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GENERAL BIOLOGY

Differences between Rodent Taxa in Fluctuating Asymmetry of Cranial Structures

I. A. Kshnyasev, E. A. Gileva, A. V. Borodin, L. E. Yalkovskaya, and S. V. Zykov

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Fluctuating asymmetry (FA) is undirected differences in the measurements or expression of characters on the right and left sides of the body (R–L). A number of studies have shown that FA, which is usually treated as a measure of ontogenetic instability, increases under the influence of various stress factors. Although this dependence is not always easily detectable, FA is frequently used for the estimation of the population state [1, 2]. At the same time, the nature and evolutionary implication of FA remain uncertain [3–5]. It is generally accepted that the genetic control of FA is mainly provided by epistatic interactions [6–8]. These interactions probably change in the course of evolution (in particular, at early evolutionary stages); therefore, it is reasonable to expect that taxa (in particular, species) would differ in the FA of the same characters. In this study, species and population features of the FA of cranial measurements in four rodent species are examined from the evolutionary point of view.

Rodent species, sampling points, pollution characteristics, and sample sizes are shown in the table. Each species examined is represented by two population samples differing in the degree of technogenic stress, including (1) a reference sample, with a relatively low stress and (2) a sample exposed to a greater impact of industries. The population stress was estimated by the average frequency of chromosome aberrations in bone marrow cells. In the reference population of each species, the frequency of aberrations was significantly lower ($\chi^2(1) = 48.7–8.1$, $p = 0.0001–0.005$); this was regarded as a result of mutagenic pollution. The age of animals (juvenile/adult) was estimated [9, 10] on the basis of the state of reproductive organs, cranial characters, and body proportions.

Each specimen was characterized by 13 measurements of the axial skull (bones of the neurocranial and facial regions, including the upper jaw) and eight measurements of the lower jaw (Fig. 1). Each parameter was measured three times on either side, using the TPS.dig software [11]. The mixed ANOVA model was applied (side was a fixed factor, specimen was a random factor, and repeated measurements of the parameter were a cell of the plan). The presence of FA was judged upon by the significance of the *side–specimen* interaction, the presence of directed asymmetry (DA) was judged upon by the significance of the factor *side* [1]. The FA of each parameter was significant in each sample ($p < 0.0001$). The analysis taking into account recommendations from [12] has shown that NA has a insignificant effect on the between-group differences in asymmetry of both skull regions.

Based on the mean value of three repeated measurements of each parameter of each specimen, the indices normalized to the measurement value were calculated, $FA' = 1 – (2RL : (R^2 + L^2))$ [13] and $FA2 = 2|R – L| : (R + L)$ [12]. Since the indices showed an extremely asymmetric distribution, the robust approach was applied, which was based on the replacement of primary indices by their ranks [14]; the ranks of two indices are identical. The rank values were analyzed using the univariate and multivariate linear models (ANOVA/MANOVA) and canonical linear discriminant analysis. The two-sided statistical hypotheses were tested at the 0.05 significance level.

The univariate model (ANOVA) was used to analyze the integrated indices of FA, which were obtained by averaging the FA ranks for the parameters of the skull regions (the axial skull and lower jaw) of each specimen. The effects of four between-group factors (species, population, sex, and age) and one within-group factor (skull region) and their interaction were estimated. Only three effects were statistically significant, i.e., *species* ($F(3; 234) = 12.7$, $p \le 1.0 \times 10^{-7}$) and two two-factor interactions, *species–population* ($F(3; 234) = 3.5$, $p \le 0.016$) and *species–skull region* ($F(3; 234) = 13.5$, $p \le 4.5 \times 10^{-8}$). The greatest differences were found between *M. musculus* and the other three species

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

Average ranks of the FA' index, calculated from 13 measurements of the axial skull and 8 measurements of the lower jaw in four rodent species; values of FA' indices that contain the same letters (a, b) differ nonsignificantly (Tukey test)

Species	Sampling point, pollution features (sample size)	Average rank of FA'	
		axial skull	lower jaw
	Family Muridae		
House mouse (*Mus musculus*)	1. Tyumen oblast: village of Sovetskii, 61°21′ N, 63°34′ E; global pollution (25)	163.7 a	134.4 a
	2. Sverdlovsk oblast: town of Kamensk-Ural'skii, 56°24′ N, 61°56′ E; chemical and radioactive pollution (36)	161.9 a	131.1 a
	Family Cricetidae, subfamily Arvicolinae		
Northern mole vole (*Ellobius talpinus*)	1. Kurgan oblast: village of Klyuchiki, 55°01′ N, 63°43′ E; global pollution (18)	136.6 b	121.1 a
	2. Chelyabinsk oblast: head of the East Ural radioactive trail, 55°45′ N, 60°54′ E; radioactive pollution (31)	131.6 b	133.3 a
Common vole (*Microtus arvalis*)	1. Sverdlovsk oblast: village of Bainy, 56°42′ N, 62°08′ E; global pollution (60)	129.5 b	146.8 a
	2. Mari El Republic: city of Ioshkar-Ola, 56°40′ N, 48°00′ E; chemical pollution (35)	117.1 b	130.2 a
East European vole (*Microtus rossiaemeridionalis*)	1. Sverdlovsk oblast: village of Bainy, 56°42′ N, 62°08′ E; global pollution (38)	115.1 b	127.9 a
	2. Mari El Republic: city of Ioshkar-Ola, 56°40′ N, 48°00′ E; chemical pollution (23)	122.2 b	125.9 a

