

The Genetic Structure of Model Species Populations of Terrestrial Mollusks in Conditions of Urbanized Landscape using the Example of *Chondrula Tridens* Müll (*Gastropoda*, *Pulmonata*)

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Abstract—At the basis of an analysis of morphological and genetic variety, which was done by the polyacrylamid gel electrophoresis method, the state of population genofond of the model species *Chondrula tridens* Müll. (the three-toothed helix) was studied in conditions of urbanized the forest—steppe landscape of the southern Mid-Russia Upland. In most of the studied groups, a real decrease of heterozigosity level is observed, as well as a decrease in allele diversity, which is caused by natural and anthropogenic factors. The automatic genetic processes are considered in populations, and natural selection vectors are determined. The abundance of the studied groups is calculated.

Keywords: population genofond, terrestrial mollusk, urbanized forest—steppe landscape.

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INTRODUCTION

For evaluation of a territory's ecological level, physical parameters of the environment (air, water, and soil characteristics) are frequently used. These indubitably important elements of monitoring nevertheless reflect only the potential degree of contamination of the ecosystem. The real picture can be only obtained with the help of a biotic component in evaluation of communities' stages, since organized matter, which has homeostatic mechanisms, eases or neutralizes harmful impacts completely. Increasing pressure from human beings leads to a lack of the factor of security, including the genetic security of populations, and a process of system disorganization is observed (Altukhov, 1995). By fixing this process, we can make a real assessment of the ecosystem's state and forecast the further course of events. In many cases, the sensitivity of bioindicating methods, including genetic methods, turns out to be higher than the resolution capability of chemical, radiation, and aerospace analysis, since they need interpretation at the level of the real state of living systems.

The goal of this work is to assess the state of the genofonds of the model species *Chondrula tridens* Müll. population in the conditions of the urbanized landscape of the southern Mid-Russia Upland.

Ch. tridens is a terrestrial gastropod classed in the Mediterranean relict group (Nikolaev, 1981) that is widespread in Europe from southwestern France to Ural; it lives in Crimea and in the Caucasus, where it inhabits steppe and semi-arid areas (Shileiko, 1984). In the areas of investigation, the species, as often as

not, forms mass assemblages on chalk slopes and in ravines, gullies, river alluvial plains. A range of works have been devoted to the investigation of *Ch. tridens* as an object of biomonitoring (Matekin, 1950; Nikolaev, 1981; Kramarenko and Sverlova, 2003, 2006). However, in all these investigations, the intraspecific variability of this shellfish was considered only with respect to the shell features. This work presents the first experience of the investigation of the population structure of *Ch. tridens* on the basis of conchiological features and biochemical markers.

MATERIALS AND METHODS

The selection of individuals from the *Ch. tridens* population was carried out at 19 points (Table 1; Fig. 1). Live individuals and empty shells were collected from the soil surface and in cover at a depth of 5 cm. In rainy weather, the shellfish were collected with the help of the sweep-net method in places where helices dwell. The peculiarities of biology of the studied species in the region of investigation were studied. Most summer-season species live underground, feeding on detritus and fungi, especially in dry periods, which last for two months in the region of the southern forest—steppe. In the summer, some of the helices often creep out onto the surface. Mass movement of shellfish onto the soil surface and plant stems is observed in the breeding period in spring (April, May) and during prolonged autumn rains in September. It is precisely in these periods that representative samples can be taken. The determination of the species relation was conducted based on conchiological features

Table 1. Description of selection points

No. of spot	Spot coordinates	N, E
1	Belgorod—remains of natural chalk gully with steppe vegetation	50°36'34.71"N, 36°36'40.91"E
2	Bekaryukovskii Bor—natural landmark of same name; alluvial plain areas in Nezhegol' River valley	50°26'15.38"N, 37°04'23.98"E
3	Rzhevka—chalk slope in Korocha River alluvial plain	50°26'32.63"N, 36°58'22.89"E
4	Afanasovo—chalk slope in Korocha River alluvial plain	50°44'06.34"N, 37°08'49.79"E
5	Zimovnoe—mixed forest margin in Nezhegol' River valley	50°29'35.80"N, 37°09'56.56"E
6	Kotenevka—area of Chufichka River alluvial plain, located near Stoilenskii MMC (mining and refinement complex); Staryi Oskol region	51°11'09.62"N, 37°31'58.93"E
7	Saprykino—forest on the bottom of gully, looking out over Dubenka River alluvial plain, zone of Stoilenskoe and Lebedinskoe MMC influence	50°36'34.71"N, 36°36'40.91"E
8	Protochnoe—chalk slope, zone of Stoilenskoe and Lebedinskoe MMC influence	50°00'18.75"N, 37°31'58.93"E
9	Kochegury—gully chalk slope, looking out over Ol'shanka River alluvial plain, zone of Stoilenskoe and Lebedinskoe MMC influence	50°59'36.59"N, 37°35'29.66"E
10	Gubkin—chalk slope in Oskoletz River alluvial plain	51°17'41.29"N, 37°32'21.99"E
11	Skorodnoe—gully slope looking out over Korocha River alluvial plain	51°04'22.77"N, 37°15'03.39"E
12	Stenki Izgor'ya—chalk slope with relict steppe vegetation, located on the territory of cognominal reserved area	50°40'44.80"N, 37°48'29.48"E
13	Borki—chalk slope in Kozinka River alluvial plain, located on the territory of a natural landmark of same name	50°08'12.03"N, 37°53'09.01"E
14	Valuiki—chalk slope at Valui River valley	50°13'24.38"N, 38°00'34.61"E
15	Lis'ya Gora—natural landmark of same name, Oskol River alluvial plain	50°13'24.38"N, 38°00'34.61"E
16	Kupyansk—chalk slope in Oskol River valley	49°42'19.24"N, 37°37'24.98"E
17	Kalyuzhnyi Yar—chalk gully looking out over Aidar River alluvial plain, territory of Aidarskii Natural Park	49°57'02.88"N, 38°53'49.32"E
18	Klimenkovo—chalk slope in Sarma River region, territory of Aidarskii Natural Park	49°59'25.30"N, 39°02'35.08"E
19	Nagol'noe—chalk slope in Sarma River region, territory of Aidarskii Natural Park	49°58'43.61"N, 38°57'33.69"E

