SHORT COMMUNICATIONS

Adaptive Morphofunctional Transformations of the Mole Vole (*Ellobius talpinus* Pall.) upon Being Moved to the Northern Border of the Area

A. G. Vasil'ev[a], *, V. N. Bol'shakov[a], I. A. Vasil'eva[a], and N. V. Sineva[a]

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, 620144 Russia*
**e-mail: vag@ipae.uran.ru*

At the beginning of the 21st century the combined influence of climatogenic and anthropogenic factors is leading to rapid changes in the living conditions of animals [1, 2], which can cause accelerated morphogenetic rearrangements and microevolutionary adaptive changes in regional populations [3]. Nevertheless, despite its significance and relevance, this issue is still very poorly studied [4, 5]. In this regard, it is necessary to carry out experiments in natural conditions to model the morphogenetic responses of local populations to probable climate changes. Such modeling is possible by moving representatives of one population to other climatic conditions in a remote population within the species range [5]. At the same time, it is important to assess the extent to which the morphological and functional features of the original population are preserved in the descendants of relocated individuals after their long-term habitation in new natural conditions and in comparison with local native individuals.

Earlier, when trying to solve this problem, we used the common mole vole as a model species (*Ellobius talpinus* Pall., 1770). The mole vole is a colonial burrowing rodent that mainly leads an underground way of life [5, 6]. Based on an evaluation of the results of an experiment on the relocation of ten families of the species from the southern initial population to a geographically remote population on the northern border of the species range, the descendants of the settlers established a specific morphogenetic reaction [5]. In the configuration of the lower jaw, which this species actively uses when digging soil and foraging, the originality of the descendants of migrants was revealed in comparison with individuals of both native populations. At the same time, the question of the functional nature of the established differences and the degree of preservation of the morphological restrictions of the parental structure remained unresolved in the popula-

tions in representatives of the colony of migrants on the northern border of the range. The application of a new method of morphofunctional mandibular indices [7, 8] makes it possible to approach the solution of this problem using the same material in the study as in our previous publication [5].

The purpose of this study is to assess the stability of the structure of the lower jaw of the common mole vole of the original southern population in the descendants of animals relocated to another population at the northern border of the species range. The main task was to compare the variability of the mandibular morphofunctional indices to characterize the intensity of the gnawing and chewing functions, in individuals of the southern and northern populations of the mole vole and the colony of descendants of settlers from the southern population.

In this work, we used the same collections of the Museum of Institute of Plant and Animal Ecology of the Ural Branch of the Russian Academy of Sciences as in the previous publication [5]. We compared the samples from the southern (Orenburg oblast, in the city of Kuvandyk, 51°28′ N, 57°17′ E, 38 ind.) and northern (Chelyabinsk oblast, in the village of Kunashak, 55°35′ N, 61°40′ E, 68 ind.) populations of the mole voles, as well as northern descendants of settlers (28 ind.) from the southern population. The samples were collected in August 2005, 3 years after the release of the founders of the migrant colony to the free area of the northern population. Settlers with brown skin, as is typical of the original southern population, formed independent family colonies. They avoided contact with local black individuals (melanists) and did not form mixed pairs with them [5]. The southern population lives on the stony steppe slopes of the Guberlinsky uplands with xerophytic steppe vegetation, while the northern population inhabits open flat areas of the northern forest-steppe, where meso-
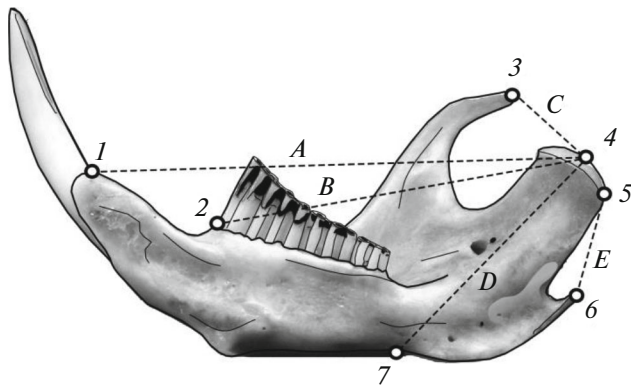
Fig. 1. Landmark placement (*1−7*) on the lingual side of the lower jaw of the common mole vole and measurements (*A−E*) to calculate morphofunctional mandibular indices.

