

Evolutionary Ecological Analysis of Coupled Geographic Variation of Two Sympatric Rodent Species in the Southern Urals

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Abstract—Geometric morphometrics has been used to reveal coupled geographic variation in the mandible shape in two sympatric rodent species, the pygmy wood mouse (*Sylvaemus uralensis* Pall.) and bank vole (*Myodes glareolus* Pall.), in the Southern Urals. It has been shown that syntopic samples synchronously collected from the local communities of these species usually display similar, parallel, and unidirectional morphological changes as demonstrated by comparison of species pairs from different localities. The degree of concordance in geographic variation of the species makes it possible to estimate their coevolutionary potential within local communities: the wider the range of ecological conditions under which parallel variation of sympatric species is observed, the higher is their coevolutionary potential.

Keywords: evolutionary ecology, geographic variation, geometric morphometrics, sympatric species, coevolutionary potential

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Traditionally, covariation is regarded as a concerted variation of different characters or even systems of characters within the same species (Mayr, 1968; Timofeeff-Ressovsky et al., 1977). A different interpretation of concordant variation is also accepted; in particular, well-known is analysis of the patterns of concerted geographic variation in characters of different species associated with the ecogeographic Bergmann–Allen rules (Mayr, 1968; Odum, 1986). This suggests that the coupled variation of the same homologous morphological structures in different sympatric species forming cenoses should also display certain regular patterns (Pianka, 1981; Giller, 1988; Vasil'ev et al., 2010; Mouillot et al., 2013).

Phylogenogenesis is necessarily associated with coevolutionary rearrangements in morphogenesis of the species constituting cenoses. There are good reasons to expect an acceleration of microevolutionary rearrangements in populations and communities under long-term climate trends and/or increase in anthropogenic impact on biomes (Zherikhin, 2003; Chernov, 2008; Vasil'ev et al., 2010). Therefore, it is natural to assume that climatic and technogenic factors not only disturb or destroy the existing biotic communities but also can have an effect on coevolutionary alterations in the emerging cenoses as well as on variation in populations of sympatric species (Bol'shakov et al., 2012).

Theoretical analysis of the problem suggests that research into morphological coupled variation of sym-

patric rodent species forming stable taxocenes (according to Hutchinson) may provide an insight into evolutionary ecological mechanisms underlying rapid coevolutionary rearrangements of biotic communities in the anthropogenically altered environment. It is necessary to emphasize that the taxocene is understood as a real fragment of a biotic community represented by taxonomically close species performing similar cenotic functions.

A case study of the rodent communities suggests that the dominant species forming the core of a taxocene and constantly maintaining a sufficiently high population size in different years are historically preadapted to the diversity of local conditions and their fluctuations to which the corresponding community is exposed. A consistently high level of population size indirectly suggests that their ecological niches do not overlap although should be rather close. On the other hand, subdominant species, which usually have a relatively low population size, can reach the level of dominant species only in individual years especially favorable from the ecological standpoint. The distinct dependence of the abundance of such species on abiotic and biotic conditions may suggest their lower fitness to local environment and, presumably, a low demand of the community for this species. The establishment of competitive biotic interactions between the dominants and subdominants is also likely. Therefore, when studying a morphogenetic response of sympatric species to similar climatic, biotic, and

anthropogenic changes in the environment and determining the tolerance limits of each species, it is possible to estimate their mutual coevolutionary potential. Hypothetically, the wider the range of environmental fluctuations under which these species give a similar morphogenetic response to the same changes, the higher should be their coevolutionary potential. Assuming that a long-term impact of similar local ecological factors leads to common coevolutionary rearrangements in the individual development of sympatric species, similarity in the manifestation of morphological between-group variation should also be definitely expected.

When assessing the coupled variation of sympatric species, it is necessary to use collection specimens from different geographic localities sampled in syntopic populations over a short period of time (in the same year and season). The degree of concordance in morphological variation of two and more species in a community can be maximally strictly estimated by comparing homologous elements in the shape of the test objects rather than commonly accepted linear dimensions.

