

Coupled Chronographic Variation of Morphofunctional Traits in Cenopopulations of Two Sympatric Rodent Species

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Abstract—Methods of geometric morphometrics have been used to assess chronographic variation of morphofunctional traits in the same cenopopulations of the bank vole (*Clethrionomys glareolus*) and pigmy wood mouse (*Sylvaemus uralensis*) in Orenburg oblast. Changes in the mandible size and shape and in morphofunctional mandibular indices that occurred over 30 years (1986–2016) have been evaluated. As a result, directional shifts in morphogenesis of the mandible and its morphofunctional transformations related to change in trophic preferences have been revealed in both voles and mice. The mandible shape has changed toward specialization for food gnawing and crushing in *C. glareolus* cenopopulation and for transverse food grinding in *S. uralensis* cenopopulation. Sexual dimorphism in the mandible centroid size (CS) in both species has increased over the 30-year period, whereas the parameter of morphological disparity (*MNND*) has decreased significantly, providing indirect evidence for an increase in developmental stability. Despite a shift in climatic regime and associated changes in vegetation, parallelism of chronographic variation has been revealed in the two taxonomically distant species, reflecting a high and positive coevolutionary potential, i.e., similar morphogenetic responses to changes in environmental conditions in different years. These results show that rapid directional modifications of morphogenesis actually occur in cenopopulations of sympatric rodent species under climate change.

Keywords: chronographic variation, rodents, cenopopulation, morphofunctional changes, climate, geometric morphometrics, Southern Urals

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Morphofunctional changes occurring in animal populations over historically short periods are of major interest for gaining insight into evolutionary-ecological processes leading to rapid microevolutionary transformations and changes in the functioning of communities [1–5]. Significant changes in ecological living conditions for species under the impact of climatic and anthropogenic factors should inevitably lead either to rapid transformation of morphogenesis (due to modifications or/and directional selection) or to their degradation and extinction. On the other hand, principally important are coupled coevolutionary changes in sympatric species, which have not yet been studied sufficiently [6, 7]. We have previously observed manifestations of positive coevolutionary potential—similar morphogenetic responses to the same environmental factors—in natural populations of sympatric species, either insectivores or rodents [8, 9].

It is particularly interesting to analyze coupled morphological variation and diversity in populations of sympatric species over historically long periods of time. The data on chronographic variation during relatively short periods (two or several successive years) mainly reflects modifications and fluctuations associ-

ated with annual climatic oscillations and cycles of population dynamics that lead to morphogenetic effects, with these effects largely depending on changes in population density. However, the mutual influence of cenopopulation densities of sympatric species on their morphogenesis has not been studied sufficiently. The same is also true of morphogenetic transformations taking place over historically long time periods, because long-term observations on the same cenopopulations have been performed very rarely [10]. Nevertheless, it is long-term monitoring that in such cases can reveal not only direct modifications but also evolutionary-ecological processes conditioned by the phenomena of diffuse coevolution of sympatric species in the community (taxocene). Of special interest is to evaluate the directions of interrelated morphogenetic rearrangements in cenopopulations of the compared species, which makes it possible to reveal manifestations of their positive or negative coevolutionary potential [9].

A positive coevolutionary potential implies that morphogenetic responses in cenopopulations of sympatric species are similar, indicating that competition between them is weak or absent. If this potential is

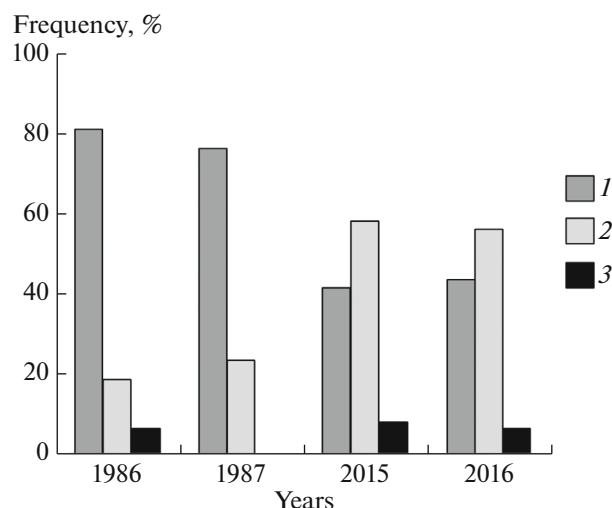


Fig. 1. Occurrence frequency ratios of three rodent species in catches of different years made in Sakmara River floodplain forest (Cherny Otrog, Orenburg oblast): (1) bank vole, (2) pigmy wood mouse, (3) yellow-necked mouse.

negative, strong competition between the species is highly probable, which makes it necessary to implement different morphogenetic programs in order to alleviate the adverse mutual influence of syntopic cenopopulations.

The collections kept at the Zoological Museum of the Institute of Plant and Animal Ecology (Ural Branch, Russian Academy of Sciences) include the serial craniological samples of two sympatric rodent species—the bank vole (*Clethrionomys glareolus*) and pigmy wood mouse (*Sylvaemus uralensis*) collected in Orenburg oblast in the late 20th and early 21st centuries, which may be used as a model for solving the problems discussed here.

The methods of geometric morphometrics [11–13] make it possible to separately evaluate variation in the size and shape of test objects. Morphological differences revealed by these methods can be interpreted in morphogenetic terms [14] and used in an attempt to solve the aforementioned evolutionary-ecological problems [13, 15, 16].

Therefore, the purpose of this study was to use the methods of geometric morphometrics in order to analyze coupled chronographic variation and within-group morphological disparity in cenopopulations of the above two sympatric rodent species from Orenburg oblast over a 30-year historical period from 1986 to 2016. It was of special interest to evaluate possible changes in morphofunctional mandibular indices characterizing trophic preferences of animals from these syntopic cenopopulations under the natural climatic conditions of the late 20th century and the second decade of the 21st century.

MATERIAL AND METHODS

The study was performed with craniological samples of bank voles (*Clethrionomys glareolus* Schreb. 1780) and pigmy wood mice (*Sylvaemus uralensis* Pall. 1811) trapped in summer (July–August) in the Southern Urals (the village of Cherny Otrog, Orenburg oblast; 51°53'59" N, 56°01'37" E). The samples were collected in 1986–1987 by A.G. Vasil'ev and I.A. Vasil'eva and in 2015–2016 by A.G. Vasil'ev and M.V. Chibiryak. The data on annual average temperatures and precipitation were obtained from the Orenburg city weather station (the nearest to Cherny Otrog).