philic meadow vegetation predominates. Mole voles of the northern and southern populations feed on both relatively hard food (dense dry rhizomes and tubers) and green parts of plants, bulbs, etc. [9, 10]. The composition of feed in both populations includes rhizomes and tubers of *Phlomis tuberosa* and *Philipendula hexapetala*, but rhizomes of *Pulsatilla patens*, *Dianthus uralensis* and bulbs *Allium stellarianum*, *A. globosum*, *A. tulipifolium*, *Gagea bulbifera*, *G. pusilla, Tulipa biebersteiniana*, *T. scythica*, as well as corms *Iris pumila*, *I. scariosa* are more characteristic in the southern one. In the northern population, wintering chambers can be located at a depth of up to 160 cm, while in the south, at up to 60−80 cm [6, 10]; therefore, the additional load during digging on the incisal part of the mandibles should probably be higher here, thus affecting their configuration. Large winter stocks (up to 2 kg) of dry rhizomes and tubers were also found in the northern population [6], which can also create an additional load during gnawing in winter. At the same time, in the southern regions, such reserves were rare and limited [10].

The age of the animals was determined by the size of the roots of the m1 first lower cheek tooth [6]. Mandible images were analyzed only in adults (1+ and 2+ years old). In all samples, 1-year-old individuals significantly predominated. Using computer programs of the TPS package [11, 12], seven landmarks were placed on the photograph of the lingual side of the right lower jaw (Fig. 1) for further measurements (*A−E*) used in the calculation of morphofunctional mandibular indices: *TI* (temporal-incisive), *TM* (temporal-molar), *MI* (masseter-incisor), *MM* (masseter-molar), and *AM* (angular-masseter). The system of measurements was slightly modified by us in comparison with the pioneer work [7]. Initially, the authors of the method proposed to measure the distance from the articular process to the upper edge of the incisor [7] (see Fig. 1). However, the constantly growing incisors of the mole vole are simultaneously mechanically

worn out under the abrasive action of the soil when digging and gnawing the underground parts of plants and are often damaged [6]. In addition, during boiling, which is necessary for further removal of soft tissues from the surface of the lower jaw, the incisor is often displaced along the incisal canal, which leads to artifacts when measuring the distance to the upper end of the incisor. Therefore, we propose to measure this measurement to the posterior edge of the alveolus of the lower incisor. We were forced to perform another initial measurement from the articular process to the upper front edge of the crown of the m1 first lower cheek tooth up to the front edge of the alveolus m1, since it is this tooth that is used in determining the age and is removed from the alveolus.

Automatic measurement was performed based on a text protocol that indicates pairs of landmarks, between which the distances were calculated using the tmorphgen6 software module of the IMP package [13]. The formulas [7, 8] were used to calculate the indices: $TI = C/A$; $TM = C/B$; $MI = D/A$; $MM = D/B$; $AM = E/D$. Comparison of the results of calculating indices using the original dental method of measurement [7] and the alveolar method that we modified in Fig. 1 made it possible to establish a high significant positive correlation between pairs of values of the dental and alveolar indices of the same name (Pearson's coefficients *r* fluctuated in the range 0.90−0.99 at $p < 0.001$). Therefore, the forced modification of the method of initial measurements *A* And *B* has little effect on the variability of the index. It is important to note that since both alveolar measurements correspond to their incisal and molar modules of the mandible (see [7]) the names of the modified indices should be considered as correct and essentially correspond to the original dental indices.

An assessment of the relationship between indices and age carried out separately for each sample did not reveal significant Pearson correlation coefficients, which made it possible to combine the age groups of 1- and 2-year-old individuals. A preliminary check using a two-way analysis of the variance did not reveal significant sexual differences for all indices (Table 1), which made it possible not to separate the samples by sex. The *MI* And *MM* indices characterize the efforts during gnawing and crushing of food objects, *TI* and *TM* reflect longitudinal chewing activity associated with longitudinal chewing and grinding of food. The *AM* index reflects the activity of transverse chewing and masticatory movements of the lower jaw [8, 14]. Statistical analysis was performed using the TPS [11, 12], IMP [13], and PAST4.04 [15] computer programs.