The recent decades have brought up new digital techniques of geometric morphometrics (GM), which make it possible to assess the shape variation of objects excluding the effect of their size (Rohlf and Slice, 1990; Zelditch et al., 2004; Klingenberg, 2011). A special advantage of GM is in the possibility to estimate the coupled variation of taxonomically close sympatric species of a local community (taxocene) based on the configuration of landmarks (homologous elements of shape). In addition, GM allows visualization of transformation in the shape of objects, thereby providing the possibility for direct morphogenetic interpretation of the manifested variation (Zelditch et al., 2004; Klingenberg, 2011).

The purpose of this study was to apply GM methods to analysis of coupled geographic variation in the mandible shape and size between the bank vole (*Myodes glareolus* Pall.) and pygmy wood mouse (*Sylvaemus uralensis* Pall.), two sympatric species dominating in the rodent communities (taxocenes) of the southern Ural, in order to evaluate their coevolutionary potential.

MATERIAL AND METHODS

Coupled geographic variation in the mandible shape was studied using syntopic and synchronous samples from the populations of pygmy wood mice and bank voles. The material for analyzing geographic variation (in two variants) consisted of museum craniological specimens collected in different years in the Southern Ural, namely, in the Orenburg oblast in a longitudinal direction (from east to west) and in the Republic of Bashkortostan in a latitudinal direction (from south to north). Synchronous sampling of both species for each variant of comparisons provided for

complete “matching” of the points with respect to both space and time.

The museum specimens from the Orenburg oblast were collected in July 1978 in three geographically distant sites in floodplain forests along the Ural and Malaya Khobda Rivers, namely, (1) Orenburg site (near the village of Kamennoozerno), (2) Ilek site (near the village of Ilek), and (3) Eginsai site (near the village of Eginsai, Pervomaiskii raion). Geographically, these trapping sites are the vertices of an isosceles triangle with the sides of 150 to 200 km. The specimens from Bashkortostan were also represented by pairs of samples collected in June 1986 in floodplain forests along the Belaya River in three distant localities, namely, (1) southern Bashkir site (near the village of Ira), (2) Kuganak site (near the Bol'shoi Kuganak railroad station), and (3) Ufa site (suburbs of Ufa). The distances between the sites in the south–north direction were about 100 km. All animals were caught in the same biotopes, namely, floodplain deciduous forests, except for the Bol'shoi Kuganak sample: it was collected in a floodplain forest near the Belaya River where the habitats of both species were exposed to pollution with organic industrial wastes during the spring floods in the mid-1980s, unlike similar habitats in other sampling areas.

Samples of young of the year of comparable age from sympatric pygmy wood mouse and bank vole populations were examined. The object for the study was the mandible as a functionally significant morphological structure most appropriate for geometric morphometrics. The lingual side of the right mandible was imaged using a flatbed scanner at 1200 dpi and digitized twice with the tpsDig2 digitizing software. Totally, 16 homologous landmarks characterizing variation in mandible shape were used (Fig. 1). The arrangement of landmarks was the same for both species and all variants of comparison. Only images of the mandibles of mature young of the year were digitized, including 69 pygmy wood mice and 60 bank voles from Orenburg oblast and 74 and 78 respective animals from Bashkortostan. The Procrustes coordinates, characterizing variation in shape, were used as a basis for the subsequent multimeric between-group comparison. The between-group differences were estimated by canonical and principal components analyses. All calculations were made using programs TPS (Rohlf, 2010a, 2010b), PAST (Hammer et al., 2001), and MorphoJ (Klingenberg, 2011).

A preliminary analysis did not reveal any bias resulting from repeated digitizing in estimates of mandible shape. When the same operator did the work, the bias proved to be very small (not exceeding 2% of the variance) and usually statistically insignificant; therefore, the images were subsequently digitized only once. No significant sex-related differences in the mandible shape were detected, which allowed us to pool the male and female samples.

