ISSN 0012-4966, Doklady Biological Sciences, 2006, Vol. 410, pp. 403–406. © Pleiades Publishing, Inc., 2006.
Original Russian Text © E.A. Gileva, V.N. Bol'shakov, L.E. Yalkovskaya, N.V. Sineva, 2006, published in Doklady Akademii Nauk, 2006, Vol. 410, No. 5, pp. 705–708.

GENERAL BIOLOGY

Chromosomal Instability and Cranial Asymmetry in the Mole-Vole *Ellobius talpinus* Pallas, 1770 Polymorphic for Coat Coloration

E. A. Gileva, Academician V. N. Bol'shakov, L. E. Yalkovskaya, and N. V. Sineva

Received May 29, 2006

DOI: 10.1134/S0012496606050176

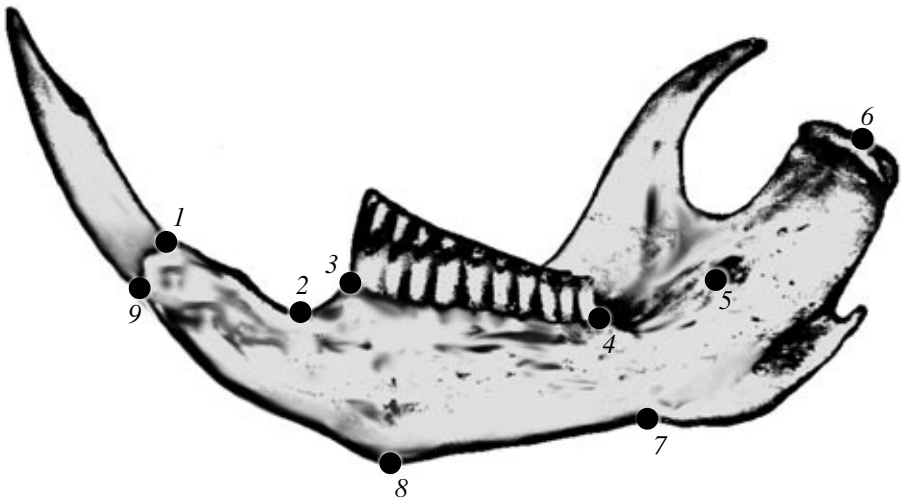
The genes that control coat color in mammals often have a pleiotropic effect: under the laboratory conditions, they have been shown to influence carbohydrate–lipid metabolism, body weight, specific behavior, etc. [1–3]. Natural rodent populations are polymorphic with respect to coat color, but associations of their polymorphism with other phenotypic characteristics that may, in particular, have an adaptive importance have been little studied.

The mole-vole (*Ellobius talpinus* Pallas, 1770), a burrowing rodent that has been found to be significantly diverse with respect to color in a considerable part of the species range, may serve as an informative model for studying these associations. Three types of mole-vole color are known: entirely black (melanists), entirely brown, and intermediate (alternating black and brown spots that widely vary in size and location [4]). Melanism is inherited as a monogenic recessive trait [5], whereas the brown and intermediate colors are apparently controlled by several genes. These most likely include *MC1R* (melanocyte-stimulating melanocortin receptor), which is characteristic of many rodents, and *Agouti,* an antagonist of *MC1R* [6, 7]. In the Southern Ural and Trans-Ural natural populations, the frequencies of the color morphs of the mole-vole display a distinct geographic trend: the frequency of melanism increases when moving from the steppe to the forest zone [4]. Thus, the polymorphism of *E. talpinus* coat color can be associated with varying adaptability. Therefore, the levels of genomic and ontogenetic instabilities were compared in various color morphs of the mole-vole.

The frequencies of chromosome aberrations in bone marrow cells served as indices of genomic instability; the ontogenetic instability was estimated by the degree of asymmetry in bilateral cranial structures, including the fluctuating asymmetry (FA), i.e., undirected differences in the trait value on the right and left sides of the skull, as well as the mandible asymmetry estimated by the method of geometrical morphometry. This method makes it possible to compare the shapes of the objects with complex configuration described by a set of landmarks on a plane projection of the object. On the basis of the landmarks of the compared objects, a common space (first, multidimensional; then, two- or three-dimensional) can be constructed for all objects and their consensus configuration. The distinctions between the object shapes are estimated from the difference between their distances from the consensus. Distances in a tangent space are recommended for comparing the shapes of biological structures. An important advantage of geometrical morphometry is that the results obtained are independent on the absolute size of the object [8].

Eighty-four mole-voles caught in 1998–2000 in the Kurtamyshskii rayon, Kurgan oblast (55°01′ N, 63°43′ E) were used in our study. Metaphase chromosome preparations were obtained from the bone marrow by the standard method. The frequency of chromosome aberrations (structural aberrations, gaps, aneuploidy, and polyploidy) was estimated in 50–100 cells of each animal. The level of ontogenetic instability was determined from the degree of cranial asymmetry with the use of Palmer's and Strobeck's approaches [9]. The FAs of ten craniometric traits (coronary lengths of I^1 and I_3; alveolar lengths of I^1, I_1, and I_3 of the upper and lower tooth rows, the length of incisor foramen, and the height and length of the mandible) were estimated four times on each skull side using an MBS-10 microscope. All traits of the compared groups were analysed using the two-way ANOVA (the mixed model: the side is a fixed factor, whereas the individual is a random factor). The FA was considered proved if the side × individual interaction was significant for all traits in all samples.

