

## The Ratio between Chronographic and Geographic Variation in the Morphofunctional Features of Bank Vole (*Clethrionomys glareolus* Shreb.) in the South of Its Range

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**Abstract**—A simultaneous analysis of the chronographic variation of the mandible of bank vole (*Clethrionomys glareolus* Shreb.) in three longitudinally distant populations that inhabit floodplain forests of Sakmara and Samara rivers in the south of the species range (Orenburg oblast) has revealed significant morphogenetic and morphofunctional changes in each of these populations over 33 years. Changes in the shape of the mandible in allochronous vole samples (1983 and 2015) have been detected using methods of geometric morphometrics. Morphofunctional rearrangements have been assessed based on mandibular indices, which characterize differences in the structure of the mandible resulting from changes in functional loads after a shift in diet. Significant changes in the mandible shape and in the values of these indices have occurred in all allochronous populations. Chronographic variation in the mandible shape in all populations has similar direction in the morphospace; its range exceeds that of geographic variation and reflects a significant historical rearrangement of morphogenesis. The authors discuss the probable relationship of rapid morphogenetic and morphofunctional changes in vole mandibles to the transformation of local trophic conditions caused by different directions of plant successions, which, in turn, are related to local trends of climate changes.

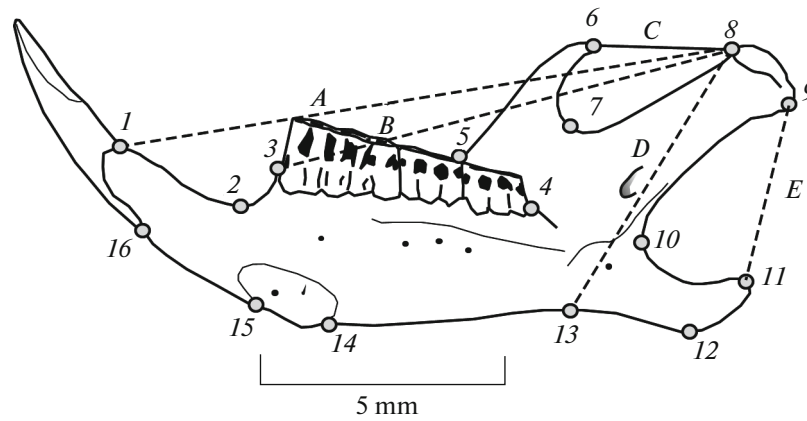
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The problem of the ratio between the chronographic and geographic variation in the assessment of historical processes in animal populations has traditionally been of particular interest to ecologists and evolutionists [1–10]. It is usually assumed that the range of geographic differences between populations is higher than that of chronographic changes in each of them. However, the assessment of changes in a population over time often covers no more than three successive years. As a rule, chronographic variation is not analyzed when long historical periods are considered. Evaluation of the ratio between the geographic and chronographic forms of variation based on parallel collections of syntopic allochronous samples over a historically long period is of particular evolutionary-ecological interest, since it is more probable to reveal the direct or indirect morphogenetic response to the effect of climate changes over historical periods of more than two–three decades than over two–three successive years. Climate trends lead to the acceleration of succession processes and influence their direction [11], which, in turn, leads changes in diet habits of first-order consumers, thereby influencing their morphofunctional features [12]. For instance, changes in

the composition of vegetation may influence the morphology of functional structures in rodents, i.e., the shape and size of their mandible and cheek teeth, prognathism of their incisors, and also the proportions of their axial skull parts [13–15]. Cases of directional changes in the shape of the mandible in rodents due to their feeding on products with different mechanical compositions have been recorded in recent years [12, 14, 16]. Parallel long-term chronogeographic analysis of variation makes it possible to assess temporally distant morphogenetic and morphofunctional changes in model morphological structures using methods of geometric morphometrics [15, 17–19].

The purpose of this study was to compare the range and direction of the chronographic and geographical forms of variation in the morphofunctional features of the same geographically distant bank vole populations at the southern boundary of their range over a historical period of more than 30 years (1983–2015). Particular attention was given to the evolutionary-ecological analysis of rapid morphogenetic and morphofunctional historical changes in the mandible in two structural and functional intrapopulation groups: mature male and female young of the year.



**Fig. 1.** Scheme of bank vole mandibular ramus (lingual side) with locations of landmarks (1–16) and measurements (A–E) necessary for calculating mandibular functional indices: TI, TM, MI, MM, and AM (formulas for their calculation see in the text).