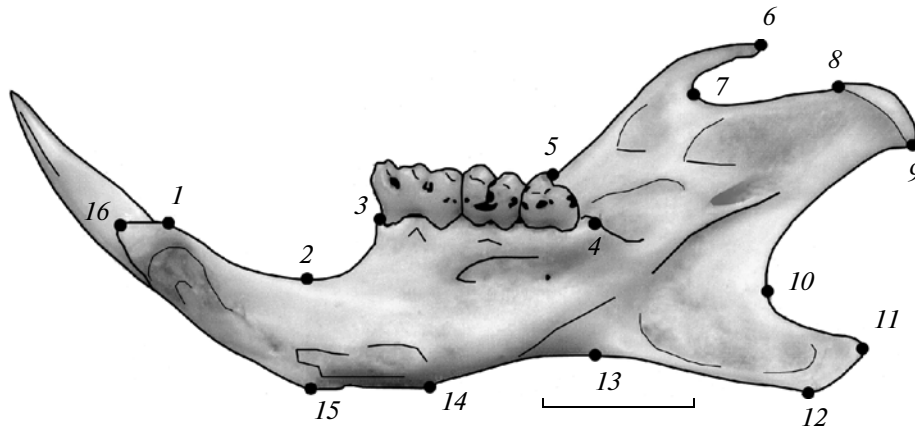


Fig. 1. Arrangement of 16 landmarks on the lingual side of the pygmy wood mouse mandible.

RESULTS AND DISCUSSION

In the first variant, we compared geographic variation of the bank vole and pygmy wood mouse in a longitudinal direction, from east to west, in Orenburg oblast. Since the sampling sites were sufficiently distant from each other (150–200 km), the samples could be regarded as belonging to different population groups.

Canonical analysis of the Procrustes coordinates characterizing the mandible shape in the bank vole and pygmy wood mouse revealed statistically significant between-group differences along all five canonical axes. As expected, interspecific differences were detectable along the first canonical variable. It is evident that the species specificity in the shape of the mandible as a “food-procuring” organ (according to Giller) in sympatric species—the bank vole and pygmy wood mouse—distinctly reflects their specific trophic preferences for green and grain foods, respectively.

Note that analysis of correlations between individual ordinates for all five canonical variables and the variable characterizing the species showed that only the coefficient of correlation with the first axis was statistically significant. Therefore, having excluded the first canonical variable from consideration, we thereby exclude the major interspecific features of the mandible, which have a strictly taxonomic meaning. Coupled geographic variation in the mandible shape was clearly observable along the second and third canonical variables for syntopic populations of both sympatric species. As is evident from Fig. 2, the sample centroids for the populations of both species from the same localities are close to each other in the planes of the second and third canonical axes (their standard errors in most cases overlap), but they are distant in a statistically significant manner for the corresponding pairs of samples from other geographic sites. Parallelism in the deviations of population centroids for both species is direct evidence for similarity in their mor-

phogenetic response to the same local conditions as well as for not only morphogenetic, but also ecological nature of coupled variation in the mandible shape along the second and third canonical variables. Interestingly, the ordinates of the centroids for syntopic species pairs form a triangle in the morphospace and reflect mutual geographic arrangement of the samples. It is also noteworthy that samples from the southernmost Egingsai taxocene (which lives under more arid conditions) are also the most distant from other samples in the common morphospace.

In the second variant, the geographic variation of the two species in a latitudinal direction was analyzed using syntopic, synchronously collected samples from Bashkortostan populations. In this case, the results of analysis of the principal components of mandible shape according to the Procrustes coordinates are presented (Figs. 3, 4). The first four principal components account for 92% of the total variance, with the proportion of the variance along the first principal component exceeding 80%. Not only to taxonomic differences but also distinctive morphofunctional features of each species associated with their specific ecological characteristics and roles in the community manifest themselves along the first principal component. That is why this component (PC1) accounts for the largest proportion of the total variance. The drastic divergence of the two sympatric species in the mandible shape along the first principal component directly indicates their different specificity in using this food-procuring structure, which allows them to successfully coexist in the same biotopes. We excluded the first principal component from further analysis, which allowed us to “level off” taxonomically and functionally determined species-specific differences in the mandible shape.