Rodent trapping was conducted synchronously and syntopically using snap-trap lines set in a floodplain forest on the right bank of the Sakmara River. In addition to the two model species, the yellow-necked mouse (*S. flavicollis*) was common in the study area. A quantitative estimation of their ratio in catches of different years showed that both model species were codominant in the local rodent community (with the dominance of *C. glareolus* in the 1980s and of *S. uralensis* in 2015–2016). According to the classification by Tischler [17], *S. flavicollis* could be classified as a subdominant species, even though it was absent in catches made in 1987 (Fig. 1).

All voles and mice used in the study were of the same age group (male and female young of the year, a total of 204 ind.); juveniles and overwintered animals were excluded from analysis. The numbers of males and females in samples taken in different years are shown in Table 1.

Analysis of chronographic variation was performed in different ways: (1) by comparing samples of different years for each species separately; (2) by comparing the same samples in the common morphospace; (3) by pooling conspecific samples into two separate groups collected in the 20th and in the 21st century, thereby leveling off differences between successive years; and (4) by using the same variant but dividing the samples by sex.

Variation in the mandible shape was assessed by methods of geometric morphometrics [11–13, 18]. The mandible as an organ directly involved in the trophic function was an appropriate model for evaluating morphofunctional trophic features of the species in different years. Moreover, the mandible is a flat object that could be effectively analyzed by the above methods. Scanned images of the right mandibular rami were digitized at 1200 dpi with tpsUtil and tpsDig2 software [19, 20] and analyzed with respect to the arrangement of 16 landmarks characterizing variation in the mandible shape (Fig. 2). A total of 204 images were included in analysis.

The functional features of mandible configurations were assessed using the following five measurements: (A) articular–incisor; (B) articular–molar; (C) temporal–articular; (D) articular–masseter, and (E) articular–angular (Fig. 2). These measurements were used to

Table 1. Comparison of mandible centroid sizes (CS \pm SE) for male and female bank voles and pigmy wood mice from allochronous syntopic samples collected in cenopopulations of Sakmara River floodplain forest (Cherny Otrog, Orenburg oblast) in the late 20th and early 21st centuries (1986–1987 and 2015–2016)

Year, animal sex	Centroid size (number of animals)	
	<i>C. glareolus</i>	<i>S. uralensis</i>
1986	931.79 \pm 6.05 (26)	880.64 \pm 7.57 (18)
Males	931.15 \pm 7.04 (15)	878.98 \pm 10.97 (8)
Females	932.66 \pm 11.01 (11)	881.97 \pm 10.94 (10)
1987	885.83 \pm 4.59 (22)	848.87 \pm 8.77 (11)
Males	882.91 \pm 5.22 (13)	848.57 \pm 9.51 (9)
Females	890.05 \pm 8.50 (9)	849.20 (2)
2015	904.85 \pm 7.21 (23)	852.85 \pm 5.48 (28)
Males	884.55 \pm 4.94 (13)	842.18 \pm 8.86 (14)
Females	931.25 \pm 10.63 (10)	862.73 \pm 6.44 (14)
2016	917.64 \pm 4.88 (34)	837.19 \pm 4.57 (42)
Males	905.64 \pm 5.28 (17)	829.78 \pm 6.06 (20)
Females	929.65 \pm 7.23 (17)	843.92 \pm 6.55 (22)

calculate the functional mandibular indices [12]: *TI*, temporal–incisor; *TM*, temporal–molar; *MI*, masseter–incisor; *MM*, masseter–molar; and *AM*, angular–masseter indices. Calculations were made by the following formulas: $TI = C/A$; $TM = C/B$; $MI = D/A$; $MM = D/B$; and $AM = E/D$ [21, 22]. The *TI* and *TM* indices characterize the intensity of lengthwise cutting-masticating movements; the *MI* and *MM* indices, the intensity of movements related to food gnawing and crushing. The proposed *AM* index indirectly characterizes the intensity of horizontal masticatory movements involved in food grinding [22].

The configurations of the landmarks were superimposed by the method of generalized least-squares Procrustes analysis (GPA) [11] with calculation of Procrustes coordinates. The centroid size (CS) characterizing the overall size of digitized objects was cal-

culated as the square root from the sum of squared distances from the center of the image to each of the landmarks [11]. Preliminary experiments on repeated landmark placement revealed no significant bias of estimates resulting from operator error [23].

Between-group differences in the mandible shape were evaluated by canonical analysis of the calculated Procrustes coordinates. Multiple comparisons of differences in separate variables between the groups were made using one-way ANOVA. The significance of between-group differences by factors “species” (S) and “year” (Y) with regard to their interaction was estimated by two-way ANOVA. The hierarchy of between-group differences revealed by canonical analysis was evaluated by UPGMA cluster analysis of a matrix of squared Mahalanobis distances (D^2). An adequate distance metric for clustering was deter-

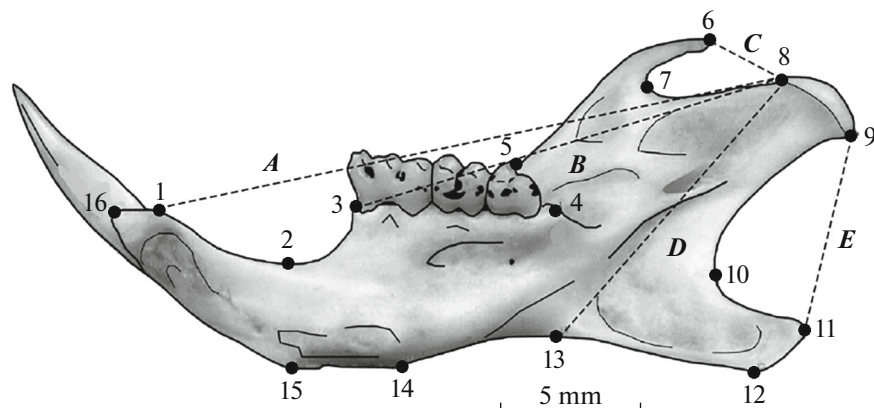


Fig. 2. Locations of 16 landmarks characterizing variation in the shape of the mandible on its lingual side and measurements (A–E) for calculating morphofunctional mandibular indices (the example of pigmy wood mouse).