(Λ (Wilks criterion) = 0. 84, $F(3; 234) = 21.8$, $p \leq 2.1 \times 10^{-9}$), while the difference between *Ellobius* and *Microtus* is nonsignificant. These differences are distinct for the FA of the axial skull, but poorly pronounced in the FA of the lower jaw (table). The greatest ontogenetic instability is characteristic of the axial skull of *M. musculus*, while the smallest instability is observed in the East European vole *Microtus rossiaemeridionalis.* A dependence of FA of the axial skull and the technogenic stress was only found in the common vole *Microtus arvalis*; moreover, FA is lower in the population exposed to the technogenic stress.

The entire set of data on 21 FA indices of the axial skull and lower jaw were analyzed using the multivariate model (MANOVA). The effects of four factors (species, population, sex, and age) and their interaction were tested. Four effects were shown to be significant, namely, *species* ($\Lambda = 0.191$; $F(63; 639.6) = 7.52$, $p \leq 10^{-16}$), *population* ($\Lambda = 0.844$; $F(21; 214) = 1.89$, $p \leq 0.013$), *age* ($\Lambda = 0.850$; $F(21, 214) = 1.80$, $p \leq 0.02$), and the interaction *species–population* ($\Lambda = 0.610$; $F(63; 639.6) = 7.52$, $p \leq 1.9 \times 10^{-4}$). Significant differences were observed between *M. musculus* and other species ($\Lambda = 0.424$; $F(21; 214) = 13.87$, $p \leq 10^{-16}$), between *Ellobius* and *Microtus* ($\Lambda = 0.576$; $F(21; 214) = 7.50$, $p \leq 2.2 \times 10^{-16}$), and between *M. arvalis* and *M. rossiaemeridionalis* ($\Lambda = 0.817$; $F(21; 214) = 2.29$, $p \leq 0.002$). Thus, multivariate analysis has revealed a greater number of significant effects (including significant population differences). This contradicts the conclusions and recommendations of [15], which followed from the comparison of univariate and multivariate analyses of population differences in FA.

Using discriminant analysis, d^2 (Mahalanobis distances) between the eight population samples under study were estimated, and the first four significant canonical axes, which were responsible for 97% of the total variance, were treated. The first canonical axis differentiates representatives of different families (Muridae and Cricetidae) (characters 1–3, 9, 11, 13, 14, 7); the second opposes the genera *Ellobius* and *Microtus* (characters 5, 6, 21, 9, 16, 18); the third opposes the chromosome sibling species of the *arvalis* group of the genus *Microtus* (characters 1, 7, 9, 16, 18, 2); and the fourth opposes two populations of *Microtus arvalis* (characters 1, 2, 5, 7, 13, 14, 18). The main contribution to the canonical discriminant functions is made by the FA indices of tooth row and skull characters that are connected with the functioning of dentition. Nonsignificant Mahalanobis distances are only recorded between the populations of *Mus musculus* and between the populations of *Microtus rossiaemeridionalis*; in addition, population 2 of the common vole does not differ from both populations of *M. rossiaemeridionalis.* The smallest contribution to the variation of FA is made by the factor *population* (the last, fourth, significant canonical axis), although conspecific populations were shown to differ in stress effect [9, 10]. The tree (Fig. 2) based on the matrix of between-group Mahalanobis distances and showing the differences between species and populations corresponds to the phylogenetic relationships of the groups examined.

Thus, multivariate analysis has been shown to be more efficient, while the classification based on the FA of cranial measurements offers new opportunities for the evolutionary approach to the study of ontogenetic homeostasis. However, this conclusion requires addi-