The degree of concordance in geographic variation along the second, third, and fourth components after excluding the first principal component (PC1) still remained high. In this case, we also observed similar

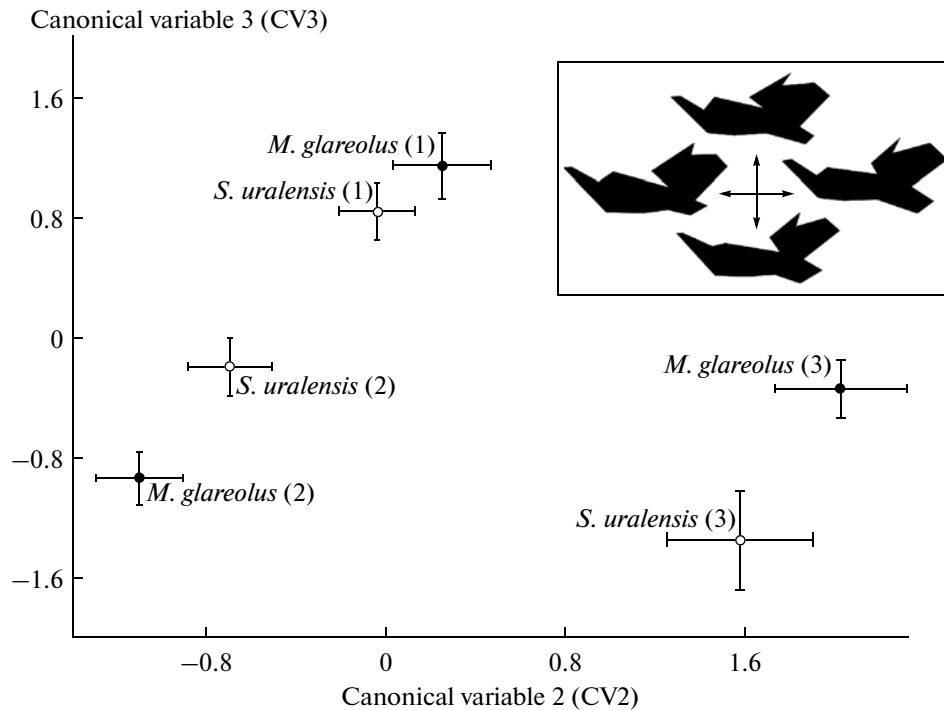


Fig. 2. Results of canonical analysis of variation in the mandible shape along the second and third canonical variables (CV2 and CV3): comparison of sample centroids (with standard errors) for three pairs of syntopic populations of the pygmy wood mouse (*S. uralensis*) and bank vole (*M. glareolus*) from (1) Orenburg, (2) Ilek, and (3) Egingsai sites (Orenburg oblast). Shadowgraphs (framed) illustrate trends of change in the mandible shape along the corresponding canonical axes.

convergence of the centroids for the samples of both model species from the same sampling sites in the spaces of the second–third and third–fourth principal components (Figs. 3, 4).

Figure 3 shows the positions of centroids for the population samples of both species. It is evident that the centroids for each pair of syntopic samples of the compared species from the same locality are in all cases located near each other. The centroids for bank vole local samples are located along the second principal component in the same order as their geographic sites in the north–south direction (Ufa → Bol’shoi Kuganak → Ira), which is indicative of latitudinal geographic variation in the mandible shape of this species. However, this sequence is distorted in the case of complementary pygmy wood mouse samples due to the deviation of the Kuganak sample (Ufa → Ira → Bol’shoi Kuganak).

Remember that the Kuganak sample of pygmy wood mice, similar to the corresponding bank vole sample, was collected in the floodplain forest of the Belaya River in the area of pollution with toxic petroleum products. Therefore, the deviation of the Kuganak sample of mice (as well as the bank vole sample) can be attributed (with some caution) to the effect of a technogenic factor. Moreover, it can be concluded that the pygmy wood mouse, compared to the bank vole, has displayed somewhat higher morphogenetic reactivity and phenotypic plasticity upon chronic exposure to this

pollutant. We have earlier demonstrated the same for the species pair northern red-backed vole (*M. rutilus*)–pygmy wood mouse (Bol’shakov et al., 2012).