## MATERIAL AND METHODS

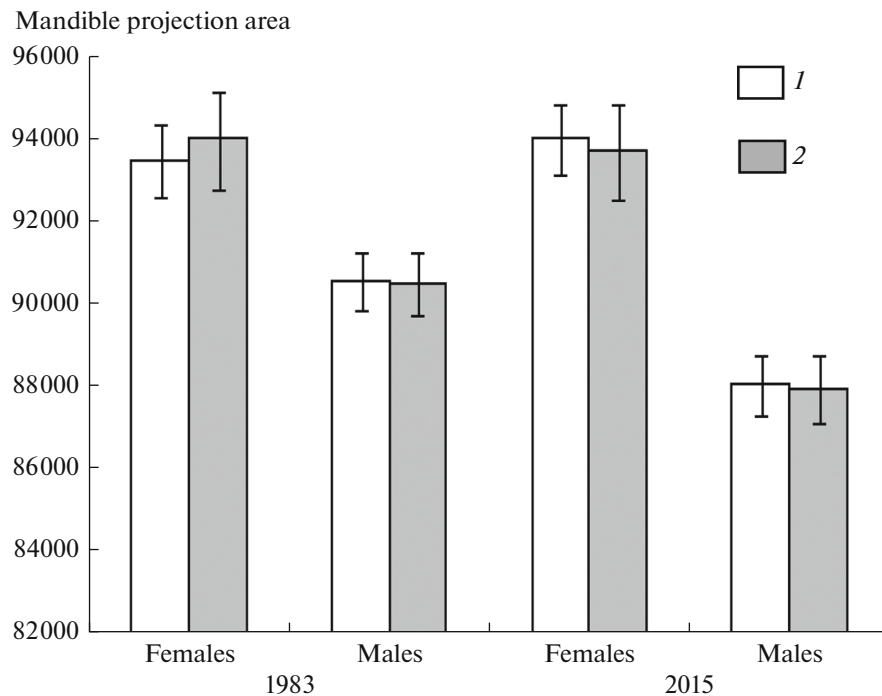
The material for the study was collected by the authors in 1983 and 2015 and kept in the Zoological Museum of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences. It consisted of samples taken in three localities of Orenburg oblast: (1) the village of Totskoe (52°31'54" N, 52°47'16" E), (2) the village of Platovka (51°56'09" N, 54°00'45" E), and (3) the city of Kuvandyk (51°28'50" N, 57°28'25" E). Sampling was carried out in the second half of summer in biotopes with similar conditions: floodplain forests of the Samara River (Totskoe and Platovka populations) and Sakmara River (Sakmara population). These sites are spatially arranged in a line running west to east: the Totskoe and Sakmara populations are in the end positions, and the Platovka population occupies an intermediate position, being geographically closer to the Totskoe population. Rodents were caught with lines of wooden snap traps exposed for 5 days. A comparative analysis was performed using syntopic samples of mature young-of-the-year bank voles of both sexes, including a total of 97 ind. collected in 1983 and 103 ind. in 2015 (35 and 39 ind. from the Totskoe population, 35 and 23 ind. from the Platovka population, and 27 and 41 ind. from the Sakmara population, respectively).

The object of the study was the mandible as an ecologically important organ that is directly connected with foraging, feeding, and the cenotic role of the species. Variation in the mandible shape was studied by methods of geometric morphometrics [15, 17–19] in scanned images of the right mandibular rami. Images of the mandible from the lingual side were digitized at 1200 dpi with tpsUtil and tpsDig2 software [20, 21]. The arrangement of 16 homologous landmarks was analyzed to describe variation in the mandible shape (Fig. 1). Variation in the mandible size was assessed based on the size of the mandibular ramus area (*SM*) within the contour drawn through the landmarks,

which was calculated using tpsUtil [20]. This indicator was chosen because the centroid size (CS), which is commonly used for indirect assessment, depends not only on the overall size of the object but also on the degree of indentation of its contour [18]. The configurations of the landmarks were superimposed by the method of generalized least-squares Procrustes analysis (GPA) [22], with calculation of Procrustes coordinates reflecting variation in shape. The methods of geometric morphometrics make it possible to analyze of variation in the shape of digitized objects excluding the influence of variation in their size and provide the possibility of morphogenetic interpretation of the observed differences [15, 18, 19].

Between-group differences in the mandible shape were assessed using canonical analysis of Procrustes coordinates. Two-way nonparametric multivariate analysis of variance (NPMANOVA) with 10 000 permutations [23] was used to estimate the significance of intergroup differences depending on factors “year” (Y) and “locality” (L) based on the values of all canonical variables.

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. 1). These measurements were used to 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 [12]:  $TI = C/A$ ;  $TM = C/B$ ;  $MI = D/A$ ;  $MM = D/B$ ; and  $AM = E/D$ . The TI and TM indices characterize the intensity of longitudinal 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 transversal masticatory movements involved in food grinding [24]. Multiple comparisons were made using one-way



**Fig. 2.** Comparison of the mandible lingual projection area in male and female bank voles (mean  $\pm$  SE) in combined samples of 1983 and 2015 based on (1) actual samples and (2) minimum random samples after rarefaction (2).

ANOVA. Between-sample pairwise differences were evaluated by Tukey's post hoc Q-test.

The levels of within-group morphofunctional disparity were estimated by analyzing the pattern of distances between the ordinates of mean values of the indices within the annual variation polygon. To this end, the mean distances between the ordinates (the measure of morphofunctional disparity, *MMFD*) were calculated, taking into account their standard errors [25]. The significance of *MMFD* differences between samples was estimated by the unequal variance *t*-test. The main calculations and statistical analysis of the material were performed in TPS [20, 21], PAST 2.17c [26], and MorphoJ 1.6d [19].