and genitalia (Shileyko, 1984). Shell measurement was conducted under MBS-10 binoculars with the help of an ocular micrometer (only shells of species were measured that were grown and had a mouth lapel). The scheme of the survey is presented in Fig. 2. We chose the most frequent shell surveys used in malacology. In addition, we calculated the ratio of shell width to height (WS/HS) and the ratio of whorl height to shell height (WH/SH), which was intended to be a determinative criterion for two forms of this species, "albolimbata" and "galiciensis" (Clessin, 1879, 1887). In addition, the degree of mouth armature was determined (denticulation index) with the help of the formula

$$\text{Index} = (\text{MH}/\text{MW}) / (a + b + c).$$

Tissues of living examples were subjected to electrophoretic analysis. Water-soluble protein extraction was conducted from a shellfish leg by freezing it at -80°C with follow-up defrosting and mechanical chopping by a Teflon homogenizator in 0.05 M HCl-buffer (pH 6.7). Electrophoresis of allozymes was conducted in 10% polyacrylamide gel in a VE-3 chamber (Helicon) Gel tris-HCl-buffer (concentrating gel pH 6.7, dividing gel

pH 8.9) and electrode tris-glycine-buffer (pH 8.3) were used. Protein painting in the case of unspecific esterase was conducted in the substrate mixture: tris-HCl (pH 7.4), α -naphthylacetate, fast red TR; to produce superoxide dismutase, a potassium-phosphorus buffer (pH 7.8), NTS, and FMS were used.

Ten zones of unspecific esterases were found in *Ch. tridens*; five monomolecular loci were interpreted, which were used as genetic markers (Fig. 2): EST1 (with two alleles), EST 5 (with six alleles), EST 8 (with three alleles), EST 9 (with three alleles), and EST 10 (with five alleles). In addition to this, four loci of superoxidized ismutases were identified and interpreted: SOD 1 (monomer with three alleles), SOD 2 (dimer with three alleles), SOD 3 (monomer with three alleles), and SOD 4 (monomer with three alleles). The inheritance of all marked loci occurs with a codominant type (Ivanova and Snegin, 2007).

During the whole investigation period, 913 shells and 1783 living species were studied. Collection was conducted from 2006 to 2010.

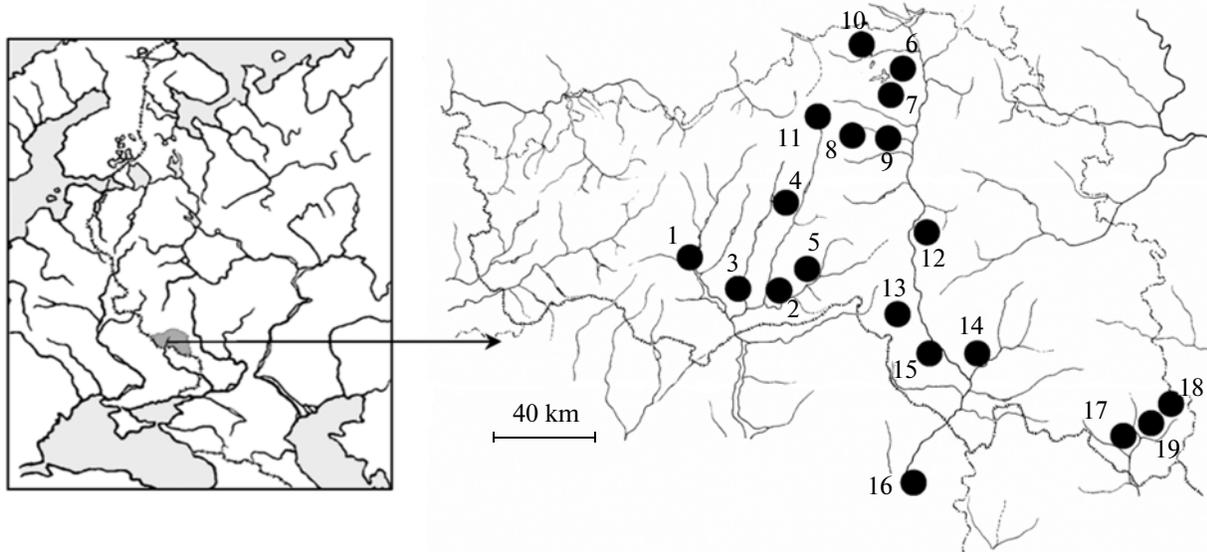


Fig. 1. Shellfish collection sites in the investigated area.