Table 2. Results of one-way ANOVA for annual average temperature values over three periods (1986–1999, 2000–2010, and 2011–2016) in Cherny Otrog, Orenburg oblast

Component of variation	Sum of squares (SS)	Number of degrees of freedom (d.f.)	Mean square (MS)	F	Significance level (<i>p</i>)
Between-group	11.999	2	5.999	7.37	0.0027
Within-group	22.781	28	0.814		
Overall	34.780	30			
Effect size, ω^2	0.291				

mined based on the highest value of cophenetic correlation coefficient (R_{coph}).

The index of sexual dimorphism was calculated by the formula $ISD = [(X_f/X_m) - 1]100$, where X_f and X_m are the mean parameter values in females and males, respectively [24]. In multivariate analysis, squared Mahalanobis distances D^2 with estimated significance levels were used for this purpose.

Morphological disparity within the groups was evaluated by nearest neighbor point pattern analysis within selected convex hulls for the first two canonical variables (CV1 and CV2) [25, 26]. In accordance with this method, index R was calculated as the ratio of the observed mean nearest neighbor distance ($MNND$) between ordinates to the theoretically expected mean distance ($ExpNND$) calculated based on the Poisson distribution. This index characterizes the mode of ordinate dispersion: $R < 1$ indicates ordinate clustering; $R = 1$, Poisson dispersion; and $R > 1$, overdispersion. In the last case, an increase in $MNND$ value may be interpreted as evidence for increasing morphological disparity within groups [26] or, in terms of geometric morphometrics, for a wider “fan” of individual morphogenetic trajectories within the sample [8, 22]. The null hypothesis that the observed $MNND$ is equal to $ExpNND$ for random point scattering at the same density of ordinates within the convex hulls was evaluated using the nearest neighbor test (Z value) [25]. The method proposed by Donnelly [20] was used to eliminate the edge effect of ordinate dispersion within the convex hulls [27].

Statistical calculations, including those related to geometric morphometrics were performed with programs TPS [19, 20], MorphoJ 1.6d [18] and PAST 3.26 [28].

RESULTS

Comparison of annual average temperatures averaged over three time intervals revealed a distinct ascending trend, with this parameter increasing from $4.58 \pm 0.35^\circ\text{C}$ in 1986–1999 to $5.62 \pm 0.30^\circ\text{C}$ in 2000–2010 and to $6.09 \pm 0.21^\circ\text{C}$ in 2011–2016. One-way ANOVA confirmed that this increase is statistically significant (Table 2). The effect size index ω^2 was above average, judging from the proposed threshold values [29], indicating that the local annual average

temperature has changed significantly over the study period. Its values in two successive years of the 20th century were lower than in the early 21st century: 4.0 and 3.4°C in 1986–1987 vs. 6.2 and 6.5°C in 2015–2016, respectively; i.e., the temperature increased by approximately 2.5°C , which is direct evidence for climate warming over the past three decades. This trend is clearly seen in the plot of annual average temperature smoothed over three successive years (Fig. 3): strong fluctuations typical of the late 20th century give way to small, low-amplitude fluctuations at a higher temperature level in the second decade of the 21st century. The annual average precipitation increased over the study period by approximately 100 mm (from 348 mm in 1986 to 456 mm in 2016), but differences in its amount between the three time intervals lacked statistical significance ($p > 0.05$). Thus, the annual average temperature has risen significantly by the second decade of the 21st century, while the annual precipitation has shown only a tendency to increase.

The average mandible centroid size (CS) in both species changed from year to year in the same direction (Table 1). Spearman's rank correlation coefficient between its values in synchronous heterospecific samples proved to be fairly high and statistically significant ($R_s = 0.61$, $p = 0.036$). The average CS values in female samples were almost always higher than in parallel male samples (Table 1). A comparison of average indices of sexual dimorphism (ISD) in CS showed that these indices in both species were below 1.0% in the late 20th century (1986–1987) but increased in the second decade of the 21st century (2015–2016), varying from 1.9 to 5.7%.

Two-way ANOVA for CS in allochronous syntopic samples (1986, 1987, 2015, and 2016) confirmed statistical significance of interspecific and interannual differences in this parameter and of the interaction of factors “species” (S) and “year” (Y) (Table 3). Factor S accounted for 44.53% of the total variance; factor Y , for 11.00%; and their interaction ($S \times Y$), for only 4.37%. In other words, the effect of this interaction is weak, even though statistically significant ($p = 0.0002$). Therefore, chronographic variation in CS is largely consistent between the two species with respect to the direction of change in values.