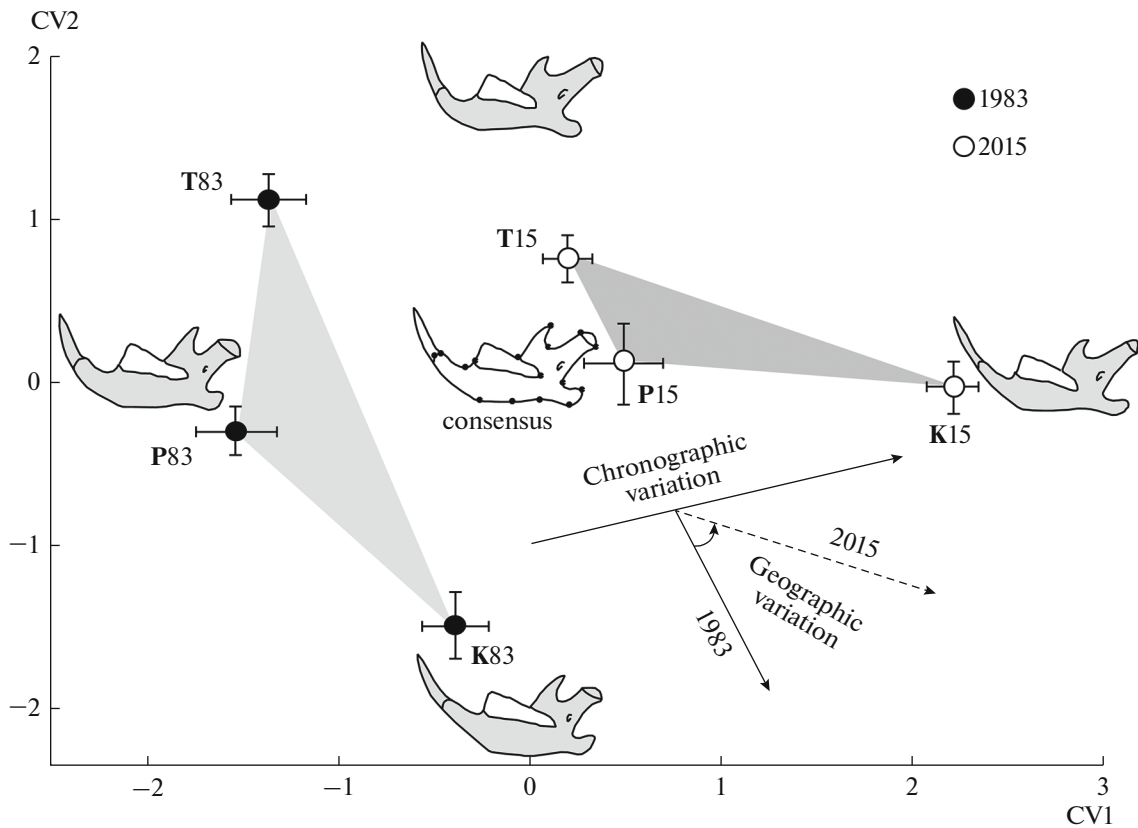
## RESULTS

Comparison of the area of the lingual projection of mandibular rami (*SM*) between male and female samples revealed similar sex-related differences both in 1983 and in 2015 (Fig. 2). Comparisons were made using the actual sample sizes and the sizes standardized by a random rarefaction procedure, and the results proved to be almost identical in both variants. The statistical significance of differences in the mean *SM* value between samples was estimated using Tukey's post hoc test. In both observation years, these values (and the habitual mandible size) were significantly higher in females than in males ( $Q = 3.66$ ,  $p = 0.041$  in 1983;  $Q = 7.47$ ,  $p \leq 0.0001$  in 2015). In other words, sexual dimorphism in the mandible size was

observed in the populations at both time points (3.2% in 1983 and 6.6% in 2015). One-way ANOVA revealed significant differences in the *SM* value ( $F = 12.21$ ;  $d.f._1 = 3$ ;  $d.f._2 = 196$ ;  $p \leq 0.0001$ ), and the Welch F-test confirmed this result ( $F = 12.16$ ;  $d.f. = 108.5$ ;  $p \leq 0.0001$ ). Cohen's  $f^2$  measure of effect size ( $f^2 = 0.17$ ) exceeded the medium level [27, 28]; i.e., the revealed sex differences in size are substantial.

Comparison of the mandible area in sex-combined samples from the three geographically distant syntopic populations in different years also revealed significant differences (one-way ANOVA:  $F = 3.28$ ;  $d.f._1 = 5$ ;  $d.f._2 = 194$ ;  $p = 0.007$ ); however, Cohen's  $f^2$  in this case was very low ( $f^2 = 0.06$ ), being indicative of a small effect size. Significant differences were revealed only in 2015 when comparing the most geographically distant Totskoe and Sakmara samples ( $Q = 4.16$ ;  $p = 0.038$ ). Geographic differences in the mandible size within each time point were not revealed and can be neglected in further analysis.

Canonical analysis of Procrustes coordinates characterizing variation in shape showed that the configuration of the mandible in syntopic populations differed significantly between 1983 and 2015 (Fig. 3, Table 1). The between-group differences were statistically significant along all canonical axes. The first three axes accounted for 81.6% of the total variance, allowing fairly complete characterization of between-group variation in the mandible shape. Figure 3 shows sample centroids with standard errors ( $\pm$ SE) along each



**Fig. 3.** Results of canonical analysis of Procrustes coordinates characterizing chronographic and geographic between-group variation in mandible shape in bank voles from allochronous (83 – 1983; 15 – 2015) syntopic populations: (T) Totskoe, (P) Platovka, (K) Sakmara.

