

# Calculation of Fluctuating Asymmetry of the biggest Caspian whipsnake population in Hungary compared to a common snake species

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**Abstract:** The Fluctuating Asymmetry (FA) is a special type of asymmetry in the populations of bilaterally symmetrical creatures. The FA gives us numerical data on the developmental instability of the populations and refers to potential genetical and environmental stressors affecting the populations. Here we give the first data on the FA of the protected Caspian whipsnake (*Hierophis caspius*) from Hungary. The FA indices of the biggest population from Villány Mts were compared to the FA indices of two differently stressed Dice snake (*Natrix tessellata*) populations [stressed (Mád) and semi-natural (Lake Balaton)]. Based on the values of the multiple and the simple indices derived from sublabial scales, we can say that the *status quo* of the highly protected Caspian whipsnake population does not represent significant deviation from the near-natural dice snake population from the Lake Balaton.

**Key words:** fluctuating asymmetry; interspecific comparisons; *Hierophis caspius*; *Natrix tessellata*

## Introduction

There can be many reasons of asymmetries (fluctuating directional and antisymmetry) occurring in bilateral organisms. Fluctuating asymmetry (FA) refers to the developmental instability of populations which originates in developmental noise and developmental stability. The first occurs during every ontogenesis, the latter characterizes the individual and population level genetic potentials (e.g., Palmer 1994; van Dongen 2006). Fluctuating asymmetry means random deviations from perfect symmetry, with a normal distribution and mean zero (Van Valen 1962; Palmer 1994) at population level. Its distribution can differ among populations, and can be derived from genetic and environmental stress (Cadée 2000). If the effective size of the population drops to a critical level, it can result in the increasing number of developmental deviations, which can be one reason of increased FA. If inbreeding of small populations increases, number of phenotypical asymmetries can increase too together with the decrease of individual fitness (Clarke 1995; Roldan et al. 1998; Stige et al. 2006). Altering habitats by human perturbation in a direct or indirect way can be such a stressor that can induce FA. In this case, an individual who lives in such habitat has to allocate substantial energy into fighting the environmental stress, and fewer energy remains for (symmetrical) growing and reproduction (Palmer &

Strobeck 1992; Palmer 1994). A substantial amount of ontogenetic potential can remain idle, which can be seen e.g. in the decreased adult sizes (Leung et al. 2000) or increased level of FA. It is important to emphasise that FA is only a pattern, thus it is supposed to be non-heritable, however its defining processes can be heritable. Dealing with FA will not provide us direct evolutionary implications but we will have some indirect information on the actual status of the population. We have to mention that there are some data on FA having a genetically determined and heritable component, but it seems to be quite small, and hard to detect (Leamy 1997; Leamy & Klingenberg 2005).

Our aim was to provide numerical information and describe the developmental instability pattern of the largest and strictly protected Caspian whipsnake population (*Hierophis caspius* Gmelin, 1789) of Hungary. Thus we could have some indirect information on its so called life quality, i.e. habitat conditions, natural status and stress. FA is biologically meaningful only if it is compared to other FA data. Regularly, authors compare populations of the same species (e.g., Herczeg et al. 2005; Stige et al. 2006; Vilisics et al. 2005) because species-level differences in the heritability of the main processes beyond FA are not known. In our situation we could not solve it, because the Caspian whipsnake is very rare in Hungary (Báldi et al. 2001). We think that it may also be informative to compare the FA

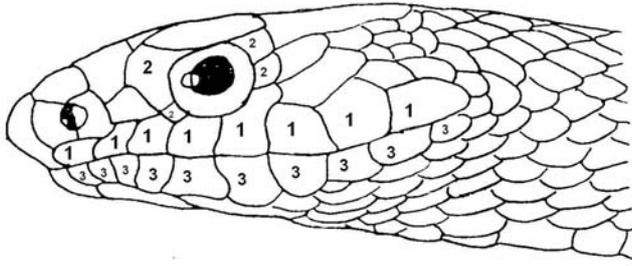


Fig. 1. Measured bilateral traits on Caspian whipsnake. 1 – supralabial, 2 – periocular, 3 – sublabial scutes.

of the whipsnake to an other unrelated, but common snake in Hungary. So as to make it, we used FA indices of Dice snake populations (*Natrix tessellata* Laurenti, 1768) from a previous study (Szabó 1999; Herczeg et al. 2005).

It is well known, that individuals of populations living in disturbed and in semi-natural habitats can show different proportion of asymmetry (Leung et al. 2000). Our hypothesis was that if the FA of the whipsnake population was significantly closer to the FA of the stressed Dice snake population, it would mean that urgent conservation actions were needed to improve its habitat. Otherwise the maintenance of habitat and environment seems to be satisfactory.