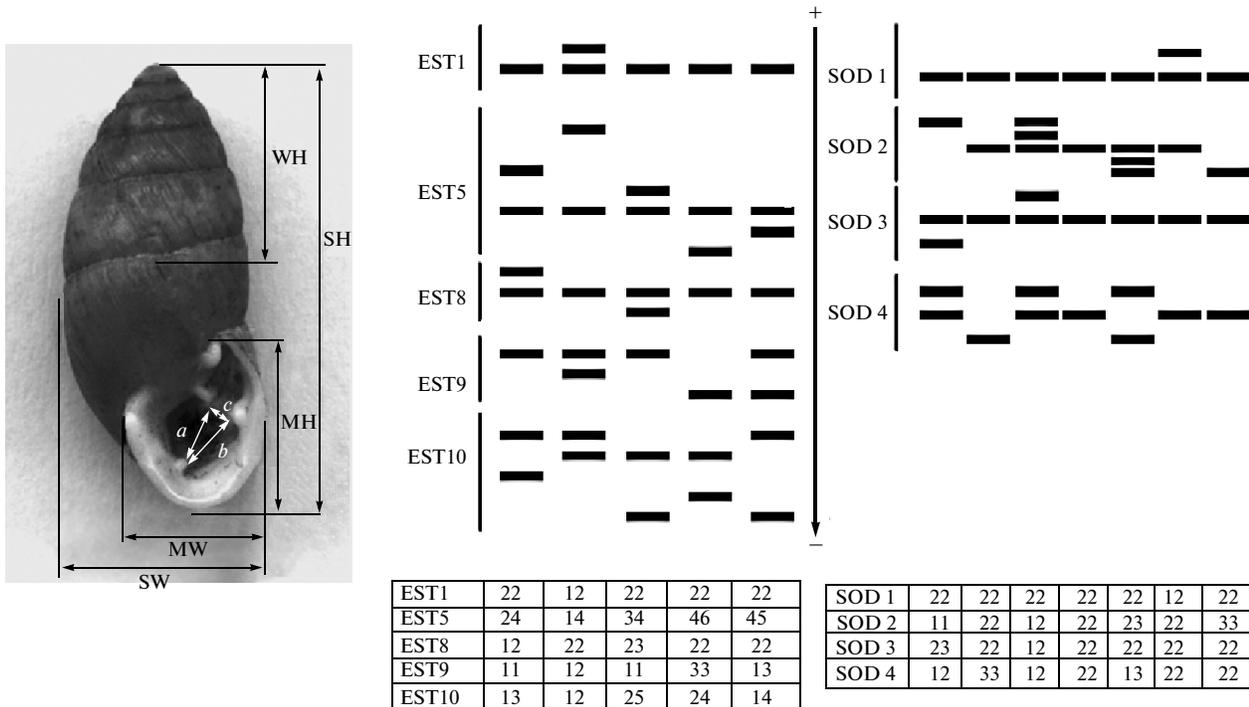


Fig. 2. From the left—*Ch. tridens* shell. From the right—activity zones of nine loci and genotypes corresponding to them. Measurements; SH—shell height; SW—shell width; WH—whorl height; MH—mouth height; MW—mouth width; Distance between tops of teeth; a—columellar and parietal; b—columellar and palatal; C—parietal and palatal.

Data elaboration was conducted with the help of the GenAlEx program (Peakal R. and Smouse, 2001) and TFPGA (Miller and Mark P., 1997).

RESULTS AND DISCUSSIONS

According to the data obtained from shell morphometric indices (Table 2), most of the investigated

Ch. tridens groups in the southern Central Russian region are part of the so-called galiciensis variant, which has a smaller shell (SH < 12 mm) in comparison with the “albolimbata” variant (only the population from the Stenki Izgor’ya reserve—point 12—is classed in the latter). Of the helices of the “galiciensis” variant, according to their shell height and weight,

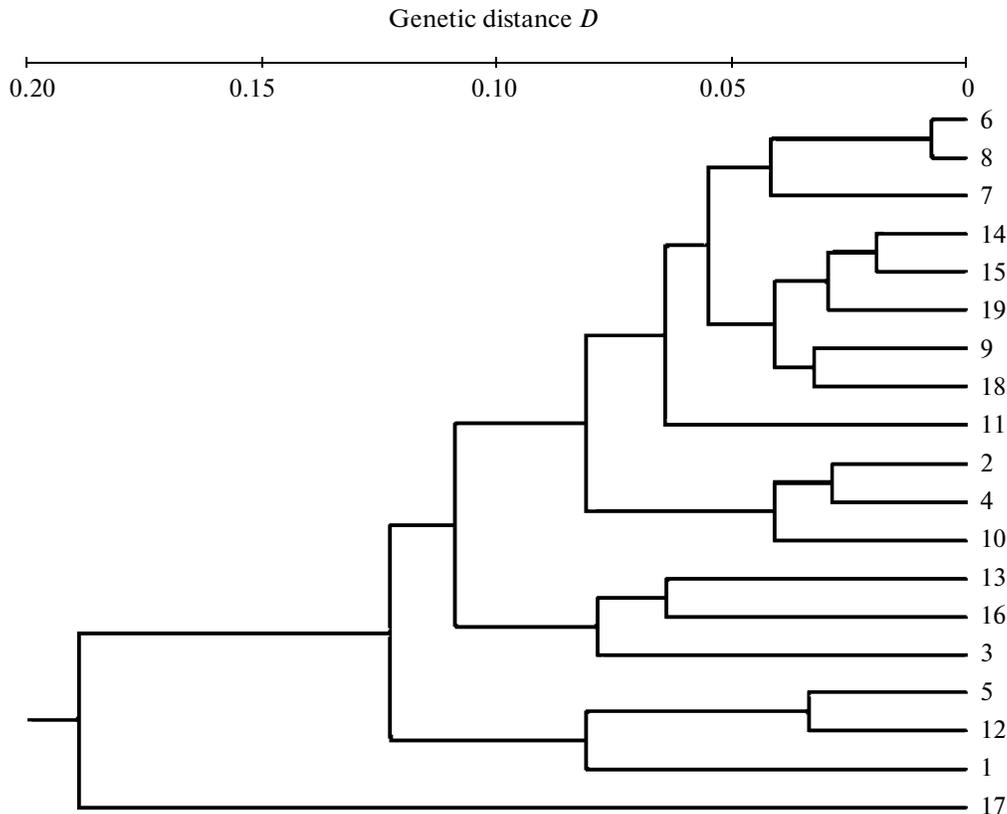


Fig. 3. Dendrogram of genetic distances (Nei, 1972) (UPGMA). Genetic distance D .

only the largest sizes relative to other investigated groups were marked on points connected with to mining and refinement complexes (6, 7, 8). The animal's reaction upon xerophytization of biotopes of this region, which is caused by rarefaction of plant communities, is a likely explanation for this phenomenon¹. However, in the same zone, groups with authentically smaller shell sizes are marked (points 9 and 10), so we do not incline to connecting the increase of animal sizes with anthropogenic factors only. It is likely that the shell sizes are mostly dependent on historically formed genofond features of populations. We also did not determine the correlation between shell size and biotope moisture degree. Species with large or small shells can be come across in moist alluvial plains, as well as on dry chalk slopes. This phenomenon can be explained through the following reasons. We noted that, in conditions of spring floods, part of the species, which inhabit an alluvial plain, dies. However, a constant supplementing of their population occurs as a result of the washing in of helices that live on the nearby chalk slopes by summer rains, so vertical shellfish migration in river valleys occurs, which reflects in similar shell parameters. The conducted monofactor

¹ According to a range of authors, in arid conditions, the increase of shellfish body volume leads to an increase in the amount of water.

dispersive analysis showed a high differentiation of populations in conditions of forest–steppe landscape. In addition, the greatest increase in transpopulation variability was brought about by such indices as the height and width of the shell (Table 3). The lowest differences are marked at the correlation index of these two features. This index may reflect the species-specific shell constitution of *Ch. tridens*, and therefore it is less exposed to variability.

