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Analysis of Genetic Variability in Populations of a Terrestrial Snail *Chondrula tridens* Müll. (Gastropoda, Pulmonata), Based on the *RAPD* and *ISSR* Markers

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Abstract—In this study we examined evolutionary processes in the populations of small mobile animal species, the terrestrial mollusks *Chondrula tridens* Müll. (*Ch. tridens*) in the urbanized landscape. We applied PCR methodology, using the *RAPD* and *ISSR* DNA markers, to define the genetic structure in the *Ch. tridens* populations, inhabiting the Mid-Russian Upland. Analysis of the obtained DNA patterns uncovered the presence of both polymorphic and monomorphic alleles. Based on this data, we defined the levels of genetic variability in the studied *Ch. tridens* populations, and determined the factors affecting formation of the population's gene pool in this species. We present our conclusions on the genome structure and gene pool distribution in the populations of the *Ch. tridens* in the urbanized landscape.

Keywords: PCR; terrestrial mollusks; population gene pool; Mid-Russian Upland
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INTRODUCTION

The present work is a continuation of our study of the population structure of a model species, the terrestrial snail *Ch. tridens* Müll. (three-toothed snail) on the territory of the Russian Plato. These studies were conducted in the forest-steppe and steppe landscapes in the southern part of the Mid-Russian Upland.

The *Ch. tridens* species are widely distributed across Europe, from southwestern France to the Urals, the Crimea and the Caucasus, where they inhabit the steppes and semi-desert land plots (Shileiko, 1984). In the regions examined in this study, the mollusk inhabits chalky slopes in the gullies, ravines, and floodplains. This species belongs to the Mediterranean relict group (Nikolaev, 1981). Thanks to its eurybiontic properties and conchological variations, *Ch. tridens* have long attracted researchers, analyzing evolutionary genetic processes in wild species (Matekin, 1950, Nikolaev, 1981; Grebennikov, 1999; Kramarenko and Sverlova, 2003, 2006; Snegin, Grebennikov, 2011; Komarova, Stojko, 2012). However, all these studies conducted only morphometric analyses of the shell parameters.

In our previous study, we examined the population structure of this species in the south of the Mid-Russian Upland, based on the analysis of conchological signs and allozymes (Snegin, 2011a). However, the use of these markers for determination of the genome structure has several limitations. First, the morphometric parameters of the shells of this mollusk are not discrete and are subject to modification variability. This presents a difficulty in tracing the genetic processes in the studied groups. Second, the protein markers reflect the variability only of the coding part of the genome, which, based on a number of estimates, constitutes about 10% of the total DNA of the snail, while the rest of the "satellite" DNA remains out of sight. To overcome this drawback, we carried out further analysis of the *Ch. tridens* population gene pool, using the *RAPD* and *ISSR* DNA markers for noncoding DNA regions, which occur in multiple copies throughout the genome.

MATERIALS AND METHODS

Tissue Samples

In this study, we used tissue samples of *Ch. tridens*, which were previously stored at the Cryobank created at the Laboratory of Population Genetics and Toxicology at the Belgorod National Research University (BelSU). These population samples were obtained during expeditions in the period from 2006 to 2010. Snails were collected by hand from the soil surface, the stems and leaves of plants, and sometimes found in the leaf litter. In total, we examined 1146 individuals from twenty five populations *Ch. tridens* (Table 1). They included 21 natural populations from the territory of the Mid-Russian Upland and two adventitious groups collected in the city of Belgorod, which contained the largest individuals[1] and formed colonies, reproduc-

[1] The height of the shell of these mollusks, which were most likely imported from the North Cucasus, can reach up to 20 mm, while the maximum height of the local species is 12 mm. The detailed description of this phenomenon can be found in our previous publication (Snegin, 2011b).