## Material and methods

### The investigated sites

Our Caspian whipsnake population is situated in the southern part of Hungary, in Baranya, in the Villányi Mts. This is the largest remnant population of this formerly common species. The Dice snake is widespread in Hungary. The studied populations live in the Northeastern part of the country (Mád 48°11' N, 21°18' E) and near Lake Balaton (Tihany 46°55' N, 18°53' E) (Szabó 1999).

Since 1998 we have had lot of field studies in the Villányi Mts, we have measured lots of bilateral traits characteristic of the whipsnake (number of periocular, supralabial, sublabial, loreal and nasal scales on the left (L) and right (R) sides of the head, see Fig. 1.). Villányi Mts is the southeast highland in Hungary located relatively far from big cities but the agricultural utilization and tourism attraction of this area is very remarkable. Therefore the human perturbation of Caspian whipsnake's habitat developed to a permanent stress factor. The gender of adult specimens is easily determined on the field, but as our traits are not depended on the sex, we used the pooled data of both sexes. Caspian whipsnake individuals show not significant difference on head scales by the genders (Bellaagh 2003). Tomović et al. (2008) attract the attention that phenotypical changes during the ontogeny could be very frequent in some snake species [e.g., *Vipera ursinii macrops* (Méhely, 1911)] so the analysis of meristic morphological data from a pooled (refer to juvenil and adult individuals, too) dataset may result unsteady statements. Because there were no available studies on this species about the phenotypical changes during the ontogenetic development we used only the morphological data of adult specimens that did not show any visible mark of serious injuries. Thirty adult animals were looked

for by active searching, captured by hand, measured just after capture, and released at the capture point. These traits can be well recognised and easily counted, it was enough to count them only once. On the other hand, we had to release them as soon as possible to minimise any disturbance to the individuals.

To make the data of the two investigations comparable we used only the basic dataset of Herczeg et. al (2005) because they used different statistical methods. The first Dice snake population lives in a small and very perturbed habitat, in an intensive fishpond near Mád, northeastern Hungary, where 33 individuals were captured. The second, larger population is from a semi-natural habitat (from Lake Balaton), here we captured 77 individuals. Balaton is the biggest lake in Hungary located in the Transdanubian region of the country. Along the shore there are a lot semi-natural habitats e.g. reedy and stony areas, but those are hard to be approached. We took into consideration only those indices which were based on the same traits (number of supralabial, sublabial, nasal and periocular head scales).

### Statistical analyses

Measurement error analysis is critical to FA studies, except in our case when precisely measurable meristic traits are used. We excluded aberrant data of FA values with Grubb's test for outliers (Grubb 1969) [*Hierophis caspius* ( $n = 27$ ):  $z^* = 2.86$ , *Natrix tessellata* Mád ( $n = 33$ ):  $z^* = 2.95$ , *N. tessellata* Balaton ( $n = 77$ ):  $z^* = 3.29$ ], and incomplete datasets. We used Chi-square test to be sure that the morphological traits used to develop FA indices are independent. Size-dependence of FA was checked with linear regression. Normal distribution was tested using *skew* and *kurtosis* (Palmer & Strobeck 1992, Palmer 1994; Leung et al. 2000.), and the expected mean zero was inspected with a *t*-test (mean absolute FA compared to zero) (Palmer 1994).

After Palmer (1994), we chose the indices FA1 (the mean absolute asymmetry:  $\text{mean } |R - L|$ ) and FA5 ( $\Sigma(R - L)^2 / N$ ,  $N = \text{number of individuals in the sample}$ ) based on single traits, and FA11 [ $(A_i) / N$ ,  $(A_i) = \Sigma |R - L|$  for all traits] and FA12 [ $(A_i) / N$ ,  $(A_i) = \text{the number of asymmetrical traits in an individual}$ ] based on multiple traits. FA1 is simple to use, it is very popular and allows us to compare FA of more than two samples. FA5 is useful at small sample sizes, and is effective in detecting small FA differences among populations. FA11 and FA12 provided us a combined comparison of multiple traits. FA indices were statistically analysed with Kruskal-Wallis and Scheffé-tests (Zar 1984).

Being aimed at comparing the asymmetry of the three populations without specific indices, we conducted a two-way ANOVA (STATISTICA 6.1, StatSoft, Inc., 2003), which is widely accepted for FA studies in the literature (e.g., Palmer & Strobeck 2003). Although the absolute asymmetry values used in the ANOVA have no normal distribution, the two-way ANOVA will not be biased too much.

