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SHORT COMMUNICATIONS

Fluctuating Asymmetry of Measurable Parameters in *Rana arvalis*: Methodology

V. L. Vershinin, E. A. Gileva, and N. V. Glotov

Institute of Plant and Animal Ecology, Ural Division, Russian Academy of Sciences, ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia

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Fluctuating asymmetry (FA), i.e., undirected deviations from bilateral symmetry, of both measurable and nonmeasurable characteristics reflects ontogenetic instability, which some authors believe increases in response to genomic or environmental stress. This allows FA to be used for estimating the state of anthropogenically affected natural populations (e.g., Parsons, 1992; Zakharov, 2001). This approach seems promising when dealing with amphibians, which, on the one hand, exhibit a global decline in numbers (Halliday, 1998; Collins and Storfer, 2003) but, on the other hand, are preserved for many years under the extreme conditions of urbanization, despite a high frequency of morphological abnormalities (Vershinin, 1995; Vershinin and Kamkina, 2001). It is known that the reliability of FA estimates depends on how strictly methodological requirements are complied with (Palmer, 1994; Van Dongen, 1999). Some of them are common to all organisms (sufficient sample sizes, representative sets of estimated characteristics, etc.); others are related to the specificity of each particular object. For example, when studying measurable characteristics of amphibians, the researcher should bear in mind that the material is usually fixed and storage in the fixative may affect the estimation of FA. We studied this possibility in two populations of the frog *Rana arvalis* Nilss. living in the city of Yekaterinburg. In one of these populations, animals live under relatively favorable conditions; in the other, they are exposed to severe, adverse environmental factors, which allowed us to expect considerable destabilization of their ontogeny.

Rana arvalis young of the year were captured on August 4 and 5, 2003, in the Kalinovskie Razrezy Park Forest in northern Yekaterinburg ($n = 26$) and near the Yuzhnaya Bus Terminal ($n = 15$), respectively. Ponds existing in the park forest had resulted from flooding desolate quarries. The vegetation was a herb–grass pine forest containing small shrubs. The results of our hydrochemical analyses performed in 2003 indicate that, according to the ecological sanitary classification of the quality of surface waters suggested by Zhukinskii et al. (1981), the water quality in one of the spawning ponds in the park forest corresponded to section 26 (fairly clean) of class 2 (clean), and in another pond, to section 36 (sufficiently clean) of class 3. The spawning pond near the Yuzhnaya Bus Terminal was located in the former floodplain of a river that passed through an underground collector. The water quality corresponded to section 46 (heavily polluted) of class 4 (polluted). With respect to some characteristics (odor, color, oxygen content, etc.), the state of the pond may be estimated as critical.

We measured the size parameters of the body that are standard for amphibians (Bannikov et al., 1977; Terent'ev, 1950; Terent'ev and Chernov, 1949), namely, the lengths of the thigh, crus, rostrum, eye, temporal spot, and fifth digit and the height of the heel tuber of recently captured frogs and in frogs that had been kept in a fixative (70% ethanol) for two months. The measurements were made by means of a caliper with a scale division of 0.1 mm. The well-known work by Palmer (1994) was used as a biometric manual. The statistical significance of FA was estimated by the ratio between mean squares for the interaction of factors and for the measurement error in the framework of ANOVA (a mixed model with two factors: *individual* and *body side*). The FA2 index calculated as the absolute difference between the values of a parameter on the right and left sides divided by the mean value of the parameter served as a measure of FA.

The results of the measurements shown in the table indicate that storage in the fixative led to a slight decrease in the mean values of most parameters measured in frogs from both populations. This decrease was the largest (and statistically significant) for the lengths of the thigh, crus, and temporal spot, i.e., the measurements at least partly involving soft tissue ($F = 11.13$–144.32, d.f. = 1/25 or 1/14, $P = 0.004$–0.0001 for the results of ANOVA with factors *individual, body side*, Size characteristics and fluctuating asymmetry in *Rana arvalis* from two populations