It is known that the mouth denticulation index may serve as a characteristic of a biotope's degree of xerophytization (index, Matekin, 1950). With this, one regularity can be deduced—the degree of mouth armature is determined not only by geographical location, but also by microrelief features. For instance, Aidarskii Natural Park (which has a steppe biome), in the relict biotope Nagol'noe (point 19), which is a chalk slope of southern orientation, surpasses adjacent regions, such as Kalyuzhnyi Yar, a chalk slope of eastern position, and Klimenkovo, where helices were found in places where ground waters exit. We observed the same picture in the northern part of the investigated region, where significant differences in the denticulation index between points 6, 9, 10, and 11, on the one hand, and points 7 and 8, on the other, were remarked. In the later points, the lowest indices of

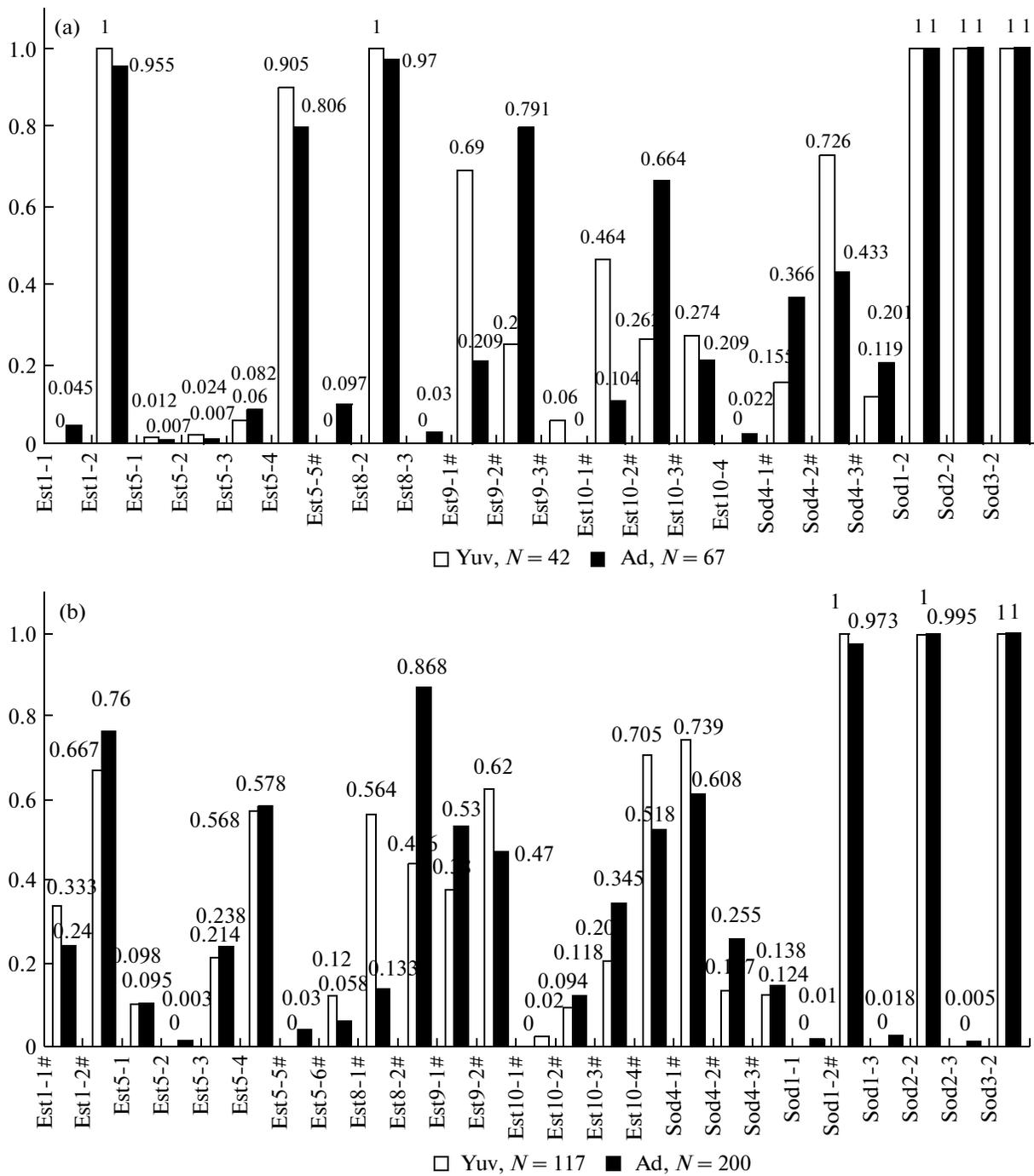


Fig. 4. Allele frequencies in different age groups of *Ch. tridens* populations. A—Bekaryukovskii Bor; B—Belgorod; Yuv—impu-beral; Ad—puberal; #—cases of authentic differences between age groups according to Fisher Exact Test.

mouth armature development in the southern part of the Mid-Russia Upland has been noted.

Allele frequencies and heterozygosity levels at the loci used are presented in Table 4. According to these data, in 40% of cases, a significant ($P \geq 0.05$) lack of heterozygotic phenotypes (heterozygotic deficit authenticity was assessed with the help of the formula

$\chi^2 = F^2N(k - l)$, $df = k - l$, where F is the inbreeding coefficient, N is the selection volume, and k is the number of alleles at the locus (Lee, 1978)) was noted and, in 33.3% of cases, full homozygosity can be seen at one of the alleles. An authentic heterozygotic surplus has been determined only in 1.7% of cases (point 1 Est 9, point 4 Est 9, point 11 Est 10). The lowest level of heterozygosity and lower values of the allele effec-