Sample centroids (with standard errors) are combined into variation polygons for each year. The outlines of mandibles correspond to the extreme values of the respective canonical axes. Consensus configuration is shown in the center. Arrows indicate the directions of chronographic and geographic variation in the general morphospace.

canonical axis. It can be seen that intergroup differences between samples from different temporal stages (chronographic variation) are manifested along the first canonical axis, CV1 (49.01% of the total variance). The polygon with samples of 2015 is displaced to the right of the polygon that combines samples of 1983. The comparison of mandible outlines along the first axis with the consensus configuration (in the center) suggests that the gracility of the mandible increased from the first time point (1983) to the present (2015) and that this increase was accompanied by the extension of the angular process, reduction of the articular process head and its posterior displacement, and shortening of the coronoid process.

The geographic variation of population samples (from the west to the east) manifested itself along the second canonical axis, CV2 (20.09% of the total variance). The geographic variation in the mandible shape is most clearly observed between samples of 1983 (from the westernmost Totskoe population to the easternmost Sakmara population). It is expressed in the relative shortening of the angular process, extension of the coronoid process, and forward displacement of the

articular process head against the background of the general increase in the mandibular height and relative tooth row length. In samples of 2015, the direction of the geographic variation in the mandible shape changed in the general morphospace and became considerably more similar to the direction of the chronographic variation.

Two-way nonparametric multivariate analysis of variance (PERMANOVA) for all five canonical variables estimated the significance of the influence of the year (Y) and locality (L) factors and their interaction (Y × L) on the between-group variation in mandible shape in allochronous syntopic bank vole populations (Table 2). The influence of both factors and their interaction proved to be highly significant ( $p < 0.0001$ ). The similar two-way PERMANOVA that took into account the year (Y) and sex (S) factors yielded a similar result; however, the level of significance of the sex factor was lower in this case ( $p = 0.024$ ), with the interaction between the year (Y) and sex (S) factors lacking statistical significance ( $p = 0.122$ ).

Changes in the configuration of mandibles are inevitably related to the modification of their func-

**Table 1.** Results of canonical analysis of Procrustes coordinates of the mandible and values of centroids of three syntopic bank vole populations (mean  $\pm$  SE) in 1983 and 2015

Names of samples and assessment of the significance of between-group differences	Canonical variables		
	CV1	CV2	CV3
Totskoe population, 1983	-1.373 $\pm$ 0.194	1.113 $\pm$ 0.159	0.690 $\pm$ 0.190
Platovka population, 1983	-1.541 $\pm$ 0.213	-0.297 $\pm$ 0.146	-1.084 $\pm$ 0.146
Sakmara population, 1983	-0.396 $\pm$ 0.172	-1.487 $\pm$ 0.203	0.598 $\pm$ 0.164
Totskoe population, 2015	0.191 $\pm$ 0.134	0.754 $\pm$ 0.143	-0.107 $\pm$ 0.147
Platovka population, 2015	0.482 $\pm$ 0.206	0.115 $\pm$ 0.248	0.754 $\pm$ 0.271
Sakmara population, 2015	2.204 $\pm$ 0.136	-0.030 $\pm$ 0.158	-0.374 $\pm$ 0.162
Eigenvalues	1.731	0.709	0.441
Wilks' $\Lambda$	0.0847	0.2314	0.3955
Number of degrees of freedom	140	108	78
Proportion of variance, %	49.01	20.09	12.48
Significance level, $p$	<0.0001	<0.0001	<0.0001

**Table 2.** Results of two-way nonparametric multivariate analysis of variance (PERMANOVA) for canonical variables characterizing variation of mandible shape in three geographically distant allochronous (1983 and 2015) bank vole populations in Orenburg oblast

Source of variation (factor)	Sum of squares	d.f.	Mean square	F	Significance level ( $p$ )
Year (Y)	261.40	1	261.400	49.762	0.0001
Locality (L)	264.01	2	132.000	25.129	0.0001
Interaction (Y $\times$ L)	121.99	2	60.994	11.611	0.0001
Residual	1019.10	194	5.253		
Total	1666.50	199			

tional load and reflected in the values of mandibular indices. The mean values of the indices were preliminarily estimated in sex-combined samples from the compared populations (Table 3). The pairwise comparison of the values of the indices by different years and different localities within each year revealed several significant differences. With respect to the temporal–incisor index (TI), all three populations are characterized by significant interannual (chronographic) changes; however, while this index decreases in the Totskoe population between 1983 and 2015 (i.e., the masticatory function weakens), it increases in the Platovka and Sakmara populations, reflecting the enhancement of the masticatory function. The Sakmara population is also characterized by a significant increase in the angular–masseter (AM) index characterizing the intensity of lateral grinding and chewing movements. A significant increase in the masseter–incisor (MI) and masseter–molar (MM) indices between 1983 and 2015 is observed in the Platovka population; i.e., the level of food grinding movements of the mandible increases against the background of the general increase in the degree of vegetation xeromorphy in the locality. The values of the indices also show clear differences between different

populations both in 1983 and 2015. It is noteworthy that the indices that characterize the masticatory function, TI (temporal–incisor index) and TM (temporal–molar index), vary in the opposite direction in geographically most distant populations, i.e., the Totskoe and Sakmara populations, in different years: the indices are significantly higher for the Totskoe population than for the Sakmara population in 1983 and significantly lower for the former than for the latter in 2015. In other words, opposite processes of functional changes in the structure of mandible and their use are observed for the animals of these populations.