## Results

We did not find any asymmetry in the nasal and supralabial head scales of Caspian whipsnake, because in such a sample size every asymmetry value proved to be outlier. After excluding outlier data and outlier asymmetry values, we had 33 Dice snake individuals from Mád and 74 from Lake Balaton, and 27 Caspian whipsnakes from the Villányi Mts. The sublabial and the periocular head

Table 1. Distribution of right minus left values of bilateral traits in the three snake populations.

Site	Sample size	Trait	Skew	$p$	Kurtosis	$P$
Villány	27	sublabial	-0.0068	0.9	-0.422	0.7
Villány	27	periocular	0.545	0.3	2.093	0.02
Mád	33	sublabial	0.3532	0.4	-0.898	0.3
Mád	33	periocular	0.3574	0.4	0.1221	0.9
Balaton	74	sublabial	0.0134	0.9	0.5231	0.4
Balaton	74	periocular	0.6269	0.05	0.8977	0.2

Table 2. Representative parameters of FA-indices in the three snake populations.

Site	Sample size	Trait	$(L + R)/2$		$(L - R)$		$ L - R $
			Mean $\pm$ SE	Median $\pm$ SE	Skew $\pm$ SE	Kurtosis $\pm$ SE	Median
Villány	27	sublabial	10.02	0.12	-0.0068	-0.422	0.33
Villány	27	periocular	5.12	0.23	0.545	2.093	0.29
Mád	33	sublabial	9.5	-0.03	0.353	-0.898	0.64
Mád	33	periocular	6.3	0.18	0.357	0.1221	0.42
Balaton	74	sublabial	9.5	0.01	0.013	0.523	0.28
Balaton	74	periocular	6.26	0.22	0.627	0.898	0.36

Explanations:  $L$  – value of the left side,  $R$  – value of the right side.

Table 3. Calculated FA values in the three snake populations.

Site	Trait	FA1	FA5	FA11	FA12
Villány	sublabial	0.33	0.33		
Villány	periocular	0.2963	0.518	0.63	0.518
Mád	sublabial	0.6364	0.696		
Mád	periocular	0.424	0.485	1.06	1
Balaton	sublabial	0.2875	0.28		
Balaton	periocular	0.3553	0.408	0.64	0.603

Table 4. Comparisons of FA1, FA11 and FA12 values (Kruskal-Wallis-test).

Index	Trait	Site	$P$	KW	Significance ( $\alpha = 0.05$ )	
FA1	sublabial	Mád vs. Villány			*	
		Balaton vs. Villány	0.004	10.831	ns	
		Mád vs. Balaton			**	
	periocular	Mád vs. Villány				ns
		Balaton vs. Villány	0.356	2.062		ns
		Mád vs. Balaton				ns
FA11		Mád vs. Villány			*	
		Balaton vs. Villány	0.01	9.216	ns	
		Mád vs. Balaton			*	
FA12		Mád vs. Villány			*	
		Balaton vs. Villány	0.006	10.09	ns	
		Mád vs. Balaton			*	

Explanations: \* significant, ns – non-significant.

scales are independent according to the Chi-square test ( $\chi^2 = 10.27$ ,  $\chi^* = 16.92$ ,  $\alpha = 0.05$ ,  $df = 9$ ).

Significant size-dependence of periocular head scales of Caspian whipsnake data was found. *H. caspius*:  $r_{\text{sublab}} = 0.0900$ ,  $P_{\text{sublab}} = 0.652$ ;  $r_{\text{perioc}} = 0.7601$ ,  $P_{\text{perioc}} < 0.001$ ; *N. tessellata*:  $r_{\text{sublab}} = 0.0673$ ,  $P_{\text{sublab}} = 0.491$ ;  $r_{\text{perioc}} = 0.0854$ ,  $P_{\text{perioc}} = 0.3432$ ,  $\alpha = 0.01$ ).

The *kurtosis* and *skew* were not significant in al-

most every datasets (Table 1) with a zero mean. *N. tessellata* Mád:  $P_{\text{perioc}} = 0.136$ ,  $P_{\text{sublab}} = 0.838$ ; *N. tessellata* Balaton:  $P_{\text{perioc}} = 0.002$ ,  $P_{\text{sublab}} = 0.829$ ; *H. caspius* Villány:  $P_{\text{perioc}} = 0.326$ ,  $P_{\text{sublab}} = 0.110$ ,  $\alpha = 0.01$ ). FA indices and their relations are shown in Tables 2 and 3 and Fig. 2, respectively. Results of Kruskal-Wallis and Scheffé-test are shown in Tables 4 and 5. The two-way ANOVA results are represented in Table 6.