On the other hand, it is evident that the range of differences between the southernmost (Ira site) and northernmost (Ufa site) samples along the second axis is wider in the bank vole than in the pygmy wood mouse. This indirectly suggests a somewhat smaller range of geographic variation per se in the pygmy wood mouse. Consequently, it appears that the latitudinal geographic variation in the mouse, compared to the vole, is relatively weak, while the technogenic component of variation is manifested more strongly.

A distinct “technogenic” morphogenetic response is observed along the third principal component, as follows from the shift of the centroids for impact Kuganak samples of both species, with approximately similar range of ordinate values, in the direction opposite to the conditionally control samples (Ira and Ufa) to the region of positive values of the third principal component (still, the differences are somewhat more distinct in the mouse than in the vole). Shadowgraphs of the mandibles show that the growth in the articular and coronoid processes in a dorsal direction is increased in the impact samples, while the growth in corpus mandibulae in both dorsal and caudal directions is inhibited; note that the diastema is shortened, with the incisor part of mandible being bent upward.

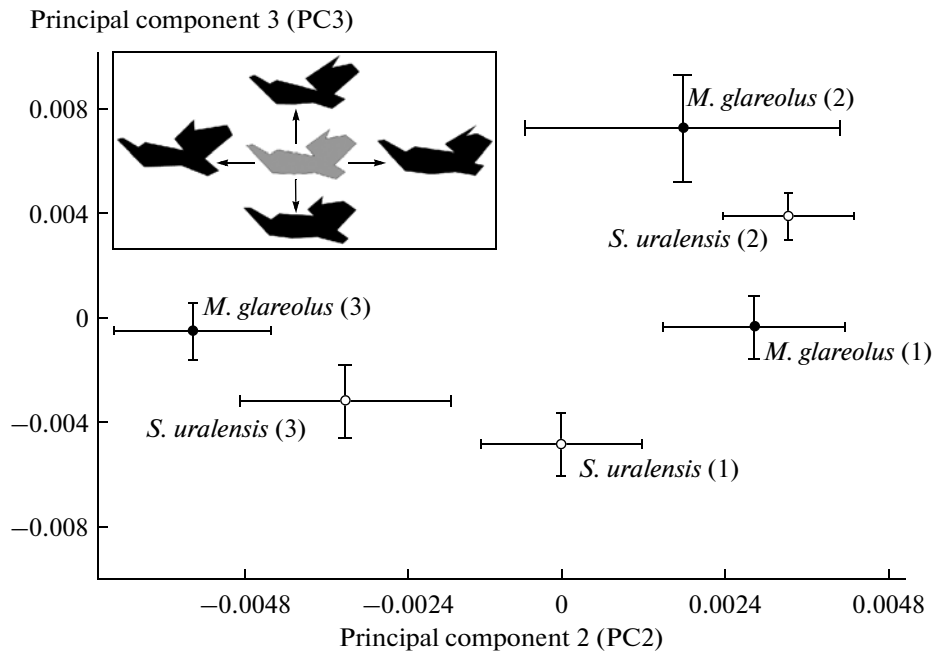


Fig. 3. Positions of sample centroids (with standard errors) for syntopic populations of the pygmy wood mouse (*S. uralensis*) and bank vole (*M. glareolus*) in the planes of the second and third principal components of mandible shape in (1) southern Bashkir, (2) Kuganak, and (3) Ufa sites (Bashkortostan). Shadowgraphs illustrate trends of change in the mandible shape along the axes; the central (gray) shadowgraph shows consensus configuration.

