster*, Green-bellied Lizard. The IUCN red list of threatened species, IUCN 2008: T164702A5919117.

Uller T. 2008. Developmental plasticity and the evolution of parental effects. *Trends Ecol Evol* l23:432-438.

Vansluys M. 1992. Aspectos da ecologia do lagarto *Tropidurus itambere* (Iguanidae) em uma área do sudeste do Brasil. *Rev Bras Biol* 52 (1): 181-185.

Whittingham LA, Schwabl H. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. *Anim Behav* 63: 63-67.