Table 5. Comparison of FA5 values of the three snake populations (Scheffé-test).

Index	Trait	Site	$S_{\alpha=0.01}$	$S$	Significance ( $\alpha = 0.05$ )
FA5	sublabial	Mád vs. Villány	2.41	2.53	*
		Balaton vs. Villány	2.41	0.36	ns
		Mád vs. Balaton	2.41	3.5	*
	periocular	Mád vs. Villány	2.41	0.15	ns
		Balaton vs. Villány	2.41	0.54	ns
		Mád vs. Balaton	2.41	0.39	ns

Explanations: \* significant, ns – non-significant.

Table 6. Results of two-way ANOVA.

	SS	df	MS	$F$	$P$
Intercept	33.632	1	33.632	120.33	0.000
Trait	0.196	1	0.196	0.700	0.407
Site	2.243	2	1.121	4.013	0.019
Trait * site	0.927	2	0.463	1.658	0.192
Error	73.775	264	0.279	–	–

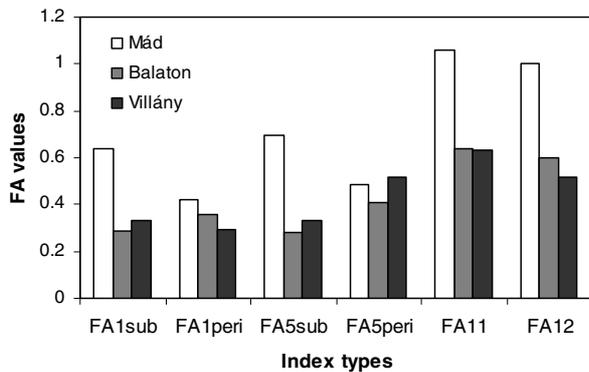


Fig. 2. Graphical comparison of FA values of the three snake populations.

**Discussion**

Two traits proved to be useful for further asymmetry analysis: the asymmetry of supralabial and periocular head scales. They also fit the requirement, that traits used in FA analyses should be developmentally independent (Palmer & Strobeck 2003).

Although significant size-dependence was found in one of the datasets, it is regarded as an artefact because of colinearity of the data.

According to the two-way ANOVA, the developmental stability of the two traits are not significantly different. The developmental stability of the samples differs significantly, but this difference is independent of the chosen trait, because the trait × sample interaction is not significant. After Scheffé and Kruskal-Wallis tests it can be seen that there is a significant sublabial head scale FA difference with the indices FA1 and FA5 between the two Dice snake populations. The Mád population differs significantly from the Caspian whipsnake population (Villányi Mts), too. The whipsnake and the semi-natural Dice snake population did not differ sig-

nificantly. The same results were obtained with multiple indices FA11 and FA12. Periocular head scale FA did not show any significant difference with the simple indices. Using of simple indices is out of date (Clarke 1998; Leung et al. 2000; Palmer & Strobeck 2003), so results of multiple indices and two-way ANOVA are regarded as representative, but in our case one trait, the sublabial head scales, does support the multiple results on it's own.

In conclusion, two-way ANOVA results and indices FA11, FA12, FA1<sub>sublabial</sub> and FA5<sub>sublabial</sub> showed us that there is no significant difference between the whipsnake population from Villány Mts and the semi-natural Dice snake population from Lake Balaton. That suggests that in spite of frequent human perturbations and habitat-degradation the Caspian whipsnakes show a quite high level of phenotypical stability. Of course, the snakes do not benefit from that conquering human attitude, after a limited size the decreasing territory will not be enough to support one of the most precious Caspian whipsnake population of Hungary, living in the Villány Mts, which now consists of hundreds of specimens.

About the traits: two of four traits (sublabial and periocular head scales) proved to be useful for fluctuating asymmetry studies of the Caspian whipsnake, according to their variance and distribution of asymmetry at population level. However, we suggest the use of all the four traits, because they can be easily, precisely (without measurement error) and fastly measured in the field. In more altered habitats it is likely that the other two traits (nasal and supralabial) will also be informative. Although periocular data did not give us much information during this study, it may be useful in other studies, so it is also worth measuring hereafter. As all the four morphological traits are meristic, easily countable without measurement error, according to literature (Palmer 1994), they would be ideal for FA

studies. They lack only one requirement: the number of the discrete units should be bigger. Here they are very small, so the asymmetry can have only a few values ( $-1$ ;  $0$ ;  $+1$  generally). Bigger stress affecting the population can increase FA but it would be best to find other suitable traits and populations to improve the method [e.g., size-distribution Herczeg et al. (2005)].

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