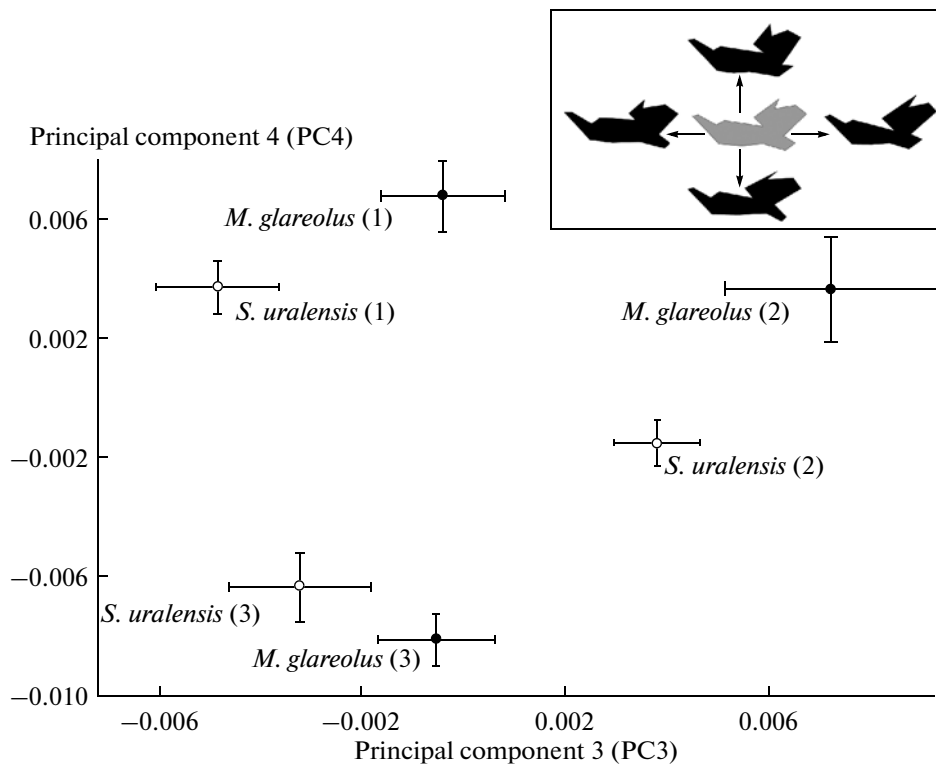


Fig. 4. Comparison of the positions of sample centroids (with standard errors) for syntopic populations of the pygmy wood mouse (*S. uralensis*) and bank vole (*M. glareolus*) in Bashkortostan in the planes of the third and fourth principal components (PC3 and PC4), characterizing variation in the mandible shape (for designations, see Fig. 3).