Two-way nonparametric multivariate analysis of variance (NPMANOVA) for the value of mandibular indices (Table 4) revealed a significant influence of the year (Y) and locality (L) factors and their interaction (Y  $\times$  L). In this case, the highest intergroup variance was found between populations, while the lowest is related to interannual differences in the indices. The intermediate value of the variance was observed for the interaction of factors; i.e., significant (sometimes, opposite) changes in the values of mandibular indices can be observed in different populations in different years.

The values of the indices were also compared taking into account animal sex. Figure 4 outlines the

**Table 3.** Mandibular indices (mean  $\pm$  SE) characterizing morphofunctional properties of the lower mandible in three allochronous populations of bank vole at two temporally distant stages (1983 and 2015) in Orenburg oblast (data combined by sex)

Population, year	Number of animals	Morphofunctional mandibular indices ( $\pm$ SE)				
		TI	TM	MI	MM	
1983:						
Totskoe	27	0.233 $\pm$ 0.004	0.299 $\pm$ 0.006	0.549 $\pm$ 0.004	0.704 $\pm$ 0.005	0.591 $\pm$ 0.005
Platovka	35	0.207 $\pm$ 0.004	0.267 $\pm$ 0.005	0.530 $\pm$ 0.003	0.684 $\pm$ 0.004	0.590 $\pm$ 0.005
Sakmara	35	0.213 $\pm$ 0.004	0.274 $\pm$ 0.006	0.538 $\pm$ 0.003	0.691 $\pm$ 0.004	0.583 $\pm$ 0.005
2015:						
Totskoe	41	0.219 $\pm$ 0.003	0.281 $\pm$ 0.003	0.543 $\pm$ 0.002	0.696 $\pm$ 0.003	0.592 $\pm$ 0.005
Platovka	23	0.222 $\pm$ 0.006	0.284 $\pm$ 0.007	0.545 $\pm$ 0.003	0.694 $\pm$ 0.004	0.587 $\pm$ 0.004
Sakmara	39	0.232 $\pm$ 0.003	0.296 $\pm$ 0.004	0.537 $\pm$ 0.003	0.686 $\pm$ 0.003	0.603 $\pm$ 0.005

**Table 4.** Results of two-way nonparametric multivariate analysis of variance (PERMANOVA) for values of mandibular indices characterizing functional features of the lower mandible in three geographically distant allochronous (1983 and 2015) syntopic populations of bank vole in Orenburg oblast

Source of variation (factor)	Sum of squares	d.f.	Mean square	F	Level of significance ( <i>p</i> )
Year (Y)	0.00980	1	0.00980	3.354	0.0191
Locality (L)	0.02108	2	0.01054	3.608	0.0022
Interaction (Y $\times$ L)	0.01723	2	0.00862	2.950	0.0001
Residual	0.56662	194	0.00292		
Total	0.61473	199			

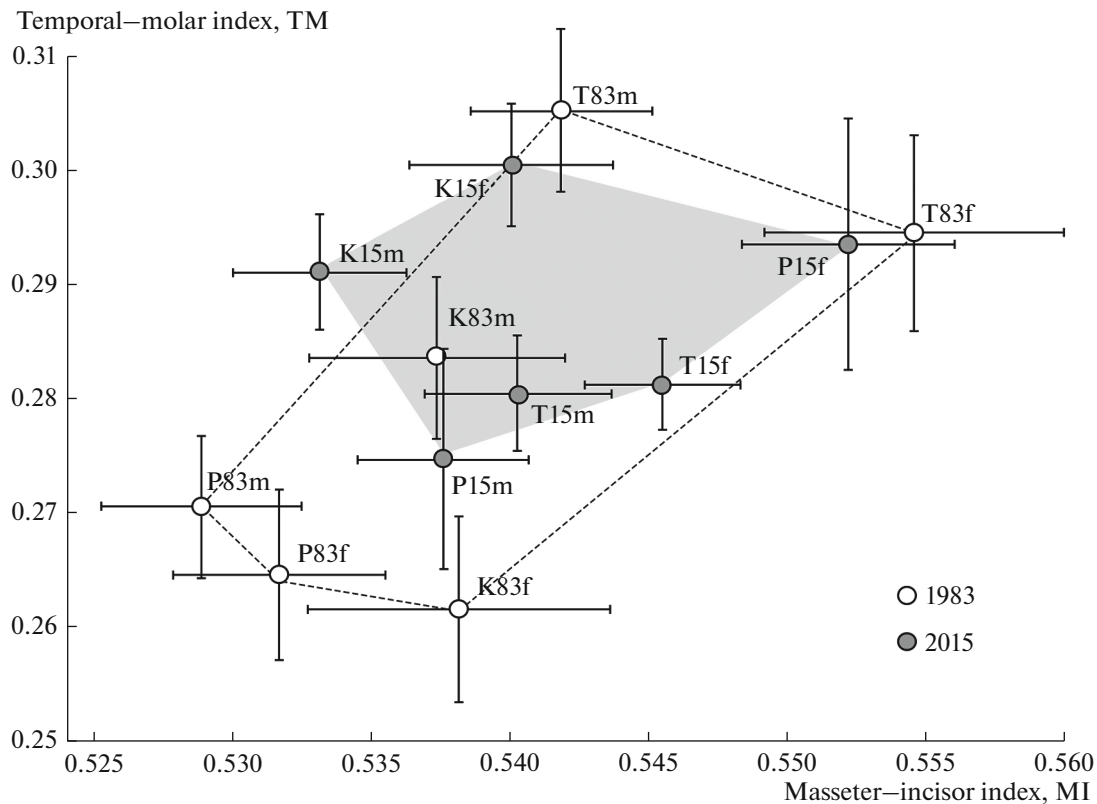
polygons of the between-group variation with respect to the TM and MI indices for the two respective temporal stages; the polygons combine male and female sample centroids, taking into account standard errors for each year. The area of the variation polygon proved to be significantly smaller for samples of 2015 than for samples of 1983, thereby reflecting a relatively higher morphofunctional similarity of geographically distant bank vole populations in the early 21st century. The same decrease in the area of the variation polygon for samples of 2015 is observed in the general morphospace with respect to the TI and MM indices (Fig. 5).