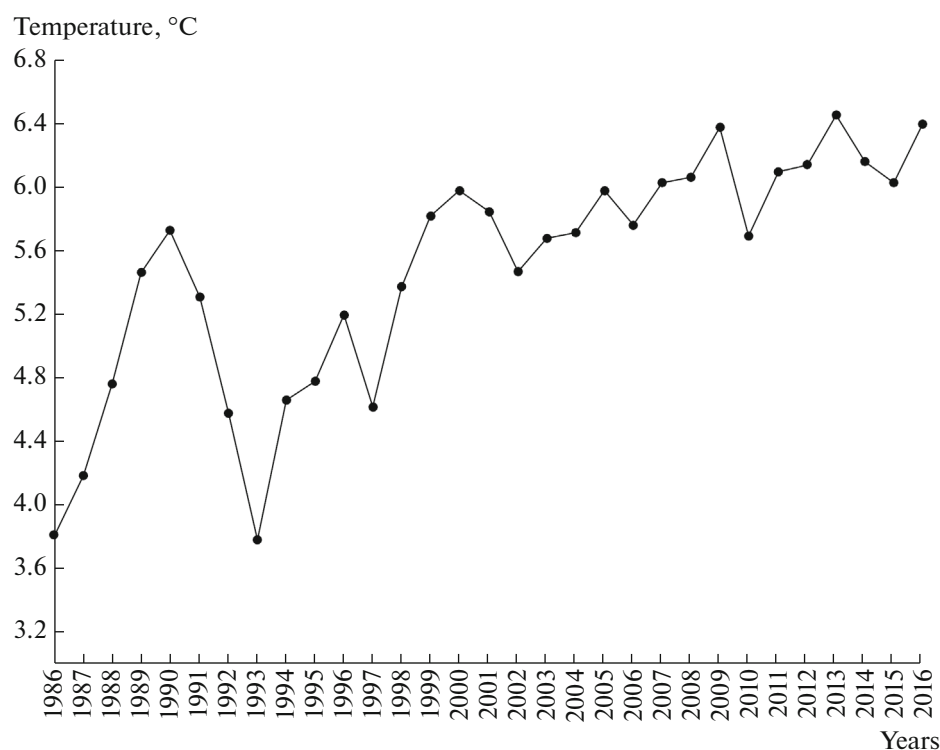


Fig. 3. Plot of annual average air temperature in the vicinity of Orenburg in 1986 to 2016 (smoothed over three successive years).

In the presence of allometry, changes in the shape of an object may be explained by changes in its size. To test for an allometric effect (as recommended in [12]), a linear regression of CS on the first principal component (PC1) of variation in mandible shape was evaluated in each sample. The permutation test showed that this regression in most samples lacked statistical significance ($p = 0.278\text{--}0.619$). The small sample of male pigmy wood mice taken in 1986 was the only exception ($p = 0.047$), which could apparently be ignored. Thus, allometry of the mandible in both species proved to be absent or insignificant and can therefore be excluded from factors responsible for the main morphogenetic transformations.

Chronographic variation in the mandible shape was preliminary evaluated in each species separately

using canonical analysis of Procrustes coordinates in samples collected in the four years. As a result, a matrix of squared Mahalanobis distances (D^2) was calculated for each species. In both cases, similar and largely parallel effects were revealed, with changes in the mandible shape being relatively slight between samples of successive years but considerable between samples more separated in time. In this regard, it was relevant to estimate proportionality of between-group chronographic differences in the mandible shape based on the pairwise D^2 matrices. Since preliminary analysis of between-group differences was performed for each species individually, these matrices did not reflect the interspecific differences themselves, unlike if all samples of both species had been analyzed simultaneously. The Mantel test for the two matrices

Table 3. Results of two-way ANOVA for mandible centroid sizes ($CS \pm SE$) for young-of-the-year bank voles and pigmy wood mice from allochronous syntopic samples collected in cenopopulations of Sakmara River floodplain forest (Cherny Otrog, Orenburg oblast) in 1986, 1987, 2015, and 2016

Factor (component) of variation	Sum of squares (SS)	Degrees of freedom (d.f.)	Mean square (MS)	F	Significance level (p)
Species (S)	190000	1	190000	217.65	<0.0001
Year (Y)	46950	3	15650	17.93	<0.0001
Interaction ($S \times Y$)	18650	3	6217	7.12	0.0002
Within-group	171100	196	873		
Overall	426700	203			

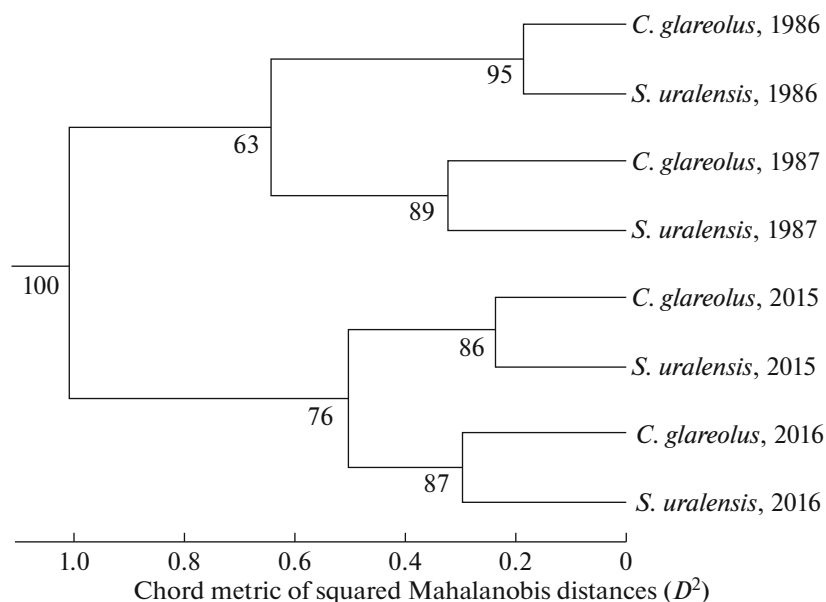


Fig. 4. Results of UPGMA cluster analysis of two matrices of squared Mahalanobis distances (D^2) between allochronous samples from syntopic bank vole (*C. glareolus*) and pigmy wood mouse (*S. uralensis*) cenopopulations based on the results of two independent canonical analyses of mandible shape in these species.

revealed a statistically significant correlation between them ($R_m = 0.47$, $p = 0.019$). Thereafter, UPGMA cluster analysis was performed for both matrices taken together to analyze the hierarchy of between-sample differences alone, excluding interspecific differences.

The results are shown in Fig. 4. Samples taken in the past and current centuries fall into two large clusters, each containing subclusters corresponding to samples taken in the two successive years (1986 and 1987 in the first and 2015 and 2016 in the second cluster). It is noteworthy that the samples of the two species are distributed in pairs over the same groups and subclusters. In other words, the clustering pattern reflects the complete parallelism of between-group variation over time in the mandible shape of the two sympatric rodent species.

For comparison, consider the results of canonical analysis of Procrustes coordinates characterizing variation in the mandible shape performed for the complete set of samples undifferentiated by sex (four samples of each species). Figure 5 shows variation ellipsoids for allochronous syntopic cenopopulations of the two species in the common morphospace, with each ellipsoid representing 95% of the within-group variance. Between-group variation along the three axes of canonical variables proved to be statistically significant. As can be seen in the figure, differences are most pronounced along the CV1 axis, which accounts for 41.3% of the total between-group variance. Chronographic differences between samples taken in the 20th and 21st centuries are clearly manifested along the CV2 axis (23.0% of the total variance). The CV3 axis (16.1% of the total variance) relates to the effect of

factor interaction “species \times year,” reflecting certain specificity in the morphogenetic reaction to living conditions from different species in different centuries. It is noteworthy that variation ellipsoids for the samples of two successive years in each species are very close to each other in the morphospace. Thus, the parallelism of between-group variation manifested itself in this case as well, but, in addition, the interaction of factors “species” and “year” was revealed, which was impossible in the previous variant where samples of different species were compared separately.

