

Asymmetry and population characteristics in dice snakes (*Natrix tessellata*): an interpopulation comparison

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Three main types of asymmetry in bilateral characters were early separated (Van Valen, 1962). Directional asymmetry in a character means a consistent bias towards always the same side as opposed to the other, whereas antisymmetry is a consistent asymmetry in a bilateral character pair where the enlarged side occurs by chance. Both directional asymmetry and antisymmetry result from normal development. Fluctuating asymmetry (FA), that is nondirectional deviation from perfect symmetry, occurs when normal development is perturbed (Palmer and Strobeck, 1986; Leary and Allendorf, 1989). These perturbances can include habitat degradation (Sarre, 1996), pollution (Bengtsson et al., 1985), hybridisation (Graham and Felley, 1985), inbreeding (Roldan et al., 1998), small population size (Hutchinson and Cheverud, 1995), and the marginal distribution of a certain population (Møller, 1995; Siikamäki and Lammi, 1998). FA was thought to be a useful tool in conservation biology indicating the level of developmental instability that results from genetic and/or environmental stresses (Parsons, 1992; Clarke, 1995). However, the concept of FA, its connection with developmental instability, its heritability, its effect

on fitness and its sensitivity to external and internal stressors have been under an ongoing debate during the last decade (e.g. Thornhill et al., 1999; Van Dongen et al., 1999; Cadée, 2000; Lens et al., 2000; Leung et al., 2000; Hermida et al., 2002; Lens et al., 2002).

Former studies have revealed that a high level of FA indicates low individual quality in lizards, i.e. low FA levels are characteristic of individuals with better chances for mating (Martín and López, 2000; López et al., 2002) and escaping (Martín and López, 2001). Forsman et al. (1994) showed that ventral scale anomalies are linked to decreased survival in *Vipera berus* (Linnaeus, 1758). Although snakes have become “popular” objects in ecological research during the last years (Shine and Bonnet, 2000), as far as we are aware of, FA is seldomly used to quantify individual quality in snakes despite that they possess a number of countable bilateral characters.

In this paper we compare FA levels in some head scale characters of European dice snake (*Natrix tessellata* (Laurenti, 1768)) individuals that colonised an artificial, small fish pond established in the mid-1970s in the vicinity of Mád (hereafter: Lake Mád), with a sample of the same species from Lake Balaton, the largest Hungarian natural lake. Although it is hard to predict the quality of colonising specimens (e.g. Lambin et al., 2001), we expected higher levels of FA in specimens from Lake Mád, according to different constraints (size of the lakes, age of the populations) that can shape population characteristics (population size, age distribution) at the two study sites. Using the dice snake as a model, our aim was to explore whether snakes

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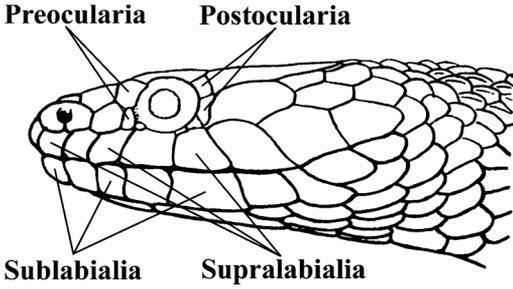


Figure 1. Head scale characters of *Natrix tessellata* examined for asymmetry. Modified after Dely (1978).

with their several countable bilateral characters were good model organisms for FA research, and if FA could be useful in conservation studies on snakes.

The dice snake is found in southern Europe, Turkey, western to central Asia, and the Middle East, typically along coasts and lake shores, or near mountain streams. The total length of the species is generally around 1 m. The breeding season lasts from May to June. One clutch consists of 10-25 eggs. The young snakes hatch from late August to early September.

Our study was conducted at two sites. Lake Mád is a fish pond, dug artificially in the mid-1970s. This water body is situated at the mountain Zemplén, eastern Hungary (48°11'N, 21°18'E), and it has a surface area of approximately 0.0054 km² with an average depth of two meters. The second study site is an approximately 0.5 km long stretch of the eastern Lake Balaton shore in the Tihany Peninsula (46°55'N, 18°53'E), western Hungary. Lake Balaton is a shallow lake with an average depth of three meters, and a surface area of 596 km². Almost all of its banks provide good habitats for dice snakes (present authors, pers. obs.). The type of human utilization (e.g. fishing, water-sports) is the same in both sites.