The levels of between-group morphofunctional disparity (MMFD) were estimated for each year by analyzing the pattern of Euclidean distances between the ordinates of male and female samples according to the mean values of all five functional mandibular indices. The mean MMFD value was  $0.0322 \pm 0.0017$  for samples of 1983 and  $0.0274 \pm 0.0012$  for samples of 2015. These data confirm that the general morphofunctional disparity in males and females significantly decreased in three model populations in 2015 ( $t$ -test = 2.25; d.f. = 130;  $p = 0.0264$  for unequal variances) compared to the previous temporal stage. The variance in between-sample distances was also significantly higher in 1983 than in 2015 ( $F = 1.77$ ;  $p = 0.0225$ ).

## DISCUSSION

It should be noted that the annual average values of temperature and precipitation in 1983 and 2015 were similar. These years were also characterized by significant values of the Pearson coefficient of correlation between the series of mean monthly temperatures ( $r = 0.99$ ;  $p < 0.0001$ ) and precipitation ( $r = 0.98$ ;  $p < 0.0001$ ). Therefore, the effects observed in these years largely reflect the historically formed differences, rather than being a direct response to specific conditions of the year. The long-term data of the Orenburg meteorological station from 1966 suggest significant differences in the values of annual average temperature between the first 25 years (until 1990) and the following 25 years (until 2015):  $4.67 \pm 0.22$  and  $5.59 \pm 0.21$ , respectively ( $t = 3.00$ ;  $p = 0.004$ ). At the same time, there were no differences in annual average precipitation ( $t = 0.33$ ;  $p = 0.741$ ).

Therefore, the annual average temperature increased over the studied historical period; however, the level of precipitation in the region remained nearly the same in the early 21st century as that in the late 20th century. Undoubtedly, the temperature increase and maintenance of the same moisture conditions were supposed to induce vegetation changes. One should note that changes were most significant in the floodplain forest of the Sakmara River near the city of