For subsequent analysis, samples of each species taken in the 20th and 21st centuries were pooled into two groups to level off the particular differences between the two successive years and evaluate the magnitude of chronographic changes in the mandible shape over 30 years. The results are shown in Table 4. Significant between-group differences along all canonical axes were revealed in this variant as well, but the first two canonical variables accounted for the greater part of the total variance. The correctness of attributing objects to their own samples was fairly high (94.6%), with the result of jackknife cross-validation test being similar (89.2%).

The CV1 axis reflects interspecific functional differences in the mandible shape and accounts for 39% of the total between-group variance. The CV2 axis reflects transformation of morphogenesis in cenopopulations over the 30-year period (almost 31% of the total variance). As in the previous variant, the effect of factor interaction “species \times year (century)” is manifested along the CV3 axis (approximately 14% of the total variance). The values and signs of centroid coor-

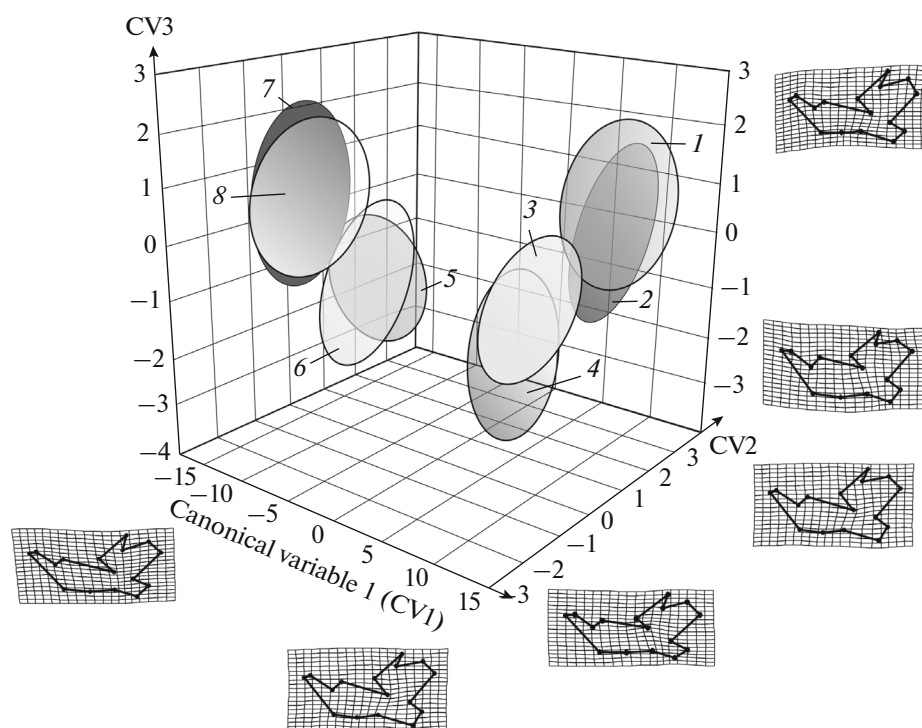


Fig. 5. Results of canonical analysis of Procrustes coordinates characterizing variation in mandible shape in allochronous syntopic samples of (1–4) bank vole and (5–8) pigmy wood mouse in the common morphospace. Variation ellipsoids cover 95% of the within-group variance, and their numbers indicate sampling years: (1, 5) 1986; (2, 6) 1987, (3, 7) 2015, (4, 8) 2016. Landmark configurations in deformation grids correspond to the extreme (maximum and minimum) values on the three canonical axes.

dinates on the first two canonical axes (Table 4) indicate the parallelism of between-group changes in the mandible shape in both species, as in the previous variants.

In the next variant, the syntopic cenopopulations of the two species were compared using pooled allochronous samples of the 20th and 21st centuries divided by sex; i.e., the canonical analysis of Procrustes coordinates was performed between eight samples. Sex-related differences in the mandible shape proved to be statistically significant in all pairwise comparisons ($p = 0.0006–0.008$), but they were greater in samples of the 20th than of the 21st century: $D^2 = 5.37$ vs. 3.53 in the bank vole and $D^2 = 8.08$ vs. 2.62 in the pigmy wood mouse. Thus, sexual dimorphism in the mandible shape decreased to a greater extent in the latter samples. As follows from the magnitude of squared Mahalanobis distances, the level of chronographic differences between the pooled samples of the 20th and 21st centuries in both species was, on average, 2.5 times higher than that of sex-related differences ($D^2 = 12.51$ vs. 4.91).

The directional and almost parallel transformation of the mandible shape over time in the two species could be accounted for by new trophic requirements consequent to climate change in the region. Therefore, it was relevant to compare allochronous samples with respect to morphofunctional mandibular indices

indirectly characterizing changes in the mode of mastication and trophic preferences of rodents.

The mean values of these indices in both species are shown in Table 5. Between-group differences in the masseter–molar and angular–masseter indices (MM and AM) are especially distinct. As follows from Fig. 6, the configurations of the mandible in both species have changed significantly over the past 30 years, reflecting modification of its functional properties and food preferences of the animals [21, 22]. The MM index in the bank vole has increased, indicating enhancement of the gnawing function, while the AM index characterizing the capacity for transverse grinding of food has decreased slightly. In the pigmy wood mouse, conversely, the latter index has increased significantly, with the former index remaining almost unchanged. Arrows in Fig. 6 indicate the directions of these changes. It can be seen that the values of the indices have gradually converged from the 20th to the 21st century, which can be interpreted as enhancement of “murine-like” features in the bank vole mandible and a tendency toward complementing the gnawing function by food grinding by transverse motion of the mandible in the pigmy wood mouse.