Table 2. Shell measurements and calculated index values for *Ch. tridens*

Colonies	SH (shell height)	SW (shell width)	MH (mouth height)	MW (mouth width)	WH (whorl height)	SW/SH	WH/SH	Index
1. Belgorod (<i>N</i> = 37)	9.6 ± 0.2	4.1 ± 0.04	3.4 ± 0.1	3.2 ± 0.06	3.9 ± 0.1	0.424 ± 0.008	0.396 ± 0.006	2.25 ± 0.08
2. Bekaryukovskii Bor (<i>N</i> = 79)	9.3 ± 0.1	4.3 ± 0.04	3.6 ± 0.05	3.0 ± 0.03	3.7 ± 0.1	0.456 ± 0.006	0.397 ± 0.007	2.31 ± 0.05
3. Rzhavka (<i>N</i> = 15)	10.1 ± 0.3	4.5 ± 0.1	4.2 ± 0.1	3.2 ± 0.08	3.7 ± 0.2	0.444 ± 0.015	0.361 ± 0.01	2.32 ± 0.08
4. Afanasovo (<i>N</i> = 20)	10.7 ± 0.2	4.6 ± 0.1	4.5 ± 0.1	4.0 ± 0.1	4.04 ± 0.2	0.433 ± 0.014	0.378 ± 0.008	2.25 ± 0.08
5. Zimovnoe (<i>N</i> = 36)	10.9 ± 0.2	4.9 ± 0.3	4.4 ± 0.08	3.6 ± 0.04	4.1 ± 0.15	0.438 ± 0.009	0.379 ± 0.008	2.37 ± 0.1
6. Kotenevka (<i>N</i> = 20)	11.5 ± 0.3	5.5 ± 0.2	4.7 ± 0.16	3.9 ± 0.1	4.3 ± 0.2	0.480 ± 0.021	0.376 ± 0.01	2.26 ± 0.08
7. Saprykino (<i>N</i> = 20)	11.1 ± 0.2	5.2 ± 0.04	4.7 ± 0.06	3.7 ± 0.03	3.9 ± 0.1	0.447 ± 0.013	0.355 ± 0.008	1.97 ± 0.05
8. Protochnoe (<i>N</i> = 22)	10.7 ± 0.2	5.1 ± 0.08	4.6 ± 0.08	3.5 ± 0.07	4.2 ± 0.1	0.475 ± 0.011	0.394 ± 0.008	1.98 ± 0.06
9. Kohegury (<i>N</i> = 21)	10.6 ± 0.2	4.9 ± 0.05	4.5 ± 0.07	3.5 ± 0.06	3.7 ± 0.2	0.466 ± 0.009	0.351 ± 0.01	2.18 ± 0.11
10. Gubkin (<i>N</i> = 79)	9.7 ± 0.1	4.5 ± 0.05	3.9 ± 0.07	3.4 ± 0.05	3.8 ± 0.1	0.472 ± 0.006	0.396 ± 0.007	2.4 ± 0.08
11. Skorodnoe (<i>N</i> = 53)	10.7 ± 0.2	4.9 ± 0.05	4.1 ± 0.07	3.8 ± 0.04	4.3 ± 0.14	0.464 ± 0.007	0.397 ± 0.007	2.5 ± 0.05
12. Stenki Izgor'ya (<i>N</i> = 150)	12.6 ± 0.1	5.3 ± 0.06	4.8 ± 0.06	3.9 ± 0.05	5.3 ± 0.1	0.432 ± 0.007	0.418 ± 0.004	2.36 ± 0.05
13. Borki (<i>N</i> = 21)	9.6 ± 0.3	4.3 ± 0.08	4.8 ± 0.06	3.9 ± 0.12	4.0 ± 0.15	0.450 ± 0.011	0.390 ± 0.01	2.34 ± 0.2
14. Valuiki (<i>N</i> = 182)	10.4 ± 0.1	5.0 ± 0.04	4.4 ± 0.04	3.5 ± 0.04	4.2 ± 0.01	0.459 ± 0.005	0.383 ± 0.005	2.34 ± 0.2
15. Lis'ya Gora (<i>N</i> = 23)	10.3 ± 0.25	4.6 ± 0.09	4.1 ± 0.1	3.2 ± 0.07	3.9 ± 0.26	0.448 ± 0.01	0.395 ± 0.012	2.1 ± 0.07
16. Kupyansk (<i>N</i> = 33)	10.5 ± 0.2	4.7 ± 0.08	4.2 ± 0.1	3.5 ± 0.06	4.05 ± 0.2	0.449 ± 0.009	0.383 ± 0.011	2.43 ± 0.08
17. Kalyuzhnyi Yar (<i>N</i> = 39)	9.7 ± 0.2	4.6 ± 0.09	4.0 ± 0.08	3.2 ± 0.07	3.8 ± 0.2	0.462 ± 0.013	0.380 ± 0.02	2.24 ± 0.08
18. Klimentkovo (<i>N</i> = 33)	10.0 ± 0.2	4.6 ± 0.07	4.0 ± 0.1	3.2 ± 0.1	3.9 ± 0.1	0.458 ± 0.012	0.390 ± 0.008	2.27 ± 0.12
19. Nagol'noe (<i>N</i> = 30)	10.7 ± 0.2	4.8 ± 0.07	4.3 ± 0.07	3.6 ± 0.06	4.0 ± 0.14	0.449 ± 0.008	0.374 ± 0.009	2.5 ± 0.11

Table 3. Results of monofactor disperse analysis by morphometric features

Index	Variability	SS	df	MS	F	P
SW (shell width)	Between groups	123.3	18	6.8	65.1	3.8×10^{-141}
	Within groups	81.1	895	0.1		
SH (shell height)	Between groups	739.1	18	41.1	83.6	2.9×10^{-167}
	Within groups	378.6	895	0.49		
Index	Between groups	11.7	18	0.65	8.0	1.2×10^{-19}
	Within groups	61.1	895	0.08		
WH/SH	Between groups	0.25	18	0.01	10.6	2.1×10^{-27}
	Within groups	1.1	895	0.001		
SW/SH	Between groups	0.11	18	0.006	4.8	3.3×10^{-10}
	Within groups	0.95	895	0.001		

tive number and Shannon index are fixed on points 5, 7, and 16, and the highest values of the inbreeding coefficient are on points 3, 6, 15, and 17 (Table 5). Moreover, in different loci, the heterozygosity level is unequal in different populations. Heterozygotic variants can be encountered more frequently in the Est 10 ($H_0 = 0.319$) and Sod 4 ($H_0 = 0.313$) loci. Precisely these loci result in the largest increase in transpopulational diversity, which is determined with the help of the inbreeding coefficient Fst (Table 6).

A result of cluster analysis against the background of genetic distances (Nei, 1978) by the unfinished pair-group method (UPGMA) is presented in Fig. 3. The analysis shows a very variegated population distribution in groups. At the same time, the geographical location of the population does not show a global influence on resemblance in the correlation of allele frequency and combinations, as in the case of shell phenotypes. Thus, populations (17, 18, 19) from Aidarskii National Park, despite the close spatial location, according to the scheme, occurred in different clusters. On the other hand the population of helices dwelling in conditions of a disordered environment created under the impact of a mining and refinement complex (points 6, 7, 8) found themselves in one group, which, perhaps, indirectly speaks in favor of a similar natural selection vector here. In terms of the degree of approach to the cluster, we may assess the degree of environmental change and the genofond stage of other populations. Earlier, the same picture in the investigated region was obtained for another representative species of terrestrial mollusk, *Bradybaena fructicum* Müll. (Snegin, 2010).