Parameter	Kalinovskie Razrezy Park Forest (n = 26)						Yuzhnaya Bus Terminal (n = 15)					
	fresh			fixed			fresh			fixed		
	mean value, mm	percentage of variance accounted for by measurement error	fluctuating asymmetry (FA2, $\times 10^3$)	mean value, mm	percentage of variance accounted for by measurement error	fluctuating asymmetry (FA2, $\times 10^3$)	mean value, mm	percentage of variance accounted for by measurement error	fluctuating asymmetry (FA2, $\times 10^3$)	mean value, mm	percentage of variance accounted for by measurement error	fluctuating asymmetry (FA2, $\times 10^3$)
Length of thigh	7.57	6.3	Nonsignificant	7.42	1.8	26.75	8.82	8.5	Nonsignificant	8.65	4.3	20.71
Length of crus	7.36	1.2	″	7.14	1.5	Nonsignificant	8.66	3.4	″	8.35	2.5	Nonsignificant
Length of rostrum	2.79	54.9	″	2.78	15.4	″	3.13	47.4	″	3.08	18.7	″
Length of eye	1.95	50.3	″	1.94	37.3	″	2.06	66.8	″	2.04	59.3	″
Length of temporal spot	3.59	40.2	″	3.46	13.9	59.09	4.02	26.7	″	3.89	18.3	62.27
Length of fifth digit	2.27	23.4	″	2.31	16.1	Nonsignificant	2.55	24.1	″	2.60	19.4	Nonsignificant
Height of heel tuber	0.81	65.0	″	0.86	25.7	″	1.03	39.2	″	1.03	35.4	″
R_S	–0.786			–0.929			–0.714			–0.929		
(P)	0.036			0.003			0.071			0.003		

Note: R_s is the coefficient of rank-order correlation between the mean value of the parameter and the measurement error (differences were considered nonsignificant at $P > 0.05$).

and *effect of the factor*; a mixed model). In frogs from the Kalinovskie Razrezy Park Forest, the height of the heel tuber somewhat increased after fixation (F = 5.13, d.f. = 1/25, $P = 0.032$). This situation, paradoxical as it may seem at first glance, was accounted for by a large measurement error, which was significantly negatively associated with the absolute size of the structure measured (the table). The heel tuber length was the smallest of the parameters measured and had, if fresh material from the control population was used, the largest measurement error. Fixation considerably decreased the proportion of variance accounted for by the measurement error (for some parameters, by a factor of two to three), and significant FA was found precisely in the fixed material from both populations, for two parameters, the lengths of the thigh and the temporal spot. For the other parameters, it did not differ significantly from zero (data not shown).

For the thigh length, we found significant interaction between the factors *body side* and *fixative effect* in both populations (F = 25.81, d.f. = 1/25, $P = 3 \times 10^{-5}$ in the Kalinovskie Razrezy Park Forest and F = 6.480, d.f. = 1/14, $P = 0.023$ near the Yuzhnaya Bus Terminal). This agrees with McCoy and Harris's (2003) assumption, based on the data on *Ambystoma maculatum*, that the storage of amphibians in a fixative may distort the proportions of their bodies and lead to artifact FA of measurable parameters involving soft tissues. However, this interaction was not observed for other parameters that we studied here, including the temporal spot (F = 1.170, d.f. = 1/25, $P = 0.323$ in the Kalinovskie Razrezy Park Forest and F = 0.141, d.f. = 1/14, $P = 0.713$ near the Yuzhnaya Bus Terminal), whose FA became significant after fixation, so McCoy and Harris's hypothesis remains unconfirmed thus far.

Thus, the FA of measurable parameters of *R. arvalis* from Yekaterinburg was not found to be associated with the strength of anthropogenic stress: for five parameters, FA was nonsignificant in both populations; for the remaining two parameters, only the FA2 of the thigh length was higher in the affected population. However, substantial distinctions between the two populations with respect to the strength of stress are evidenced by a significant difference in the frequency of major morphological abnormalities in underyearlings: 38.7% (n = 24) near the Yuzhnaya Bus Terminal and 9.1% (n = 66) in the Kalinovskie Razrezy Park Forest ($\chi^2 = 15.6$, d.f. = 1, $P = 0.0001$). Apparently, the FA of nonmeasurable characteristics is a more sensitive indicator of negative effects on amphibian populations, as was observed in representatives of the genus *Rana* from central Russia (Chubinishvili, 1997; Ustyuzhanina, 2002). In stressed *R. pipiens*, the frequency of abnormalities was also increased, this being accompanied by an increase in the FA of nonmeasurable characteristics, whereas the data on the FA of measurable characteristics were contradictory (Gallant and Teather, 2001).

Nonetheless, we believe that much more measurable parameters and larger samples should be studied to make a well-grounded conclusion that the FA of measurable parameters is ineffective as an indicator of population stress in amphibians; to minimize the measurement error, fixed material should be used. Our finding that the ontogeny of frogs from Yekaterinburg was not destabilized noticeably may have been accounted for by the small number of parameters studied and the insufficient sample size, especially in the case of the population living near the Yuzhnaya Bus Terminal. In addition, it is possible that only amphibians with a high degree of ontogenetic homeostasis survive in the course of adaptation to long-term technogenic stress, which decreases the population mean FA (Severtsova, 2002; Vershinin, 2004).

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