The data on five mandibular indices (TI , TM , MI , MM , AM) in males and females of both species in the most time-distant samples (1986 and 2016) were processed by canonical analysis, and the resulting D^2

Table 4. Results of canonical analysis of Procrustes coordinates characterizing mandible shape variation in bank voles and pigmy wood mice from pooled samples of successive years collected in cenopopulations of Sakmara River floodplain forest (Cherny Otrog, Orenburg oblast) in the late 20th and early 21st centuries

Sample	Canonical variable		
	CV1	CV2	CV3
Sample centroids ($CS \pm SE$)			
Bank vole (1986–1987)	1.544 ± 0.167	0.425 ± 0.199	0.361 ± 0.184
Bank vole (2015–2016)	0.798 ± 0.148	0.234 ± 0.130	-0.497 ± 0.150
Pigmy wood mouse (1986–1987)	-0.176 ± 0.154	-1.045 ± 0.143	-0.246 ± 0.149
Pigmy wood mouse (2015–2016)	-0.378 ± 0.135	-0.807 ± 0.126	0.371 ± 0.150
Results of canonical analysis			
Wilks' Λ	0.0930	0.2137	0.4322
Eigenvalue	1.2981	1.0220	0.4692
Canonical correlation	0.75	0.71	0.57
Proportion of variance, %	39.28	30.92	14.20
χ^2	533.22	346.41	188.34
Number of degrees of freedom (d.f.)	205	160	117
Significance level (p)	<0.0001	<0.0001	0.00003

Table 5. Mean values (with standard errors) of morphofunctional mandibular indices in allochronous syntopic samples of bank voles and pigmy wood mice from cenopopulations of Sakmara River floodplain forest (Cherny Otrog, Orenburg oblast)

Year (number of animals)	Morphofunctional mandibular indices				
	$TI \pm SE$	$TM \pm SE$	$MI \pm SE$	$MM \pm SE$	$AM \pm SE$
Bank vole					
1986 (26)	0.205 ± 0.004	0.262 ± 0.005	0.515 ± 0.004	0.660 ± 0.005	0.607 ± 0.009
1987 (22)	0.212 ± 0.006	0.269 ± 0.007	0.542 ± 0.003	0.689 ± 0.004	0.609 ± 0.006
2015 (23)	0.217 ± 0.004	0.280 ± 0.006	0.532 ± 0.003	0.687 ± 0.004	0.597 ± 0.005
2016 (34)	0.213 ± 0.004	0.273 ± 0.004	0.550 ± 0.002	0.704 ± 0.002	0.585 ± 0.005
Pigmy wood mouse					
1986 (18)	0.171 ± 0.005	0.237 ± 0.007	0.567 ± 0.003	0.785 ± 0.004	0.542 ± 0.006
1987 (8)	0.157 ± 0.006	0.220 ± 0.009	0.568 ± 0.006	0.792 ± 0.007	0.558 ± 0.007
2015 (28)	0.160 ± 0.005	0.222 ± 0.007	0.569 ± 0.003	0.788 ± 0.004	0.562 ± 0.005
2016 (42)	0.164 ± 0.004	0.229 ± 0.005	0.559 ± 0.003	0.780 ± 0.003	0.571 ± 0.004

matrix was subjected to UPGMA clustering. The results are shown in Fig. 7. The samples of bank voles and pigmy wood mice fall into two different clusters (not surprisingly, since the configuration of the mandible a priori differ between the species). The bank vole cluster is divided into two subclusters, each comprising males and females sampled in the same year (1986 or 2016), whereas in the pigmy wood mouse each subcluster comprises either males or females sampled in different years. Thus, chronographic morphofunctional differences in the bank vole mandible shape are expressed more strongly than sex-related differences. In the pigmy wood mouse, conversely,

sexual dimorphism in mandibular indices manifests itself to a greater extent than chronographic changes.

Finally, consider changes in the parameter of within-group morphological disparity ($MNND$) in pooled samples of the late 20th and early 21st centuries from bank vole and pigmy wood mouse cenopopulations (Table 6). In both species, parameter $MNND$ in the 20th century was significantly higher than in the 21st century; index R exceeded 1.0, with the Z value indicating that these effects were statistically significant (i.e., ordinate overdispersion was observed). This index in samples of the 21st century was close to 1.0, and the corresponding effects lacked statistical significance according to the Z value, which was evidence

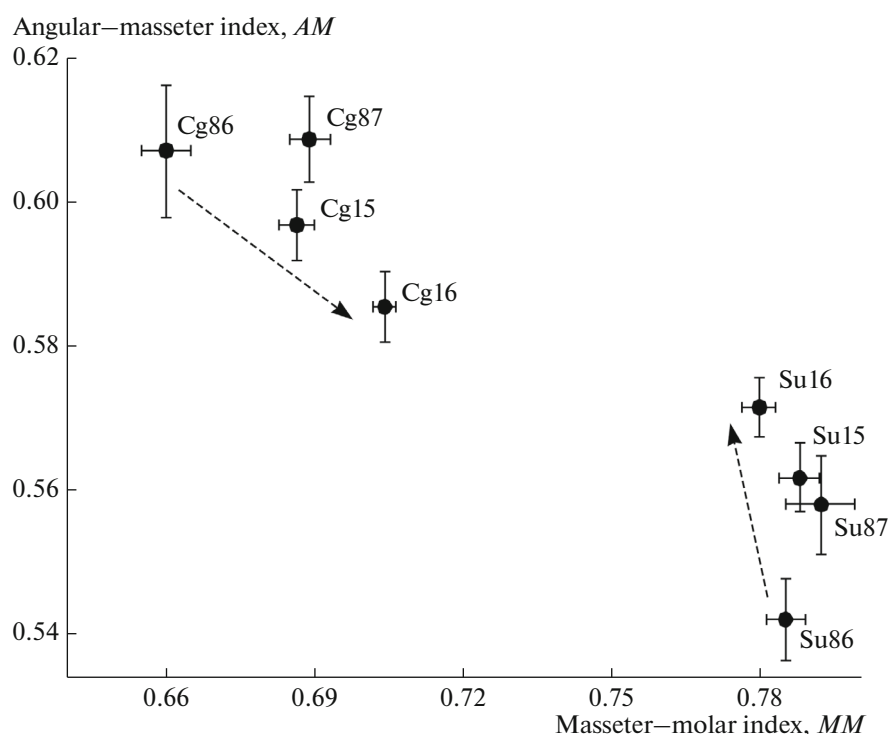


Fig. 6. Chronographic changes in the mean values of masseter–molar (MM) and angular–masseter (AM) morphofunctional mandibular indices in allochronous samples from bank vole (Cg) and pigmy wood mouse (Su) cenopopulations taken in 1986, 1987, 2015, and 2016 (indicated by the last two digits). Error bars show standard error of the mean.

for a random pattern of ordinate scattering in both species.