It should be noted that the level of allele and phenotypic population diversity of *Ch. tridens* may be affected not only by modern geomorphologic processes caused by human activity, but also by historical factors. In the period of helix settlement on the territory of the forest–steppe, in virtue of the southern Mid-Russia Upland’s peculiarities of landscape, a natural split of the species population into isolated

groups occurred. If such populations were affected by additional pressure on the part of human beings as a result of tillage of the territory, overgrazing, or burning, a constant oscillation of their number would have taken place while living in the same place, or during forced processes caused by a bottleneck effect or founder effect, the monomorphism of these groups would have increased. In addition, the division of the species’ area in forest–steppe conditions could provoke the so-called “genetic revolution” effect that has been described for groups in small areas (Mayr, 1968). According to this hypothesis, in isolated conditions, i.e., in the conditions in which most of the studied populations of the region live, the genes were selected for that were most effective in a homozygotic state and rare in new populations because of the so-called “good-mixing genes” that dominate there. When so-called “loners” get to another genetic environment, they find themselves in a more favorable situation. Notably, according to Mayr, this process may concern a large number of loci simultaneously. Such “revolutionary” alleles are, perhaps, *EST 1–2*, *Est 5–4*, *Est 8–2*, *Est 9–2*, *Est 10–2*, *Sod 1–2*, *Sod 2–2*, *Sod 3–2*, and *Sod 4–2*. Precisely these alleles contribute most of all to the homozygosity level (Table 4). We may purpose that the species’ adaptive reaction to existence in urbanized conditions dominates in most of the populations of the investigated region of these alleles. If the group, due to the peculiarities of the landscape, stayed for a long time in an undisturbed advantageous biotope, despite recent emergence from isolation, it retains an allele potential that is peculiar to primeval populations; i.e., at the present time, we encounter a change of the allele content in such a group in an interim stage. As an example, we can use the population that inhabits the urban part of Belgorod (point 1) on a small site with chalk denudations with relict steppe vegetation. This group has a great abundance and a high value of heterozygosity and allele diversity. In this group, alleles were observed that are rare for the forest–steppe: *Est 1–1*, *Sod 1–1*, *Sod 1–3*,

Table 4. Shell measurements and calculated index values for *Ch. tridens*

Locus	Allele	Population																		
		1 N = 317	2 N = 137	3 N = 20	4 N = 46	5 N = 110	6 N = 155	7 N = 76	8 N = 33	9 N = 60	10 N = 78	11 N = 60	12 N = 74	13 N = 21	14 N = 310	15 N = 44	16 N = 83	17 N = 42	18 N = 101	19 N = 16
Est 1	1	0.274	0.022	0.000	0.000	0.005	0.019	0.000	0.015	0.000	0.109	0.000	0.054	0.000	0.024	0.000	0.060	0.000	0.000	0.000
	2	0.726	0.978	1.000	1.000	0.995	0.981	1.000	0.985	1.000	0.891	1.000	0.946	1.000	0.976	1.000	0.940	1.000	1.000	1.000
	H_0	0.435	0.044	0.000	0.000	0.009	0.039	0.000	0.030	0.000	0.218	0.000	0.027	0.000	0.010	0.000	0.120	0.000	0.000	0.000
Est 5	H_e	0.398	0.043	0.000	0.000	0.009	0.038	0.000	0.030	0.000	0.194	0.000	0.102	0.000	0.047	0.000	0.113	0.000	0.000	0.000
	F	-0.093	-0.022	-	-	-0.005	-0.020	-	-0.015	-	-0.122	-	0.736	-	0.795	-	-0.064	-	-	-
	1	0.096	0.007	0.000	0.000	0.000	0.065	0.039	0.136	0.233	0.000	0.000	0.020	0.143	0.298	0.102	0.000	0.060	0.248	0.094
Est 8	2	0.002	0.011	0.000	0.000	0.009	0.013	0.000	0.000	0.183	0.000	0.020	0.000	0.000	0.005	0.011	0.000	0.000	0.000	0.000
	3	0.229	0.077	0.000	0.022	0.064	0.010	0.000	0.015	0.000	0.000	0.367	0.155	0.071	0.232	0.341	0.018	0.060	0.045	0.000
	4	0.574	0.832	0.425	0.967	0.900	0.906	0.961	0.833	0.583	0.994	0.633	0.486	0.452	0.400	0.375	0.813	0.155	0.465	0.750
Est 9	5	0.019	0.073	0.575	0.011	0.027	0.006	0.000	0.015	0.000	0.006	0.000	0.318	0.333	0.063	0.170	0.169	0.726	0.243	0.156
	6	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000
	H_0	0.470	0.153	0.050	0.065	0.091	0.097	0.079	0.242	0.633	0.013	0.067	0.392	0.476	0.494	0.432	0.096	0.286	0.475	0.313
F	H_e	0.602	0.296	0.489	0.064	0.185	0.174	0.076	0.287	0.572	0.013	0.464	0.638	0.659	0.693	0.704	0.310	0.442	0.661	0.404
	F	0.219	0.483	0.898	-0.026	0.509	0.443	-0.041	0.154	-0.108	-0.006	0.856	0.385	0.277	0.288	0.386	0.689	0.353	0.281	0.227
	1	0.292	0.000	0.075	0.054	0.000	0.139	0.105	0.227	0.092	0.000	0.033	0.189	0.476	0.092	0.068	0.000	0.000	0.015	0.000
F	2	0.708	0.985	0.925	0.946	0.886	0.774	0.862	0.667	0.750	0.981	0.667	0.723	0.524	0.868	0.761	0.964	1.000	0.985	1.000
	3	0.000	0.015	0.000	0.000	0.114	0.087	0.033	0.106	0.158	0.019	0.300	0.088	0.000	0.040	0.170	0.036	0.000	0.000	0.000
	H_0	0.306	0.029	0.150	0.065	0.082	0.103	0.211	0.333	0.283	0.013	0.467	0.284	0.667	0.197	0.341	0.072	0.000	0.030	0.000
F	H_e	0.413	0.029	0.139	0.103	0.201	0.374	0.245	0.493	0.404	0.038	0.464	0.434	0.499	0.237	0.387	0.070	0.000	0.029	0.000
	F	0.260	-0.015	-0.081	0.366	0.594	0.724	0.141	0.323	0.299	0.660	-0.005	0.346	-0.336	0.170	0.118	-0.037	-	-0.015	-
	1	0.473	0.416	0.000	0.315	0.014	0.255	0.145	0.348	0.058	0.308	0.642	0.047	0.000	0.197	0.341	0.054	0.798	0.292	0.219
F	2	0.527	0.522	1.000	0.641	0.982	0.700	0.855	0.652	0.833	0.577	0.333	0.953	0.976	0.790	0.614	0.940	0.190	0.629	0.625
	3	0.000	0.062	0.000	0.043	0.005	0.045	0.000	0.000	0.108	0.115	0.025	0.000	0.024	0.013	0.045	0.006	0.012	0.079	0.156
	H_0	0.596	0.307	0.000	0.717	0.018	0.290	0.184	0.455	0.100	0.359	0.467	0.041	0.048	0.171	0.159	0.048	0.310	0.297	0.375
F	H_e	0.499	0.551	0.000	0.487	0.036	0.443	0.248	0.454	0.290	0.559	0.477	0.090	0.046	0.337	0.505	0.114	0.327	0.513	0.537
	F	-0.195	0.443	-	-0.472	0.493	0.345	0.256	-0.001	0.656	0.358	0.021	0.550	-0.024	0.492	0.685	0.577	0.055	0.421	0.302