Table 1. Description of the collection sites

Site	Biotype description	Coordinates
1. Belgorod	Remains of a natural chalky beam with steppe vegetation within the city of Belgorod (near the car market)	50°36′34.71″ N 36°36′40.91″ E
2. Bekariukovskij Bor	Nature preserve Bekariukovsky Bor, floodplain areas in the Nezhegol' r iver valley	50°26′15.38″N 37°04′23.98″ E
3. Rzhevka	Western exposure chalk slope, floodplain of the Korocha river near Rzhevka village	50°26′32.63″ N 36°58′22.89″ E
4. Afanasovo	Western exposure chalk slope in the floodplain of the Korocha river near the village Afanasovo	50°44′06.34″ N 37°08′49.79″ E
5. Zimovnoe	Nezhegol' river valley, edge of the upland oak woods near the village of Zimovnoe	50°29′35.80″ N 37°09′56.56″ E
6. Kotenevka	Land floodplain of the Chufichka river, near the dumps of the Stoilensky Mining plant, Starooskol'ski district	51°11′09.62″ N 37°31′58.93″ E
7. Saprykino	Mixed forest at the bottom of the beam, exiting in the floodplain of the Dubenka river near the village of Saprykino; zone of influence of the Stojlensky and Lebedinsky Mining Plants	50°36′34.71″ N 36°36′40.91″ E
8. Protochnoe	Chalky hillside opposite the village of Protochnoe, zone of influence of the Lebedinsky and Stojlensky Mining Plants	50°00′18.75″ N 37°31′58.93″ E
9. Kochegury	Chalky beam hillside overlooking the floodplain of the river Olshanka opposite the village of Kochegury, zone of influence of the Stojlensky and Lebedinsky Mining Plants	50°59′36.59″ N 37°35′29.66″ E
10. Gubkin	Western exposure chalky slope in the floodplain of the river Oskolec within the city of Gubkin	51°17′41.29″ N 37°32′21.99″ E
11. Skorodnoe	Beam slope overlooking the floodplain of the river Korocha near the village of Skorodnoye	51°04′22.77″ N 37°15′03.39″ E
12. Stenki Izgorya	Nature preserve Stenki Izgorya, chalky slope with southern exposure and relict steppe vegetation, located in the preserve	50°40′44.80″ N 37°48′29.48″ E
13. Borki	Nature preserve Borka. Chalky hillside in the floodplain of the river Kozinka, northwestern exposure	50°08′12.03″ N 37°53′09.01″ E
14. Valujki	Valley of the river Valuy, foot of the southeastern chalk slope near the city of Valuyki, close to the highway	50°13′24.38″ N 38°00′34.61″ E
15. Lisya Gora (Fox Mountain)	Nature preserve Lisya Gora near the village of Yablonovo, floodplain of the river Oskol, edge of oak forest	50°13′24.38″ N 38°00′34.61″ E
16. Kupiansk	Chalky slope with western exposure in the valley of the river Oskol near the city of Kupyansk (Ukraine)	49°42′19.24″ N 37°37′24.98″ E
17. Kaliuzhnyj Yar	Chalk beam with the eastern exposure opening in the floodplain of the river Idar, nature park Aydarsky	49°57′02.88″ N 38°53′49.32″ E
18. Klimenko	Chalky slope with southern exposure in the valley of the river Sarma, territory of the nature park Aydarsky	49°59′25.30″ N 39°02′35.08″ E
19. Nagol'noe	Chalky slope with southern exposure in the valley of the river Sarma, territory of the nature park Aydarsky	49°58′43.61″ N 38°57′33.69″ E
20. Mining plant	Reclaimed dumps of the Stoilensky Mining Plant	51°17′18.18″ N 37°40′56.29″ E
21/1 Vodstroj	City of Belgorod, motorway embankment descending into the floodplain of the river Gostyanka, mixed forest of willow and maple with addition of spruce	50°35′23.32″ N 36°33′ 58.20″ E
21/2	The same habitat where the large introduced individuals cohabit with aboriginal snails	
22. BelSU (Belgorod National Research University)	City of Belgorod, lawn near the old university building, plantings of chestnuts and firs, habitat of large introduced snails	50°37′ 16.58″ N 36°34′ 36.25″ E.
23. Arakaevo	Right bank of the Michael's pond on the river Serga, Sverdlovsk region, Nizhneserginski district, village Arakaevo; foot of the slope and a steep slope, overgrown with grass; on the slope, there are few rocky outcrops of limestone with rocky talus at the base	56°26′45.00″ N 59°12′ 56.00″ E
24. Nikolaev	Wasteland near the water utility pumping station (HCB-3) in the city of Nikolaev (Ukraine), grassy lawn along the concrete fence, snails were collected in the leaf litter	46°57′ 55.82″ N. 32°02′17.09″ E

Table 2. Characteristic of the primers used in the study

Method	Primer designation	Nucleotide sequence	Number of loci
RAPD	OPC 8	5'-GGGATATCGG-3'	13
ISSR	UBC 827	5'-$(AC)_8$G-3'	18
	SAS 1	5'-$(GTG)_4$GC-3'	18

Table 3. The levels of heterozygosity in the detected loci (data averaged for 25 *Ch. tridens* populations)