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



The scheme of mandibular marks: *1*, the upper edge of the incisor alveole; *2,* the lowest point of the diastem; *3,* the frontal alveolar edge of I_1; *4*, the dorsal alveolar edge of I_3; *5*, the lower edge of the mandibular foramen; *6*, the apical point of the articular process; *7*, the highest point of the mandibular incisure; *8,* the lowest point of the angular process; *9*, the lower edge of the incisor alveole.

There was no antisymmetry in any sample; when a directed asymmetry was found, the results were properly corrected. The FA indices of individual traits were determined using the dimensionless FA2 index [9], which is the ratio of the trait differences on the right and left sides to the average size of the trait; this index excludes the dependence of skull size on FA estimates. To determine an integrated FA index, the FA2 values of individual traits were averaged first for each individual and then for all individuals in a sample [10].

Geometrical morphometry of the mandible was based on digital images obtained with a Nikon Coolpix 990 digital camera and the eyepiece of a Stemi 2000-C microscope (Carl Zeiss) at a constant magnification of 6.5×. Using the TPS software package [11], marks were put on the right and left mandible branches (nine marks on each branch) at a resolution 300 points per inch (figure). On the basis of these marks, consensus configurations were constructed and the tangent distances of the right and left branches from the common consensus were estimated. All measurements were made in triplicate, the values of tangent distances were averaged for each side, and the difference between them was calculated. This difference served as the index of mandible asymmetry. Statistical hypotheses were tested at a 5% significance level.

Note that, in *E. talpinus* inhabiting the Ural and Trans-Ural areas, only melanists can be unambiguously identified, whereas dividing of the animals into brown and intermediate ones is largely subjective, because they often form a continuous series with respect to coat color. Therefore, two ways of statistical comparison were used: the comparison with respect to all the three morphs separately and the comparison of black animals with nonblack ones, i.e., the voles with brown and intermediate coat colors were pooled into a single group. The annual differences in the frequencies of chromosomal aberrations (1998, 1999, 2000) and the FAs of the measured traits (1998, 1999) proved to be nonsignificant (p = 0.082–0.937); therefore, data on different years were pooled (Table 1). Both methods of color morph comparison showed that the differences in the frequencies of structural and numerical mutations were nonsignificant at the 5% significance level, although, when melanists were compared with the pooled group of brown and intermediate mole-voles, the genomic instability of melanists tended to be higher. The same tendency was observed when the frequencies of aberrant cells and those with gaps were summed; this approach was often used by other authors, because recent data suggest the same origin of gaps and structural chromosomal aberrations (e.g., [12]). The combined index is significantly higher in melanists than in nonblack mole-voles, i.e., in two other morphs ($\chi^2 = 4.217$, $df = 1$, $p = 0.040$).

The differences in the FAs of craniometric measures were insignificant. The FA2 index averaged over ten traits tended to be higher in black mole-voles as determined using both methods of comparison and especially when the data on brown and intermediate animals were pooled. Nevertheless, the 5% level of significance was not reached in the latter case.

As determined by geometrical morphometry, differences were more distinct in the mandible of the color morphs (and, hence, in their developmental stability) (Table 2). The annual distinctions in the tangent index of asymmetry were statistically significant, but, in both 1998 and 1999, the mandible configuration was significantly more asymmetrical in melanists than in animals with other colors from the same population. The same ratio was determined by both methods of comparison, irrespective of whether brown and intermediate animals

Table 1. The frequency of chromosome aberrations and FA2 indices averaged for 10 craniometric traits of mole-voles differing in coat color

Coat color	Chromosome aberrations, mean percentage of cells with				Fluctuating asymmetry	
	number of animals (cells)	chromosome aberrations	aneu- and polyploidy	gaps	number of animals	mean FA2 · 10^3
Comparison variant I						
Black	42 (2840)	1.94	0.46	3.17	14	18.18
Brown	20 (1700)	1.29	0.71	3.00	13	14.33
Intermediate	23 (2050)	1.37	0.44	2.24	16	15.31
χ^2 (df = 2)		3.782	1.619	3.915	F = 1.807 (df = 2/40)	
P		0.151	0.445	0.141	P = 0.177	
Comparison variant II						
Black	42 (2840)	1.94	0.46	3.17	14	18.18
Nonblack	43 (3750)	1.33	0.56	2.59	29	14.87
χ^2 (df = 1)		3.751	0.329	1.988	t = 1.858 (df = 41)	
P		0.053	0.566	0.159	P = 0.070	

Table 2. Tangent distances characterizing the levels of the mandible bilateral asymmetry in mole-voles caught in different years (subscript A) or with different coat colors (subscript B). The factors used in ANOVA are indicayed in parentheses