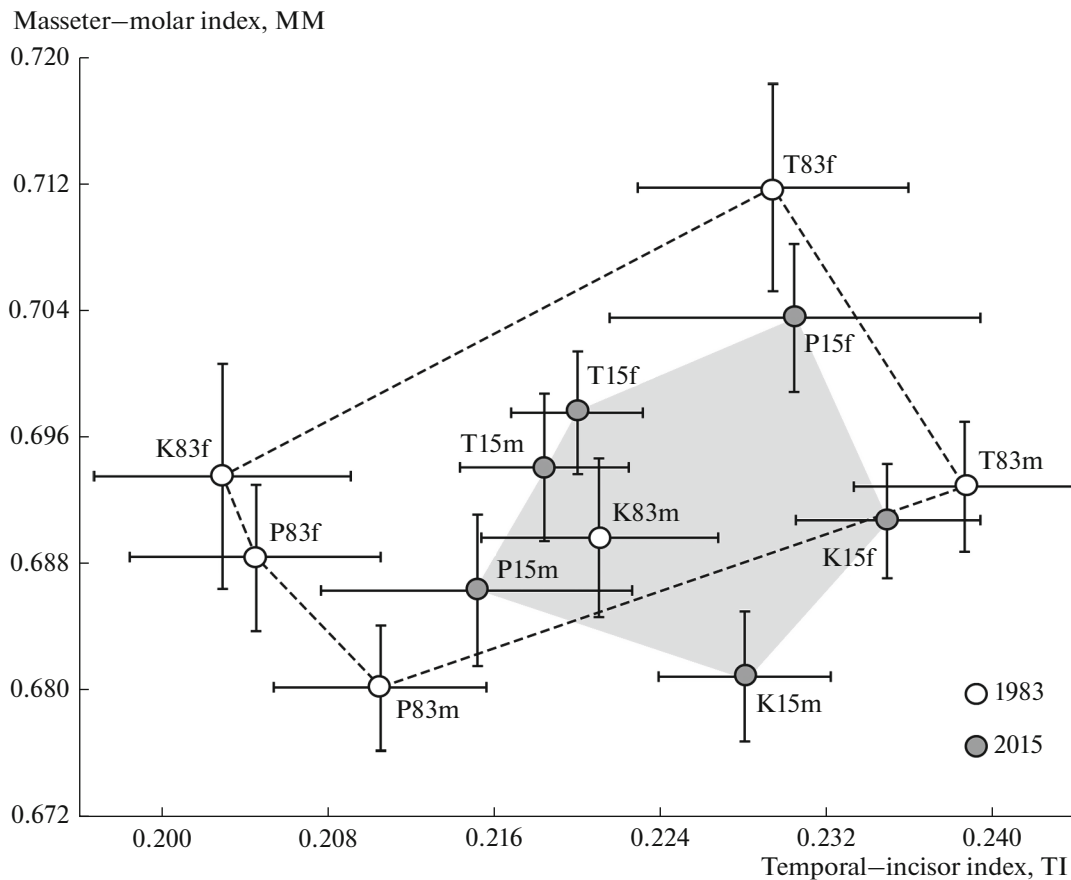


**Fig. 4.** Comparison of mean values of morphofunctional mandibular indices, MI and TM ( $\pm$  SE) in male (m) and female (f) samples from the Totskoe, Platovka, and Sakmara populations of bank vole in 1983 and 2015. Designations are as in Fig. 3

Kuvandyk (the habitat of the Sakmara bank vole population), which has transformed into a dense bottomland forest during the past 10–15 years [29]. Changes also covered the floodplain forest of the Samara River near the village of Platovka (Platovka population), where anthropogenic denudation of the forest and grass canopy, degradation of berry fields (blackberry and wild cherry), and general enhancement of vegetation xerophytism were observed. The floodplain forest near the village of Totskoe (Totskoe population) has been continuously exposed to uncontrolled felling and recreational impact over the past 10–15 years, which has led to the almost complete disappearance of oak stands, as well as to the degradation of berry fields and changes in the composition of a number of oak-connected associations on the left bank of the Samara River. At the same time, the proportion of an invasive species, ash-leaved maple, and wych-elm increased in the floodplain forest. In turn, changes in climate conditions and vegetation composition should have influenced the trophic and other ecological conditions of bank vole floodplain populations in these localities of Orenburg oblast and, indirectly, also variation in the shape and functional properties of the mandible.

The sexual dimorphism in the size of mandibles in the bank vole averaged 5.01%. The average size (area) of the mandible in both periods was significantly

smaller in males than in females; however, their ratio can vary in different years and in different populations. Therefore, it can be assumed that both the size of mandibles and habitual size of the animals are adaptively flexible and have a functional significance. In the Platovka population, sexual dimorphism in the size of mandibles was completely absent in both observation years; in the Sakmara population, significant sex-related differences were revealed only in 2015. It is noteworthy that the comparison of the area of female mandibles in different populations and in different years showed no statistically significant differences. The same comparison of allochronous allotopic samples revealed a significant peculiarity only in males from the Platovka population of 1983, which were of the same large body size as females in that year, unlike in the Totskoe and Sakmara populations. However, as in other populations, the mandibles of males from the Platovka population became relatively small in 2015; i.e., sexual dimorphism in the size of mandibles was also observed in this case. Therefore, the fact that sexual dimorphism in size can be expressed at different levels in different populations (in addition, its significance alternately appears and disappears in populations in different years) indirectly confirms the functional nature of sex differences in size.



**Fig. 5.** Comparison of mean values of morphofunctional mandibular indices, TI and MM ( $\pm$  SE) in male (m) and female (f) samples from the Totskoe, Platovka, and Sakmara populations of bank vole in 1983 and 2015. Designations are as in Fig. 3.

The assessment of regression between the values of the logarithms of the centroid size (CS) and first principal component of the shape (PCS1) revealed a linear relationship ( $p < 0.001$ ); however, the proportion of explained variance in this case proved to be low, only 14%. This suggests a very weak, although statistically significant, allometric dependence of the shape of mandible on its size, which can be neglected. The absence of significant differences in the area of mandibles between populations in the corresponding years also suggests that weak allometric effects do not influence the observed between-population differences in the mandible shape, provided the average size of the objects is similar.

The results of canonical analysis of the mandible shape suggest that significant morphogenetic changes occurred between 1983 and 2015, which were accounted for by the accumulation of numerous small nonlinear changes in the mandible shape, rather than by allometric effects. The chronographic variation in the value of the between-group variance along the first canonical axis is more than two times higher than the geographical variation in its value along the second axis (for samples in 1983). In the initial year of 1983,

the geographic variation in the mandible shape from the western Totskoe population to the easternmost Sakmara population in the general morphospace is almost at straight angle ( $77^\circ$ ) to the direction of the chronographic variation. The cosine of this angle in radians corresponds approximately to the Pearson correlation coefficient  $r = 0.22$  in the case under consideration, thereby showing a weak correlation. In 2015, the directions of the geographic and chronographic variation are significantly consistent with each other. In this case, the angle between the directions of the chronographic and geographic variation is acute ( $30^\circ 30'$ ) and corresponds to the coefficient  $r = 0.86$ , thereby showing a strong correlation between them.