Fieldwork was carried out from 4 August until 24 September 1998. Snakes were captured by hand and measured for length. Four different types of head scales (fig. 1) were counted on both sides: the preocularia (PrO), postocularia (PoO), supralabialia (SpL) and sublabialia (SbL). Ventral scale patterns were drawn for individual identification to avoid pseudoreplication, and to estimate population sizes from recaptures. Processed animals were released at the place of capture. Altogether 115 snakes were captured; 81 individuals at Balaton, and 34 at Mád. Some individuals could not be measured because of injuries. Thus, scale data from 102 snakes were used for the present study.

Body length (snout-vent length) was used as a rough proxy of age (Lenz and Gruschwitz, 1993). The differences in median body length among populations were tested with a Mann-Whitney U-test, while the distribution of the size groups among populations were compared with a χ^2 test. We used rough population size estimations based on our mark-recapture data using the modified Petersen-Lincoln index for multiple recaptures (see Bailey, 1952).

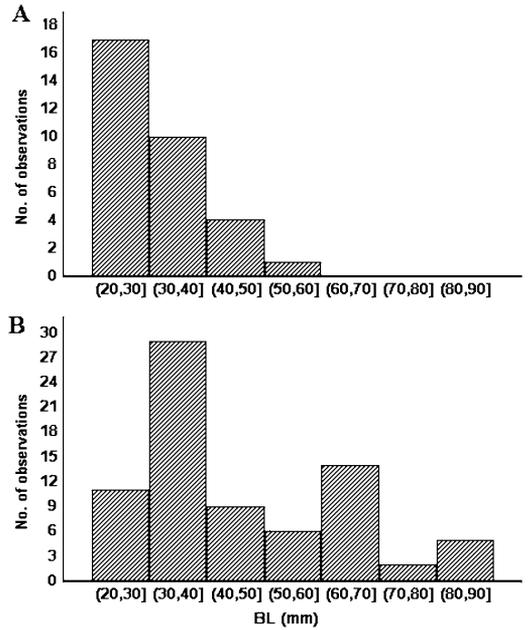


Figure 2. Body length distribution of *Natrix tessellata*, representing age (Lenz and Gruschwitz, 1993) in the Mád (A) and the Balaton (B) populations.

The found asymmetry was interpreted to represent FA in PrO, SpL and SbL, because the signed differences between the left and right-hand counts were normally distributed (Kolmogorov-Smirnov tests; $d < 0.06$; $p > 0.05$) around a mean of zero (one-sample t-tests: all $p > 0.05$). The presence of directional asymmetry was found in PoO, where the mean of the signed differences between the left and right-hand counts differed significantly from zero (one sample t-test: $p = 0.04$), while the distribution was normal (Kolmogorov-Smirnov test: $d = 0.06$; $p > 0.05$). Thus, we excluded PoO from further analyses. We measured the level of asymmetry by the index $A = 100 * \{ |L - R| / (N/2) \}$ (Werner et al., 1991), where L is the left-hand scale count, R the right-hand count, and N is the sum of the scales on both sides. We calculated this index first for the three head scale types separately, and then for the sum of the three types between the left and right side ($A = 100 * \{ \Sigma |L - R| / (N/2) \}$).

We tested for FA differences among the populations first using a MANCOVA model where FA levels of the different scales were the dependent variables, population origin the factor and body length the covariate (to test for possible year effects). Second, we used an ANCOVA with population origin as factor, body length as covariate and the summed level of FA as dependent variable. For statistical analyses, we used the program STATISTICA 4.5 for WINDOWS (StatSoft Inc., Tulsa, Oklahoma, 1994).