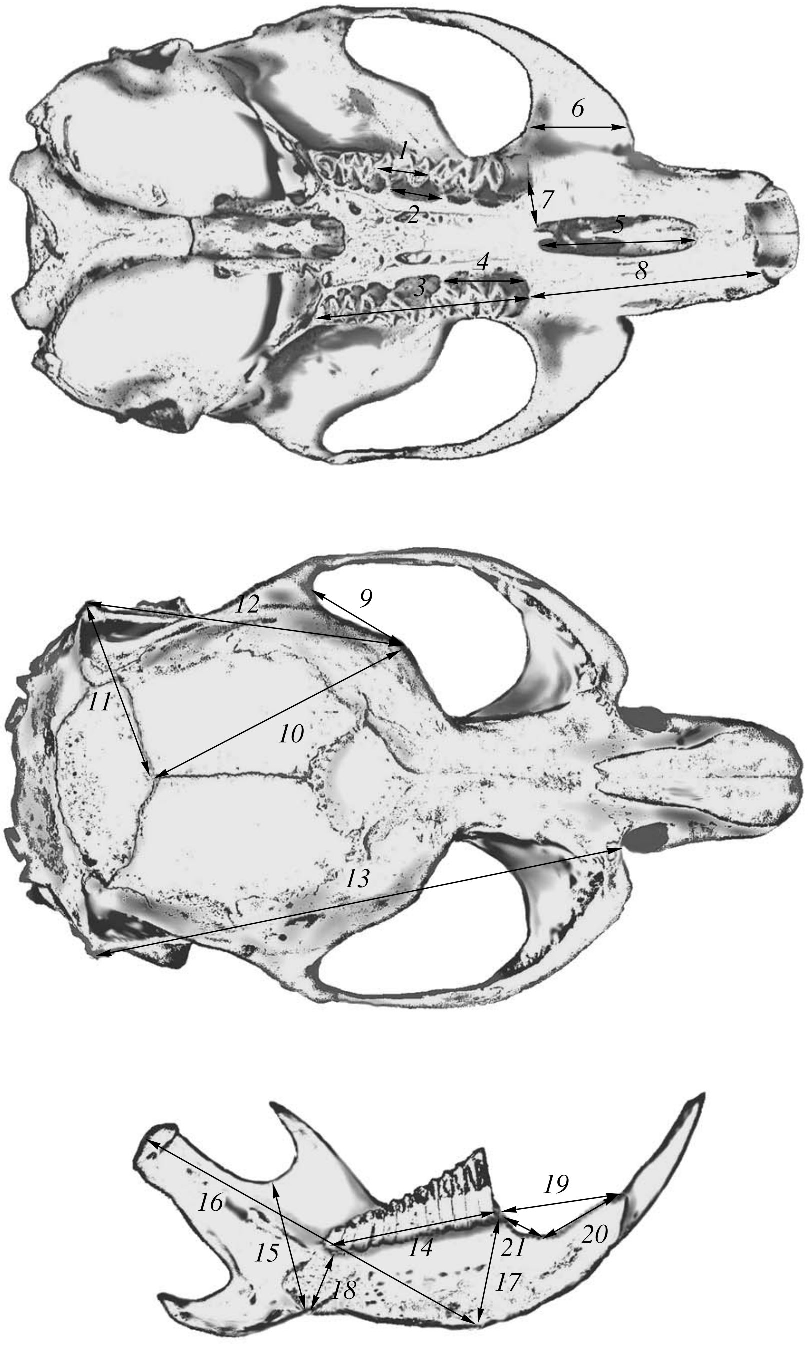

Fig. 1. The scheme of cranial measurements: axial skull: (*1*) crown length of M^2, (*2*) alveolar length of M^2, (*3*) alveolar length of the upper tooth row, (*4*) alveolar length of M^1, (*5*) length of the incisive foramen, (*6*) greatest width of the projection of the zygomatic arch, (*7*) distance from the alveolus of M^1 to the edge of the incisive foramen, (*8*) distance from the alveolus of M^1 to the alveolus of incisor, (*9*) distance from the posterior edge of the zygomatic arch to the postorbital tubercles, (*10*) distance from the middle of the interparietal to the postorbital tubercles, (*11*) distance from the middle of the interparietal to the edge of the suture of the occipital, (*12*) distance from the edge of the suture of the occipital to the postorbital tubercles, and (*13*) distance from the edge of the suture of the occipital to the anterior edge of the zygomatic arch; lower jaw: (*14*) alveolar length of the lower tooth row, (*15*) mandibular depth, (*16*) distance from the apex of the articular process to the symphysial tubercle, (*17*) distance from the anterior edge of the alveolus of M^1 to the symphysial tubercle, (*18*) mandibular depth at the alveolus of M^3, (*19*) diastematic length, (*20*) distance from the alveolus of incisor to the lowest point of the diastema, and (*21*) distance from the anterior edge of the alveolus of M^1 to the lowest point of the diastema.

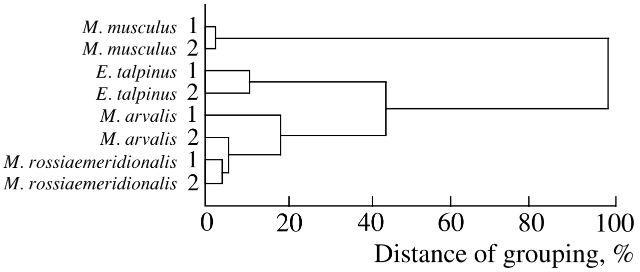

Fig. 2. The similarity tree of populations of four rodent species based on the matrix of Mahalanobis distances estimated from the ranks of the FA' indices of 21 cranial measurements.

tional corroboration based on a greater number of rodent populations and species Rodentia.

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