Table 4. (Contd.)

Locus	Allele	Population																		
		1 N = 317	2 N = 137	3 N = 20	4 N = 46	5 N = 110	6 N = 155	7 N = 76	8 N = 33	9 N = 60	10 N = 78	11 N = 60	12 N = 74	13 N = 21	14 N = 310	15 N = 44	16 N = 83	17 N = 42	18 N = 101	19 N = 16
Est 10	1	0.013	0.248	0.025	0.120	0.082	0.294	0.428	0.303	0.175	0.071	0.000	0.162	0.071	0.058	0.023	0.012	0.250	0.332	0.125
	2	0.109	0.449	0.125	0.120	0.686	0.526	0.053	0.379	0.508	0.731	0.717	0.595	0.143	0.879	0.659	0.024	0.155	0.366	0.750
	3	0.292	0.270	0.300	0.489	0.232	0.058	0.513	0.045	0.317	0.154	0.283	0.243	0.500	0.058	0.295	0.964	0.452	0.158	0.063
	4	0.587	0.026	0.325	0.217	0.000	0.074	0.007	0.273	0.000	0.026	0.000	0.000	0.143	0.003	0.000	0.000	0.071	0.094	0.063
H ₀	5	0.013	0.007	0.225	0.054	0.000	0.048	0.000	0.000	0.019	0.000	0.000	0.000	0.143	0.002	0.023	0.000	0.071	0.050	0.000
	H ₀	0.328	0.445	0.400	0.457	0.382	0.277	0.250	0.545	0.150	0.051	0.567	0.203	0.381	0.106	0.273	0.024	0.476	0.376	0.375
	H _e	0.565	0.663	0.738	0.682	0.468	0.626	0.551	0.688	0.611	0.436	0.406	0.561	0.684	0.221	0.477	0.070	0.699	0.719	0.414
F	F	0.419	0.329	0.458	0.331	0.185	0.557	0.546	0.207	0.754	0.882	-0.395	0.639	0.443	0.517	0.429	0.657	0.318	0.477	0.094
	1	0.006	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.214	0.000	0.000
	2	0.983	1.000	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.968	1.000	1.000	0.786	1.000	1.000
Sod 1	3	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H ₀	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000
	H _e	0.034	0.000	0.180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.062	0.000	0.000	0.337	0.000	0.000
Sod 2	F	0.908	-	1.000	-	-	-	-	-	-	-	-	-	-	-0.033	-	-	1.000	-	-
	1	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.053	0.034	0.000	0.024	0.000	0.000
	2	0.997	0.985	1.000	1.000	1.000	0.990	1.000	1.000	1.000	1.000	1.000	1.000	0.976	0.944	0.966	1.000	0.976	0.985	1.000
Sod 3	3	0.003	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.015	0.000
	H ₀	0.000	0.015	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.081	0.068	0.000	0.148	0.030	0.000
	H _e	0.006	0.029	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.107	0.066	0.000	0.146	0.029	0.000
Sod 4	F	1.000	0.493	-	-	-	-0.010	-	-	-	-	-	-	-0.024	0.245	-0.035	-	-0.024	-0.015	-
	1	0.000	0.000	0.000	0.000	0.000	0.058	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.000	0.000	0.000	0.000	0.000
	2	0.998	1.000	1.000	1.000	1.000	0.942	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.956	1.000	1.000	1.000	1.000	1.000
H ₀	3	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H ₀	0.003	0.000	0.000	0.000	0.000	0.103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.000	0.000	0.000	0.000	0.000
	H _e	0.003	0.000	0.000	0.000	0.000	0.109	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000
H _e	F	-0.002	-	-	-	-	0.056	-	-	-	-	-	-	-	-0.046	-	-	-	-	-
	1	0.656	0.296	0.175	0.326	0.900	0.029	0.079	0.212	0.033	0.173	0.000	0.736	0.024	0.287	0.125	0.428	0.631	0.084	0.156
	2	0.211	0.504	0.250	0.185	0.027	0.774	0.888	0.742	0.942	0.564	1.000	0.216	0.810	0.658	0.818	0.446	0.167	0.827	0.688
H ₀	3	0.132	0.201	0.575	0.489	0.073	0.197	0.033	0.045	0.025	0.263	0.000	0.047	0.167	0.055	0.057	0.127	0.202	0.089	0.156
	H ₀	0.382	0.489	0.550	0.543	0.145	0.316	0.092	0.333	0.017	0.423	0.000	0.419	0.238	0.390	0.136	0.482	0.214	0.287	0.500
	H _e	0.507	0.619	0.576	0.620	0.184	0.361	0.204	0.402	0.112	0.583	0.000	0.409	0.316	0.482	0.312	0.602	0.533	0.301	0.479
F	F	0.247	0.209	0.046	0.124	0.209	0.124	0.548	0.170	0.851	0.274	-	-0.025	0.247	0.189	0.563	0.200	0.598	0.048	-0.045
	F	0.247	0.209	0.046	0.124	0.209	0.124	0.548	0.170	0.851	0.274	-	-0.025	0.247	0.189	0.563	0.200	0.598	0.048	-0.045

Note: H₀—mean observed heterozygosity; H_e—mean expected heterozygosity; F—inbreeding coefficient.