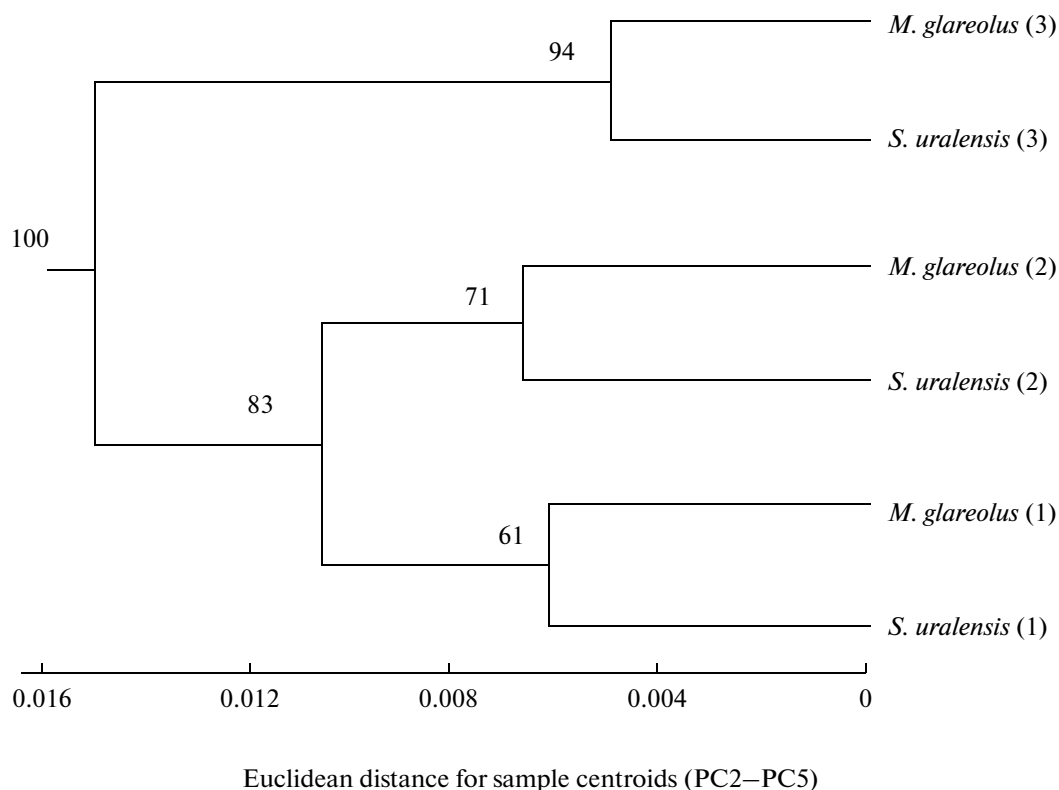


Fig. 5. Cluster analysis (UPGMA) of the ordinates of centroids for syntopic populations of the bank vole (*M. glareolus*) and pygmy wood mouse (*S. uralensis*) from (1) southern Bashkir, (2) Kuganak, and (3) Ufa taxocenes in Bashkortostan (1986) based on four principal components (PC2–PC5).

Let us analyze the coupled between-group variation along the third and fourth principal components. It is evident from Fig. 4 that the between-group differences in this case are also correlated: the centroids of the samples of two species from the same localities converge most closely in the plane of the third and fourth axes, indicating distinct parallelism of between-group variation in the mandible shape in both species.

The between-group variation along the third axis, PC3 (Fig. 4), which may be interpreted as a coupled technogenic variation, has been considered above (Fig. 3). This aspect reflects the parallel *technogenic variation* of two sympatric species as their nonspecific morphogenetic response to general technogenic pollution of habitats. The coupled latitudinal geographic variation per se manifested itself along the fourth principal component, PC4: the projections of sample centroids on this axis strictly match the sequence of geographic locations of samples from the southernmost to northernmost taxocenes. Note that the bank vole displays a slightly wider range of geographic variation. It is noteworthy that the coupled technogenic variation of these species is characterized by somewhat greater variance than the latitudinal geographic variation (the variance along PC3 is slightly greater than that along PC4), or at least they are comparable in this respect (PC2 variance comprises both the geographic and

technogenic components of variation). This agrees with the conclusion we arrived at when comparing a series of local population groups of *S. uralensis*, the species considered in this study (Bol'shakov et al., 2012).

Cluster analysis of the ordinates of sample centroids also demonstrates parallelism in geographic variations of the mandible shape in the compared syntopic population groups of the bank vole and pygmy wood mouse in Bashkortostan (Fig. 5). The calculations were based on the Euclidian distance for the values of centroids for four principal components (PC2–PC5) using an UPGMA algorithm. The calculated cophenetic correlation value, $Coph = 0.91$, reflects a good coupling of the initial matrix and the final one, obtained by clustering. As shown in Fig. 5, all pairs of syntopic samples of both sympatric species group together in common subclusters at a sufficiently high statistical bootstrap support (61 to 94%) based on 100 iterations. Note that cluster analysis, which formally confirms coupled geographic variation of these sympatric species, also demonstrates a hierarchy at the level of local “taxocenes” that unite these species according to the similarity of their mandible shapes. The general structure of the cluster suggests that the southern Bashkir (Ira site) and Kuganak (Bol'shoi Kuganak site) species complexes are closer to each other by this criterion than to the northern taxocene

(Ufa site). This result is somewhat unexpected, since, according to the data considered above, the total deviation of the centroids for Kuganak syntopic samples of both species along the third principal component should have resulted in a high specificity of the Kuganak taxocene as well. Therefore, taking into account the set of ordinate values of the sample centroids along four principal components (PC2–PC5), which characterize variation in the mandible shape of sympatric species used here for cluster analysis, it is possible to infer that the coupled latitudinal geographic variation (that is, geographic variation at the level of taxocene) has proved to be higher than the coupled technogenic variation.