DISCUSSION

Due to climate change, annual average temperatures in the study region have risen by almost 2.5°C , which could lead to certain changes in the composition and productivity of vegetation and, hence, have a direct or indirect effect on trophic resources for rodents, including the abundance of food invertebrates and availability of biotopes suitable for them. Such changes in forage resources for the bank vole and pigmy wood mouse could be responsible for morphogenetic transformations revealed in these species over the 30-year period.

The abundance and proportion of the bank vole in the rodent community of the study region have decreased significantly by the second decade of the 21st century, and the pigmy wood mouse (formerly subdominant) has become a dominant species. Shannon's diversity index (H) in the rodent community has increased significantly, from 0.650 in the 20th to 0.880 in the 21st century ($t = 4.31$; $p = 0.000021$), with Simpson's dominance index (D) decreasing from 0.624 to 0.449 ($p < 0.001$) and Margalef's species richness index (Mg) remaining almost unchanged (0.362 vs. 0.327, $p = 0.999$).

A general tendency toward sexual dimorphism in the mandible has been revealed in both species, with its size being greater in females than in males. The index of sexual dimorphism (ISD) in the mandible

Table 6. Comparison of empirical parameters of within-group morphological disparity ($MNND$) and their theoretically expected values ($ExpNND$) between pooled samples of the late 20th and early 21st centuries (1986–1987 and 2015–2016) from syntopic bank vole (*C. glareolus*) and pigmy wood mouse (*S. uralensis*) cenopopulations (Sakmara River floodplain forest, Cherny Otrog, Orenburg oblast)

Sample	$MNND \pm SE$	$ExpNND \pm SE$	R (number of animals)	Z	Significance level (p)
<i>C. glareolus</i> , 20th century	0.379 ± 0.039	0.313 ± 0.022	1.21 (49)	2.56	0.0105
<i>C. glareolus</i> , 21st century	0.227 ± 0.027	0.216 ± 0.014	1.04 (56)	0.52	0.6028
<i>S. uralensis</i> , 20th century	0.471 ± 0.049	0.344 ± 0.030	1.37 (29)	3.43	0.0006
<i>S. uralensis</i> , 20st century	0.234 ± 0.019	0.230 ± 0.014	1.02 (69)	0.27	0.7892

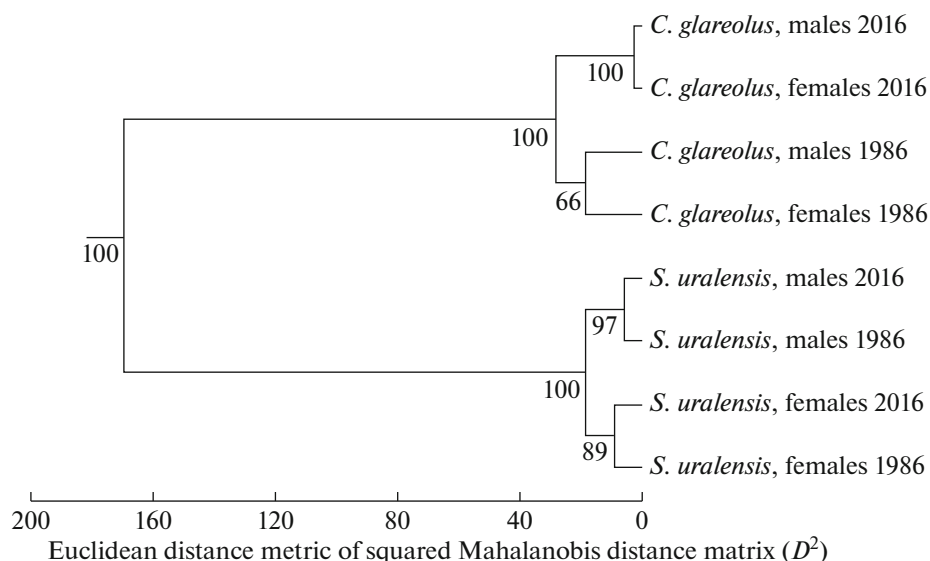


Fig. 7. Results of UPGMA cluster analysis of squared Mahalanobis distance matrix (D^2) based on Euclidean distance between the extreme allochronous samples (1986, 2016) of males and females from syntopic bank vole and pigmy wood mouse cenopopulations according to the results of canonical analysis of the values of morphofunctional mandibular indices (TI , TM , MI , MM , AM).

centroid size (CS) has increased severalfold by the 21st century, while sex-related differences in the mandible shape have decreased slightly in samples of both species. Compared to the bank vole, sexual dimorphism in the mandible shape in the pigmy wood mouse was higher in the 20th century but has become lower by the 21st century. As shown in our previous studies [22, 30], sex-related differences in the mandible shape in the bank vole as a dominant species become more pronounced in unfavorable years, at generally low population density and relatively low abundance of other species in the rodent community.

The pigmy wood mouse has increased in abundance and become a dominant species by the 21st century. However, this increase has been accompanied by a slight decrease in body size (judging from CS), indirectly indicating growth suppression due probably to certain deterioration of traditional trophic conditions for the species, compared to those in the 20th century. Moreover, the pigmy wood mouse at the phase of high population abundance may face increasing competition for resources from larger rodents such as the yellow-necked mouse and bank vole. It is worth reminding that the values of mandibular indices in the bank vole provide evidence for the enhancement of murine-like features and morphofunctional properties of the mandible, due to which this species can in part utilize food resources characteristic of mice. This especially applies to the increase in MM index (see Fig. 6), i.e., enhancement of the ability to gnaw and crush food, as mice do. Intensification of interspecific competition in the rodent community of the Sakmara river floodplain forest may also be due to the fact that oak grove in this forest were almost completely destroyed by fires

at the turn of the 20th and 21st centuries, and acorns—previously a major food source for all rodent species—became no longer available. Oak (*Quercus robur*) has been replaced by invasive ash-leaved maple (*Acer negundo*), and ash-leaved maple and caragana (*Caragana arborescens*) seeds and blackberries currently serve as the main seasonal food for dominant rodent species. Therefore, it can be hypothesized that modifications of mandibular morphogenesis in the bank vole and pigmy wood mouse have occurred in order to alleviate possible inter- and intraspecific competition caused by restriction of traditional food resources, with consequent changes in the shape of the mandible providing for the improvement of its morphofunctional properties.