Year of trapping	Coat color	Tangent index of asymmetry*
	Comparison variant I	
1998	Black (N = 4)	0.0217
	Brown (N = 3)	0.0043
	Intermediate (N = 8)	0.0097
1999	Black (N = 10)	0.0080
	Brown (N = 10)	0.0067
	Intermediate (N = 6)	0.0042
	F_A; P (df = 1/35)	4.433; 0.043
	F_B; P (df = 2/35)	4.694; 0.016
	F_{AB}; P (df = 2/35)	2.754; 0.077
	Comparison variant II	
1998	Black (N = 4)	0.0217
	Nonblack (N = 11)	0.0083
1999	Black (N = 10)	0.0080
	Nonblack (N = 16)	0.0057
	F_A; P (df = 1/37)	8.836; 0.005
	F_B; P (df = 1/37)	8.332; 0.006
	F_{AB}; P (df = 1/37)	4.190; 0.048

* The difference between the tangent distances of the right and left mandibular branches from the common standard.

were examined separately or pooled. The significance of the interaction between the year of trapping and coat color was related to the fact that the asymmetry index in the black animals was in different years increased to a different degree as compared to nonblack voles.

Thus, a higher level of ontogenetic and, with some reservations, chromosomal instability is characteristic of melanist mole-voles. The most convincing are the data suggesting a more asymmetrical shape of the mandible in black mole-voles as compared to that in animals with other coat colors. The differences between color morphs in the degree of genomic and ontogenetic homeostasis are likely to be related to the involvement of melanin metabolism into numerous metabolic processes (including hormonal ones) that are important for both mutagenesis regulation and cranial structure development. Bol'shakov et al. [13] have demonstrated that melanist mole-voles differ from other animals in some biochemical and physiological characteristics. In particular, the background level of the stress hormones 11-OCS in the blood plasma of black voles was noticeably higher than in those with the brown and intermediate coat colors. The chromosomal instability of melanists was presumably accounted for by the intensity of their strained hormonal status. The mutagenic effect of stress hormones was observed in both laboratory and natural populations of rodents [14, 15].

ACKNOWLEDGMENTS

We thank N.G. Evdokimova, A.V. Borodina, and S.V. Zykova for the assistance in collecting and treating the material. This study was supported by the Russian Foundation for Basic Research (project no. 05-04-

48373) and Russian Foundation for Basic Research–Ural (project no. 04-04-96124) and the Federal Program "Leading Scientific Schools" (project no. 2006-RI-112.0/001/337).

REFERENCES

1. Hayssen, V., *J. Comp. Psychol.*, 1997, vol. 111, no. 4, pp. 419–423.
2. Sviderskaya, E.V., Novak, E.K., Swank, R.T., and Bennett, D.C., *Genetics*, 1998, vol. 148, no. 1, pp. 381–390.
3. Shevchenko, A.Yu., Makarova, E.N., Bazhan, N.M., et al., *Ros. Fiziol. Zh.*, 2003, vol. 89, no. 7, pp. 906–908.
4. Evdokimov, N.G., *Populyatsionnaya ekologiya obyknovennoi slepushonki* (The Population Ecology of *Ellobius talpinus*), Yekaterinburg: Ekaterinburg, 2001.
5. Cheprakov, M.I., Evdokimov, N.G., and Glotov, N.V., *Genetika,* 2005, vol. 41, no. 11, pp. 1552–1558.
6. Barsh, G.S., *Trends Genet.*, 1996, no. 12, pp. 299–305.
7. Hoekstra, H.E. and Nachman, M.W., *Mol. Ecol.*, 2003, no. 12, pp. 1185–1194.
8. Pavlinov, I.Ya., *Zh. Obshch. Biol.*, 2000, vol. 61, no. 6, pp. 583–600.
9. Palmer, A.R. and Strobeck, C., *Developmental Instability: Causes and Consequences*, New York: Oxford Univ. Press, 2003, pp. 279–319.
10. Zakharov, V.M., Baranov, A.S., Borisov, V.I., et al., *Zdorov'e sredy: Metodika otsenki* (Environment Health: The Method of Assessment), Moscow: Tsentr Ekol. Politiki Rossii, 2000.
11. Rohlf, F.J., *TPS. Program Vers. 1.39*, New York: Dept. Ecol. Evol., State Univ. New York, 2003, pp. 11794–5245.
12. Paz-y-Mino, C., Davalos, M.V., Sanchez, M.E., et al., *Mut. Res*., 2002, vol. 516, nos.1–2, pp. 57–61.
13. Bol'shakov, V.N., Evdokimov, N.G., Moshkin, M.P., et al., *Dokl. Akad. Nauk SSSR*, 1989, vol. 308, no. 2, pp. 500–502.
14. Skorova, S.V., Nazarova, G.G., and Gerlinskaya, L.A., *Izv. Sib. Otd. Ross. Akad. Nauk,* 1986, issue 3, no. 18 (429), pp. 91–95.
15. Dyuzhikova, N.A., Bykovskaya, N.V., Vaido, A.I., et al., *Genetika*, 1996, vol. 32, no. 6, pp. 851–853.