Comparison of morphofunctional mandibular indices showed (Table 2, 3) that intergroup differences are most clearly manifested in the *TI, TM, MI*, and *AM* indices. The intergroup differences according to the *MM* index were small and insignificant (see Table 3); therefore, in all samples, the general ability for vertical

Table 1. A two-way analysis of variance of the values of the mandibular indices (*TI*, *TM*, *MI*, *MM*, *AM*) by factors: group (G), sex (S) and their interaction (G × S)

Source of variability (factor)	Sum of squares (SS)	Number of degrees of freedom (df)	Middle square (MS)	F	Significance level (*p*)
TI — temporal-incisor index					
Group (G)	0.006485	2	0.003242	13.51	****
Sex (S)	0.000115	1	0.000115	0.48	ns
Interaction (G × S)	0.003626	2	0.001813	7.55	***
Intragroup	0.03072	128	0.00024		
General	0.04065	133			
TM — temporal-molar index					
Group (G)	0.015440	2	0.007720	17.34	****
Sex (S)	0.000208	1	0.000208	0.47	ns
Interaction (G × S)	0.005091	2	0.002545	5.72	**
Intragroup	0.05699	128	0.000445		
General	0.07725	133			
MI — masseter-incisor index					
Group (G)	0.002966	2	0.001483	6.49	**
Sex (S)	0.000891	1	0.000891	3.90	ns
Interaction (G × S)	0.000983	2	0.000492	2.15	ns
Intragroup	0.02924	128	0.000228		
General	0.03348	133			
MM — masseter-molar index					
Group (G)	0.00098	2	0.00049	1.25	ns
Sex (S)	0.000763	1	0.000763	1.95	ns
Interaction (G × S)	0.000502	2	0.000251	0.64	ns
Intragroup	0.05009	128	0.000391		
General	0.052234	133			
AM — angular-masseter index					
Group (G)	0.006926	2	0.003463	5.44	**
Sex (S)	0.001714	1	0.001714	2.69	ns
Interaction (G × S)	0.005874	2	0.002937	4.62	*
Intragroup	0.08143	128	0.000636		
General	0.09640	133			

ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

crushing of food objects by cheek teeth is similar. The ability for more intense gnawing, judging by the size of the masseter-incisor index *MI*, is expressed in the northern population of melanists (see Table 2). At the same time, the migrants retain the functional properties of the parent population, since the values *MI* are significantly less than for the animals of the northern population (in both cases of comparison $p < 0.001$). The value of the temporal-incissor index *TI*, which characterizes the ability for longitudinal chewing and grinding of food objects, is also significantly higher in northern animals (see Tables 2, 3). However, in this case, the group of migrants did not retain the low value of the index that is typical for the animals of the origi-

nal southern population. In contrast, its value approached that of individuals of the northern population. At the same time, the angular-masseter index *AM* in the group of immigrants is significantly higher (see Tables 2, 3) than in individuals from both aboriginal populations (correspondingly, for Tukey's a posteriori test in pairwise comparison with the northern sample, the value of the criterion was 4.42 ($p = 0.0051$), and from the south—4.11 ($p = 0.0102$).

Thus, the descendants of the settlers, having been placed in new habitat conditions in the north of the range, faced the need to feed on harder food, which was successfully used by individuals of the local popu-

Table 2. Comparison of the average values of the mandibular indices (taking into account the standard error $\pm SE$) between three compared samples of the common mole vole