We found a significant difference between the two study populations both in the median body length ($U_{31,71} = 564$; $p < 0.001$) and in the size class distribution ($\chi^2_6 = 135.75$;

Table 1. Fluctuating asymmetry levels in the head scales preocularia (PrO), sublabialia (SbL), supralabialia (SpL), and the sum of them (sumFA) derived from *Natrix tessellata* individuals originating from populations (Mád and Balaton) with different characteristics, histories, and habitat parameters. Sample size (N) and mean \pm SD of the fluctuating asymmetry levels are shown. See text for the indices used to calculate the fluctuating asymmetry levels. The signs (*) and (**) denote significant differences at $p < 0.05$ and $p < 0.01$ levels, respectively.

	PrO		SpL*		SbL**		sumFA**	
	Mád	Balaton	Mád	Balaton	Mád	Balaton	Mád	Balaton
N	31	71	31	71	31	71	31	71
mean	10.32	9.41	5.16	2.58	6.81	3.46	6.61	3.88
SD	17.79	16.95	6.6	5.66	5.77	5.09	4.99	4.15

$p < 0.001$; fig. 2). The estimated maximum age of snakes in the Lake Mád population was three years, whereas that in the Lake Balaton population was eight to nine years. Many newly hatched juveniles were captured at both sites. We recorded 34 captures at Lake Mád with 10 recaptures during seven days of sampling, and 81 captures at Lake Balaton with only three recaptures during 13 days. Although population size estimation was impossible for the Balaton population and only approximate regarding the Mád population ($N = 89.9$; $SD = 30.1$) according to the low number of recaptures, differences in population sizes are evident.

We note that two-population comparisons must be handled with caution but that, in cases where population characteristics are clearly distinguishable, such comparisons are still used (e.g. Van Damme et al., 1990; Uller and Olsson, 2003). Taking the differences in the histories, habitat parameters and in the detected population characteristics between our study populations into consideration, we concluded that the two populations were comparable. Results from MANCOVA suggested significant between-population differences in levels of FA (Wilks' $\lambda_{3,97} = 0.92$; $p = 0.04$). Pairwise comparisons for SpL and SbL values revealed that FA was significantly higher in the Mád population (Scheffé tests; PrO: $p = 0.81$; SpL: $p = 0.048$; SbL: $p = 0.004$; table 1). The FA level was independent from body length (within-cell regressions: $R^2 < 0.26$; $F_{1,99} < 2.68$; $p > 0.11$) irrespective of population origin (test of parallelism: Wilks' $\lambda_{3,96} = 0.99$; $p = 0.96$). The summed FA level was higher in the Mád

population ($F_{1,99} = 7.75$; $p = 0.006$; table 1) and independent from body length too (within-cell regression: $R^2 = 0.001$; $F_{1,99} = 0.11$; $p = 0.74$), irrespective of population origin (test of parallelism: $F_{1,98} = 0.02$; $p = 0.89$).

Our results support those derived from previous studies which compared FA among populations differing in their sizes (Soulé, 1979; Hutchinson and Cheverud, 1995). The between-population differences in median length (and thus age) did not directly affect the level of FA; therefore, no year effects were detectable. The presumably many times smaller and younger dice snake population of Mád showed significantly higher rates of head scale FA than the Balaton population. However, in this study, we cannot clearly determine the kind(s) of (environmental and/or genetic) stress factor(s) causing FA differences between the investigated populations.

In conclusion, we found that dice snakes from two different locations showed significant differences in levels of FA. We interpret this observation as showing that the snakes are sensitive to environmental and/or genetic stresses. Therefore, we can recommend snakes and other squamates to be used as model organisms in FA research, and we support the use of FA as an indicator of stress in reptile conservation biology. A great advantage of snakes and other squamates as models in asymmetry studies is that they have many countable bilateral characters (scales), which can be recorded without error. We note that, although the lack of postembryonic changes in scalation is a limiting factor in intrapopulation comparisons because FA of

scales does not reflect individual success during postnatal ontogeny (scale determination finishes during embryogenesis; Maderson, 1965); this causes no problems at the interpopulation level.

Acknowledgements. We thank Imre Bodnár for kindly allowing us to make investigations at his fish pond at Mád, and the Balaton Limnological Research Institute of the Hungarian Academy of Sciences for the permission to conduct research at the Tihany region of Balaton. We are highly indebted to Juha Merilä and Jarmo Perälä for their helpful comments leading to improvements of the manuscript.

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Received: April 26, 2004. Accepted: June 7, 2004.