Table 5. The indices of effective abundance in studied populations of *Chondrula tridens*

Population	<i>N</i>	<i>P</i> %	<i>A_e</i>	<i>l</i>	μ	<i>H₀</i>	<i>H_e</i>	<i>F</i>	<i>N_e</i>	<i>N_e/N</i>
1	317	100.0	1.7	0.567	2.19	0.280	0.338	0.172	270.6	0.854
2	137	77.78	1.6	0.442	1.97	0.165	0.248	0.335	102.6	0.749
3	20	55.56	1.61	0.406	1.82	0.128	0.236	0.458	13.7	0.686
4	46	55.56	1.55	0.393	1.86	0.205	0.217	0.055	43.6	0.948
5	110	66.67	1.18	0.231	1.53	0.081	0.120	0.329	82.8	0.752
6	155	88.89	1.46	0.456	2.07	0.138	0.242	0.427	108.6	0.701
7	76	55.56	1.25	0.263	1.55	0.091	0.147	0.384	54.9	0.723
8	33	66.67	1.57	0.448	1.90	0.215	0.261	0.176	28.1	0.850
9	60	55.56	1.46	0.392	1.78	0.131	0.221	0.405	42.7	0.712
10	78	66.67	1.42	0.362	1.77	0.120	0.203	0.409	55.3	0.710
11	60	44.44	1.37	0.305	1.51	0.174	0.201	0.135	52.9	0.881
12	74	66.67	1.52	0.442	1.92	0.152	0.248	0.389	53.3	0.720
13	21	66.67	1.63	0.448	1.96	0.206	0.250	0.175	17.9	0.851
14	310	100.0	1.51	0.473	2.09	0.178	0.252	0.294	239.5	0.773
15	44	66.67	1.61	0.485	2.04	0.157	0.272	0.425	30.9	0.702
16	83	66.67	1.26	0.259	1.55	0.094	0.142	0.341	61.9	0.746
17	42	66.67	1.59	0.480	2.04	0.148	0.265	0.441	29.2	0.694
18	101	66.67	1.67	0.463	2.04	0.166	0.250	0.337	75.6	0.748
19	16	44.44	1.38	0.367	1.75	0.174	0.204	0.148	13.9	0.871

Note: *P*—% of polymorphic loci; *A_e*—mean effective number of alleles in locus; μ —mean phenotype number; *l*—Shannon index; *N_e*—effective abundance (μ index is assessed according to Zhivotovskii, 1991, p. 113).

Table 6. Allele values of heterozygosity and inbreeding coefficients in studied *Chondrula tridens* populations

	<i>Est1</i>	<i>Est5</i>	<i>Est8</i>	<i>Est9</i>	<i>Est10</i>	<i>Sod1</i>	<i>Sod2</i>	<i>Sod3</i>	<i>Sod4</i>	Average
<i>H_o</i>	0.049	0.259	0.191	0.260	0.319	0.004	0.016	0.010	0.313	0.158
<i>H_e</i>	0.051	0.407	0.242	0.343	0.541	0.032	0.018	0.010	0.400	0.227
<i>F_{is}</i>	0.043	0.363	0.209	0.242	0.410	0.890	0.119	0.012	0.217	0.278
<i>F_{it}</i>	0.174	0.510	0.321	0.408	0.545	0.905	0.140	0.057	0.459	0.391
<i>F_{st}</i>	0.138	0.230	0.142	0.218	0.229	0.141	0.025	0.046	0.309	0.164

Note: *F_{it}*—species inbreeding coefficient relative to large population *F_{is}*—species inbreeding coefficient related to subpopulation; *F_{st}*—subpopulation inbreeding coefficient related to large population.

Sod 2–3, and *Sod 3–3*. We cannot explain this fact by appeal to selection size alone, because other populations did not show any correlation between selection size and the level of detected genetic variability.

To determine the directions of natural selection vectors, we analyzed the changes in allele frequencies in different age groups. The peculiarities of the biology of the species did not allow us to collect representative samples for impuberal individuals; as examples, we took data only for two populations—Belgorod and Bekaryukovskii Bor—in which we managed to obtain representative material. To match allele frequencies, we used a Fisher Exact Test (Fisher, 1958). According to the data obtained, in the Bekaryukovskii Bor popula-

tion, in the pubescent age group, a significant increase in allele number for *Est 5–5*, *Est 9–2*, *Est 10–2*, and *Sod 4–1* was observed. The same changes were observed in the Belgorod population for *Est 1–2*, *Est 5–5*, *Est 8–2*, *Est 9–1*, *Est 10–3*, and *Sod 4–2* allele frequencies. A significant decrease of frequencies in pubescence has been determined in the Bekaryukovskii Bor population in *Est 9–1*, *Est 9–3*, *Est 10–1*, and *Sod 4–2* alleles, and in the Belgorod population in *Est 1–1*, *Est 5–6*, *Est 8–1*, *Est 9–2*, *Est 10–4*, and *Sod 4–1* alleles. It may be that such a change in allele frequency correlation is connected with changes in feeding preferences and with changes of metabolic reactions in the period of pubescence (esterases are responsible for complex ethers

maceration, and superoxidized ismuthases neutralize free oxygen radicals).

In conclusion, we calculated the effective number of investigated helix groups. This was calculated on the basis of a formula that considered the level of inbreeding in the population (Lee, 1978):

$$N_e = \frac{N}{1 + F}$$

The results of calculation are presented in Table 4. Due to some complexities connected with determination of total species abundance in the investigated populations, to obtain comparable data, we calculated the ration of the effective selection size to its total volume. In the following, the indices obtained may be used for N_e calculation in large populations when their abundance is to be determined. According to the data obtained, on average, the N_e/N ratio is 0.772 ± 0.03 , this staying within the usual N_e range (Crow and Morton, 1955; Crow J.F. and Kimura M., 1970). The authors determined that, for most organisms, the N_e number is on average 0.75, while for many human populations it lies within the 0.69–0.95 range.

CONCLUSIONS

The results obtained give an idea of the migration structure and population genofond stage of *Ch. tridens* in the conditions of the urbanized forest–steppe landscape of the Mid-Russia Upland. According to the data obtained, the population of this shellfish, despite pressure from human beings, is reasonably well off, perhaps because of living in a wide range of conditions, including chalk slopes with sharp daily oscillations of temperature and moisture. It should be remarked that the presented results should be considered a starting point for further monitoring of this model species in order to investigate the peculiarities of the evolutionary processes occurring in its population.

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