Sample	Mandibular indices				
	$TI \pm SE$	$TM \pm SE$	$MI \pm SE$	$MM \pm SE$	$AM \pm SE$
Northern	0.215 ± 0.002	0.287 ± 0.003	0.527 ± 0.002	0.694 ± 0.003	0.281 ± 0.004
Settlers	0.220 ± 0.003	0.291 ± 0.004	0.514 ± 0.003	0.689 ± 0.004	0.299 ± 0.004
Southern	0.203 ± 0.002	0.268 ± 0.003	0.512 ± 0.002	0.688 ± 0.002	0.283 ± 0.003

lation. Since the migrants "inherited" a weak ability for this function from the parental population (see Table 2), they were forced to compensate for this shortcoming by strengthening the functions of transverse chewing and crushing food items characterized by the index *AM*. Apparently, in order to use other foods (bulbs and green parts of plants), which were also eaten by local melanistic individuals, the shape of the lower jaw of the migrants adapted, approaching the index values *TI* and *TM* to northern animals. In the steppe conditions, animals of the southern population, which were feeding on bulbs of irises and garlic, as well as tubercles of meadowsweet, did not need such morphological features, so the indices *TI* And *TM* here were relatively low.

As a result, it can be concluded that in just 3 years the settlers compensated for the morphological features in the structure of the lower jaw that was inherent in the parental population due to the formation of a new functional ability for transverse grinding of food objects, which is weakly expressed in animals of both native populations. Settlers who "inherited" limitations in their ability to chew (*MI* index), developed specific morphological and functional features that provided compensation for this deficiency and successful adaptation to new trophic conditions in the north of the range. In terms of other morphological and functional properties, the phenotype of the settlers approached the northern animals, reflecting the high phenotypic plasticity and the ability of the mole voles to quickly adaptive rearrangements of morphogenesis in the new climatic and trophic conditions of the northern forest-steppe. The greatest phenotypic

Table 3. The results of a one-way ANOVA (One-way ANOVA) of the values of the mandibular indices when comparing the samples of the mole voles from the northern and southern populations and the descendants of its settlers ($n = 135$)

Mandibular indices	F	Significance level p
TI	12.43	<0.0001
TM	16.36	<0.0001
MI	6.37	0.0023
MM	1.24	0.2941(ns)
AM	5.07	0.0076

changes in the descendants of migrants were manifested by the *TI* and *TM* indices, which indirectly may indicate their greater specialization in feeding on the green parts of plants (stems and rhizomes of cereals and bulbs), in which the function of longitudinal chewing is enhanced.

It can be assumed that the overall morphogenetic effect ("cost") of the formation of rapid morphofunctional modifications in a group of migrants is high, but at the same time it is of a forced nature, since, along with an increase in the function of transverse grinding of food (the *AM* index) the level of morphodiversity in this group, as noted earlier (see [5]), turned out to be significantly higher than in both aboriginal populations. In a previous publication [16], we showed that the morphogenetic differences between the southern and northern populations reflect the stages of gradual microevolutionary changes in the mole vole during the expansion of the Southern Trans-Urals from south to north.

Thus, with the rapid transfer of animals to a new environment from south to north (to simulate a sharp cooling and humidification of the climate), the morphogenetic "cost" of rearrangements should be greater than with the gradual historical introduction in new natural zones by the species and the morphological functional differentiation of populations at each time stage and spatial step of settlement. All this points to the important role of changes in climatic conditions in the rapid adaptive rearrangements of morphogenesis in *E. talpinus*. Nevertheless, this species, which leads an underground lifestyle, is sheltered from bad weather and is able to regulate the microclimate of the burrow and the climatic effect on morphogenesis is not direct, but should be mediated primarily by trophic changes due to a change in the composition of plant foods in the north of the range. The additional load on the incisal part of the mandible of northern animals may also be affected when digging deeper wintering chambers. There are grounds to suggest that the unusually high rate of directed morphogenetic rearrangements in the mole vole under new conditions in the north of the species range may be due to the mechanisms of transgenerational inheritance of stress-induced rapid epigenetic rearrangements of the genome [17, 18] associated with the assimilation of

certain adaptive modifications of individual development.

FUNDING

REFERENCES

1. Crutzen, P.J. and Stoermer, E.F., The Anthropocene, *Global Change News,* 2000, vol. 41, pp. 17−18.

2. Sutherland, W.J., Freckleton, R.P., Goodfray, H.Ch.J., et al., Identification of 100 fundamental ecological questions, *J. Ecol.,* 2013, vol. 101, pp. 58−67.