CONCLUSIONS

This study has demonstrated distinct parallelism of geographic variation in synchronously collected syntopic samples of two sympatric species, the pygmy wood mouse and bank vole. Concordance of geographic variation in the mandible shape in both longitudinal and latitudinal directions demonstrates a high coevolutionary potential of the two species. Concurrently, coupled technogenic variation has been observed as one of the aspects of geographic variation of these sympatric species associated with remote morphogenetic consequences of chronic technogenic pollution impact on individual development of the rodents inhabiting impact area. The coevolutionary potential of the two species has also been revealed, since they have displayed similar morphogenetic responses (though differing in amplitude). Although the geographic variation per se exceeds the technogenic variation, the latter can approach it in amplitude and, hence, should be also taken into account as an ecological factor of evolution.

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REFERENCES

- Bol'shakov, V.N., Vasil'ev, A.G., Vasil'eva, I.A., Gorodilova, Yu.V., Kolcheva, N.E., Lyubashevskii, N.M., and Chibiryak, M.B., Technogenic morphological variation of the pygmy wood mouse (*Sylvaemus uralensis* Pall.) in the Urals, *Russ. J. Ecol.*, 2012, no. 6, pp. 448–453.
- Chernov, Yu.I., *Ekologiya i biogeografiya: izbrannye raboty* (Ecology and Biogeography: Selected Works), Moscow: KMK, 2008.
- Giller, P.S., *Community Structure and the Niche*, London: Chapman and Hall, 1984. Translated under the title *Struktura soobshchestv i ekologicheskaya nisha*, Moscow: Mir, 1988.
- 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.
- Klingenberg, C.P., MorphoJ: An integrated software package for geometric morphometrics, *Mol. Ecol. Resour.*, 2011, vol. 11, pp. 353–357.
- Mayr, E., *Animal Species and Evolution*, Cambridge: Harvard Univ. Press, 1963. Translated under the title *Zoologicheskii vid i evolyutsiya*, Moscow: Mir, 1968.
- Mouillot, D., Graham, N.A.J., Villéger, S., et al., A functional approach reveals community responses to disturbance, *Trends Ecol. Evol.*, 2013, vol. 28, no. 3, pp. 167–177.
- Odum, E., *Basic Ecology*, Philadelphia: Saunders, 1983. Translated under the title *Ekologiya*, Moscow: Mir, 1986.
- Pianka, E.R., *Evolutionary Ecology*, New York: Harper and Row, 1978. Translated under the title *Evolutsionnaya ekologiya*, Moscow: Mir, 1981.
- Rohlf, F.J., *TpsUtil, File Utility Program, Version 1.47*, Stony Brook, NY: Department of Ecology and Evolution, State University of New York, 2010a.
- Rohlf, F.J., *TpsDig2, Digitize Landmarks and Outlines, Version 2.16*, Stony Brook, NY: Department of Ecology and Evolution, State University of New York, 2010b.
- Rohlf, F.J. and Slice, D., Extension of the Procrustes Method for the Optimal Superimposition of Landmarks, *Syst. Zool.*, 1990, vol. 39, no. 1, pp. 40–59.
- Timofeeff-Ressovsky, N.V., Vorontsov, N.N., and Yablokov, A.V., *Kratkii ocherk teorii evolyutsii* (A Brief Essay on the Theory of Evolution), Moscow: Nauka, 1977.
- Vasil'ev, A.G., Vasil'eva, I.A., Gorodilova, Yu.V., and Chibiryak, M.V., Relationship between morphological disparity and taxonomic diversity in rodent communities in the zone of influence from the Eastern Ural Radioactive Trace in the Southern Urals, *Russ. J. Ecol.*, 2010, no. 2, pp. 153–158.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D., et al., *Geometric Morphometrics for Biologists: A Primer*, Amsterdam: Elsevier, 2004.
- Zherikhin, V.V., *Izbrannye trudy po paleoekologii i filotsenogenetike* (Selected Works in Paleoecology and Phylogenetics), Moscow: KMK, 2003.

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