The similarity between geographic variation of the mandible at the turn of the 21st century and its chronographic variation indirectly indicates that the spatial gradient of habitat conditions for the species is largely consistent with its historical changes, thereby reflecting the directional change in climate and, accordingly, its influence on floodplain vegetation and the fauna of invertebrates serving as food for bank voles. As was noted above, changes in trophic conditions should



also affect the morphofunctional properties of vole mandibles.

Based on an experimental study on laboratory mice, Anderson et al. [12] have shown that long-term rodent feeding on foods of different textures (hard or soft) leads to statistically significant changes in the values of their mandibular indices. We also found that the long-term introduction of southern and northern muskrat populations (*Ondatra zibethicus* L.) was accompanied by temporal and spatial changes in the values of the indices due to changes in animal diet [30]. Another research that we carried out using mandibular indices [14] revealed morphofunctional differences between color morphs of mole voles (*Ellobius talpinus* Pall.) and specific features of feeding in monomorphic and polymorphic populations. Therefore, mandibular indices are considered in this study as morphofunctional indices (analogs of indices used in the method of morphophysiological indicators [31]) that characterize the feeding habits of voles and method of food processing by corresponding organs.

Comparison of the values of morphofunctional mandibular indices suggests a specific change in these characteristics for males and females in each studied population over more than 30 years (see Figs. 4, 5): some groups enhance their grinding capacity, while others are characterized by the enhancement of the mastication-related configuration. Allochronous syntopic populations underwent differently directed changes in the mandibular indices, which concerned mainly the masticatory function (TI and TM indices). The largest functional differences in the TI and TM indices were observed between allochronous female samples from the Sakmara population: the masticatory function in females was enhanced between 1983 and 2015. This is not characteristic of males; at the same time, sex-related differences in these indices leveled off by 2015. Sex-related differences in the TI and TM indices and, on the other hand, MI and MM indices in the Platovka population were not expressed in 1983, but the values of both groups of indices in females from this population significantly increased by 2015. In other words, the values of the indices remained unchanged in males but significantly changed in females due to the enhancement of their gnawing and chewing capacity. Sex-related differences in both groups of indices were observed in the Totskoe population in 1983: both gnawing and chewing functions were enhanced in females, while males were characterized only by the enhancement of the masticatory function in this population in that year. Animals from this population were trapped in that year in a sparse floodplain oak forest; therefore, the values of the indices suggest that males and females were specialized in different food items: the diet of large-size females consisted mainly of acorns, while smaller males probably preferred grains, berries (blackberry), and soil invertebrates. In 2015, ecological conditions changed in the Totskoe population and differences in

both groups of indices leveled off. Therefore, males and females from different populations in different years can partially change the morphogenesis of their mandible and implement a set of mandible configurations for consuming food objects differing in texture and means required for their mechanical processing.

Since 1998, the amplitude of interannual fluctuations of annual average temperature in Orenburg oblast has decreased significantly, with this temperature being on average higher than during the previous 30 years. This long-term situation should have been conducive to stabilization of the diversity of vegetation and composition of soil invertebrates, including those eaten by bank voles. Presumably, it is this factor that has accounted for the decrease in the scattering of values of mandibular indices and in the measure of morphofunctional disparity (MMFD) at the second stage of sample comparison in 2015.

## CONCLUSIONS

The results of this study show that a unidirectional change in the morphogenesis of the mandible has occurred in geographically distant bank vole populations at the southern boundary of the species range, which was conditioned by the upward trend in the annual average temperature and consequent transformation of vegetation a three-decade period (1983–2015). This historical process of directional chronographic variation has been accompanied by similar modification in the direction of geographic variation of local populations in the general morphospace. Changes in the shape of the mandible are coupled with changes in its functions related to food processing, which may be different in males and females. Animals of different sexes are actually representatives of different functional morphs in the population; these morphs differ in their potential for food processing, which is clearly observed in the values of morphofunctional mandibular indices in males and females (Figs. 4, 5). Under favorable conditions, which in particular were observed in 2015, sex-related morphofunctional differences are leveled off and animals of both sexes begin to perform similar trophic functions in the population and local rodent community; i.e., they begin to feed on the same products. Under different conditions, as in 1983, these differences are enhanced, thereby increasing the functional potential of intrapopulation structural groups (they may include males and females, age cohorts, functional physiological groups [see 32], biotypes [see 33], etc.). Indeed, animals of both sexes in the population can turn into specialized morphs that provide for effective utilization of food resources and, at the same time, make it possible to alleviate trophic competition.

Therefore, the morphofunctional mandibular indices actually complement the method of morphophysiological indicators proposed by Shvarts et al. [31], thereby providing adequate functional interpretation

of differences at the intra- and interpopulation levels of comparison. Our research has revealed the relationship of rapid morphogenetic and morphofunctional changes in vole mandibles with changes in local trophic conditions due to different trends of plant successions, which, in turn, are related to local trends of climate changes. These rapid directional changes in the morphogenesis of voles can be interpreted as an example of adaptive microevolutionary processes on the historical rather than geological time scale.

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#### COMPLIANCE WITH ETHICAL STANDARDS

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

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

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