3. Salamin, N., Wuest, R.O., Lavergne, S., et al., Assessing rapid evolution in a changing environment, *Trend Ecol. Evol.,* 2010, vol. 25, no. 12, pp. 692−698.

4. Vasil'ev, A.G., Bol'shakov, V.N., Vasil'eva, I.A., and Sineva, N.V., Aftereffects of muskrat introduction in Western Siberia: Morphological and functional aspects, *Russ. J. Biol. Invasions,* 2017, vol. 8, no. 1, pp. 1−9.

5. Vasil'ev, A.G., Bol'shakov, V.N., Vasil'eva, I.A., et al., et al., Morphogenetic effects of resettlement of mole voles (*Ellobius talpinus* Pall., 1770) from the southern population to the northern boundary of the species range, *Dokl. Biol. Sci.*, 2018, vol. 478, pp. 26−28.

6. Evdokimov, N.G., *Populyatsionnaya ekologiya obyknovennoi slepushonki* (Ellobius talpinus Pall., 1770) *Yuzhnogo Urala i Zaural'ya* (Population Ecology of the Northern Mole Vole *Ellobius talpinus* Pall., 1770, in the Southern Urals and Transural Region), Yekaterinburg: Izd. Yekaterinburg, 2001.

7. Anderson, Ph.S.L., Renaud, S., and Rayfield, E.J., Adaptive plasticity in the mouse mandible, *BMC Evol. Biol.,* 2014, vol. 14, no. 85, pp. 2−9.

8. Vasil'ev, A.G., Vasil'eva, I.A., Gorodilova, Yu.V., and Chibiryak, M.V., Coupled chronographic variation of morphofunctional traits in cenopopulations of two sympatric rodent species, *Russ. J. Ecol.,* 2020, vol. 51, no. 4, pp. 363−375.

9. Kirikov, S.V., *V lesakh i stepyakh Yuzhnogo Urala: putevye zapiski zoogeografa* (In Forests and Steppes of the Southern Urals: Travel Notes of a Zoogeographer). Moscow: Geografgiz, 1953.

10. Ognev, S.I., *Zveri SSSR i prilezhashchikh stran* (Mammals of the Soviet Union and Bordering Countries), vol. 7: *Gryzuny* (Rodents), Moscow: Akad. Bauk SSSR, 1950.

11. Rohlf, F.J., *TpsUtil, File Utility Program, Version 1.60.* Department of Ecology and Evolution, State University of New York at Stony Brook, 2013.

12. Rohlf, F.J., *TpsDig2, Digitize Landmarks and Outlines, Version 2.17,* Department of Ecology and Evolution, State University of New York at Stony Brook, 2013.

13. Zelditch, M.L., Swiderski, D.L., Sheets, H.D., and Fink, W.L., *Geometric Morphometrics for Biologists: A Primer,* New York: Elsevier, 2004.

14. Vorontsov, N.N., Lower Cricetidae of the world Fauna: 1. Morphology and ecology, *Fauna SSSR. Mlekopitayushchie* (Fauna of the Soviet Union: Mammals), vol. 3, Moscow: Nauka, 1982.

15. Hammer, Ø., Harper, D.A.T., and Ryan, P.D., PAST: Paleontological Statistics software package for education and data analysis, *Palaeontol. Electron.,* 2001, vol. 4, no. 1, pp. 1−9.

16. Vasil'ev, A.G., Bol'shakov, V.N., Vasil'eva, I.A., and Sineva, N.V., Analysis of geographical variability of morphogenetic trajectories using the mole vole (*Ellobius talpinus* Pall.) as an example, *Dokl. Biol. Sci.,* 2020, vol. 493, no. 1, pp. 132−135.

17. Duncan, E.J., Gluckman, P.D., and Dearden, P.K., Epigenetics, plasticity and evolution: How do we link epigenetic change to phenotype?, *J. Exp. Zool. B. Mol. Dev. Evol.,* 2014, vol. 322, pp. 208−220.

18. Donelan, S.C., Hellmann, J.K., Bell, A.M., et al., Transgenerational plasticity in human-altered environments, *Trends Ecol. Evol.,* 2020, vol. 35, no. 2, pp. 115−124.