Anderson et al. [21] have obtained experimental evidence that rapid morphofunctional modification of the mandible in rodents can occur upon long-term feeding of young rodents with soft or hard food. Therefore, relatively rapid shifts in the program of mandibular morphogenesis followed by their fixation in subsequent generations are quite possible.

Studies of recent years have demonstrated the possibility of rapid microevolutionary changes occurring within historical (rather than geological) periods of time based on stress-induced epigenetic rearrangements (changes in DNA methylation pattern, transposition of mobile genetic elements, etc.) that are closely associated with certain shifts in morphogenesis and can be inherited in a series of generations [5, 31–33]. Therefore, the basis for the observed rapid modifications of mandibular morphogenesis may be found in epigenetic changes fixed as a result of selection.

Shifts in the species ratio toward complete dominance of the pigmy wood mouse and reduced proportion of the bank vole in the rodent community provide indirect evidence for aridization and xerophytization of their habitats, with living conditions becoming more favorable for mice than for voles. Nevertheless, relatively high parameters of within-group morphological disparity (*MNND*) and statistically significant effects of nonrandom ordinate overdispersion observed in both species at the end of the 20th century are indicative of stress load on morphogenesis and symptoms of developmental instability. The level of developmental instability during this period was higher in the pigmy wood mouse, because a significant excess of parameter *MNND* over its expected value *ExpMNND* ($t = 2.24$; $p = 0.027$) was observed only in this species. The transition from the 20th to the 21st century was accompanied by a significant decrease in morphological disparity (*MNND*), and within-group ordinate dispersion acquired a random pattern. The latter indicates alleviation of stress load on morphogenesis and increasing developmental stability in cenopopulations of both species.

The range of shifts in morphogenesis revealed by comparing pooled samples taken at the end of the 20th and in the second decade of the 21st century is fairly broad, and the chronographic variance of the mandible shape within groups is almost comparable with that between species (Table 4). This is evidence for a considerable transformation of morphogenesis in both species over the above period. Chronographic changes are more distinct in the bank vole than in the pigmy wood mouse. Apparently, the new ecological conditions that have developed to date in the study region are more favorable for the latter species than those in the 20th century. Morphofunctional modifications of the mandible in the pigmy wood mouse are also expressed to a lesser extent. Nevertheless, morphofunctional differences of the mandible between males and females (see Fig. 7) provide the possibility for this species to broaden the spectrum of available trophic resources, with males and females acting as “instrumental morphs” that can accomplish primary mechanical break up of food in different ways using the musculoskeletal system of the mandible as a tool. Changes in the morphofunctional mandibular indices of the bank vole (Fig. 6) indicate that modifications of the mandible shape broadening the trophic spectrum of this species are common to males and females.

It is of special interest to compare the results of cluster analysis of squared Mahalanobis distance matrices (D^2) calculated for each species independently. They provide evidence for high congruence of morphogenetic transformations that have occurred in bank vole and pigmy wood mouse cenopopulations over the historical time interval from the late 20th to the second decade of the 21st century. The almost complete parallelism of changes in the mandible shape between the species is indicative of their high coevolu-

tionary potential [9]. As follows from the results of cluster analysis, inevitable interspecific competition is reduced to a minimum in this case, since obvious signs of increasing developmental stability have been observed in samples of the 21st century, after parallel morphogenetic transformations in cenopopulations of both species.

Climatic parameters in the 21st century have stabilized at a new level (Fig. 3). Under these conditions, adaptive morphofunctional modifications of the mandible have probably been fixed in bank vole and pigmy wood mouse cenopopulations, providing for minimization of probable competitive relationships both within the species (due to sexual dimorphism in mandible size and changes in the shape and proportions of the mandible related to its functional properties) and between the species (Table 4). Therefore, despite transformation of the environment under the impact of climatic and anthropogenic factors (fires, recreation, felling operations, the establishment of a forest hunting reserve), both species show a high level of adaptation (and coadaptation) to the changing environmental conditions of the 21st century.

CONCLUSIONS

The results of this study confirm the possibility of rapid transformations of morphogenesis in sympatric rodent species over a 30-year historical period. The living environment for the model codominant species—the bank vole and pigmy wood mouse—has changed during this period against the background of shifts in climatic parameters (primarily annual average temperatures) and consequent transformation of vegetation structure and productivity. Analysis of both species has revealed almost parallel modifications in the shape of the mandible, the organ that plays a major instrumental and functional role in the trophic provision of the animals. However, species-specific changes in the morphofunctional mandibular indices have led to certain convergence of their values between the species. The result of this process in the bank vole may be defined as the development of murine-like features in the mandible structure, which probably allows this species to broaden the spectrum of available trophic resources and modes of mechanical break up of food objects. Rapid morphogenetic transformations that have provided for almost parallel modification of mandible structure and shape in both species during a relatively short period of time (30 years) are evidence for their high coevolutionary potential [9]. Approximately 90 generations of rodents have passed during this period, which appears to be sufficient for fixation of morphogenetic transformations.

Thus, the proposed ecomorphological approach to the analysis of long-term collection material with the use of geometric morphometrics makes it possible to reveal transformations of morphogenesis and consequent morphofunctional changes occurring in ceno-

populations of model sympatric species during relatively short historical periods and also to estimate developmental stability and evolutionary-ecological status of cenopopulations in different years. This approach appears to be promising for regional ecological monitoring of populations and communities.

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COMPLIANCE WITH ETHICAL STANDARDS

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest. The authors declare that they have no conflict of interest.

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