Locus no.	He		
	OPC8	SAS1	UBC 827
1	0.212 ± 0.013	0.083 ± 0.009	0.035 ± 0.007
2	0.238 ± 0.012	0.03 ± 0.004	0.061 ± 0.010
3	0.406 ± 0.007	0.102 ± 0.010	0.095 ± 0.010
4	0.390 ± 0.010	0.118 ± 0.009	0.254 ± 0.015
5	0.301 ± 0.010	0.179 ± 0.011	0.137 ± 0.011
6	0.415 ± 0.012	0.167 ± 0.011	0.378 ± 0.011
7	0.385 ± 0.009	0.357 ± 0.012	0.254 ± 0.012
8	0.405 ± 0.007	0.176 ± 0.011	0.296 ± 0.011
9	0.374 ± 0.010	0.398 ± 0.012	0.261 ± 0.015
10	0.411 ± 0.008	0.316 ± 0.013	0.222 ± 0.013
11	0.212 ± 0.013	0.318 ± 0.011	0.355 ± 0.012
12	0.156 ± 0.014	0.355 ± 0.009	0.307 ± 0.012
13	0.068 ± 0.010	0.368 ± 0.010	0.358 ± 0.012
14	–	0.342 ± 0.010	0.343 ± 0.014
15	–	0.243 ± 0.011	0.269 ± 0.014
16	–	0.384 ± 0.011	0.202 ± 0.014
17	–	0.264 ± 0.014	0.147 ± 0.012
18	–	0.041 ± 0.008	0.073 ± 0.010

tively isolated from the aboriginal forms present in the same site. This is particularly clear for site 21 (Table 1), where large individuals coexist in the same biotope as the small aboriginal specimens. Thus, for our analysis, we subdivided site 21 into two groups, 21/1 and 21/2. In addition, for the outgroup comparison, we took samples from populations living in the Urals (site 23 in Table 1), and along the Black Sea coast (site 24 in Table 1).

PCR Analysis

Analysis of genome variability was carried out using the two polymerase chain reaction (PCR) methodologies: the *RAPD* (*Random amplified polymorphic DNA*) (Welsh, McClelland, 1990) and *ISSR* (*Inter simple sequence repeats*) (Zietkiewicz et al., 1994). PCR reactions were carried out using three primers (Table 2). Amplification was performed using thermal cyclers, MJ Mini and MyCycler (Bio-Rad, USA).

RAPD method. Reactions were carried out in a 20 μL mixture containing 20 ng of genomic DNA, PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$), 0.25 mM dNTP, 0.5 μM of the primer (Table 2), and 1 unit of Taq DNA polymerase (inhibited for a hot start). The reactions were conducted under the following conditions: hot start, 2 min at 94°C, followed by 35 cycles (denaturation for 45 s at 94°C, primer annealing for 15 s at 36°C or 15 s at 45°C, primer annealing for 1 min 72°C), additional synthesis after cycles for 10 min at 72°C, and cooling to 4°C.

ISSR method. Reactions were performed in a 25 μL mixture, containing 20 ng of genomic DNA, PCR buffer (67 mM Tris-HCl (pH 8.8), 16 mM $(NH_4)_2SO_4$, 5 mM β-mercaptoethanol, 7 mM EDTA, 3 mM $MgCl_2$), 0,25 mM dNTP, 0,5 mM of each primer (table 2), and 1 unit of Taq DNA polymerase (inhibited for a hot start). The reactions were carried out as follows: hot start for 2 min at 94°C, 40 cycles (denaturation for 30 s at 94°C, primer annealing for 30 s at 55°C, synthesis for 2 min at 72°C), additional synthesis for 10 min at 72°C, and cooling to 4°C. The PCR products were separated by electrophoresis in a 2% agarose gel in a TAE buffer, at 100 V for 45 min. The gels were then stained with ethidium bromide.

Analysis of the Data

We constructed binary matrices based on the pictures of the amplified fragments, obtained using electrophoresis. The presence of the band was designated as "1" (allele *p*) and absence, as "0" (allele *q*). Application of the *RAPD* method can result in a portion of nonspecific amplification products. Thus, only the amplicons that were clearly visible and reproducible were used in our analysis. The criterion for reproducibility was the manifestation of the same amplicons after repeated PCR on samples from the same individuals.

We found 49 loci in this snail species: 13 with the use of the *OPC 8* primer (*RAPD* method), and 18 loci, using the primers *SAS 1* and *UBC 827* (ISSR method). The obtained DNA patterns and their interpretation are presented in Fig. 1.

Data processing was carried out using the GenAlEx (Peakall, Smouse, 2001), POPGENE 32 (Yeh et al., 2000), and MEGA5 (Tamura et al., 2011) programs. The Debets polygons were constructed using Statistica 6.0 software.

RESULTS AND DISCUSSION

Table 3 shows the levels of heterozygosity for 49 loci, calculated based on the analysis of 25 populations. Based on the obtained data, the most polymorphic for the *RAPD* marker were loci, 3, 4, 6, 8 and 10. Analysis for the *ISSR* markers, showed that loci 7, 9, and 16 were the most variable for *SAS 1*, and loci 6, 11, 13, and 14 for marker *UBC 827*. The most monomor-

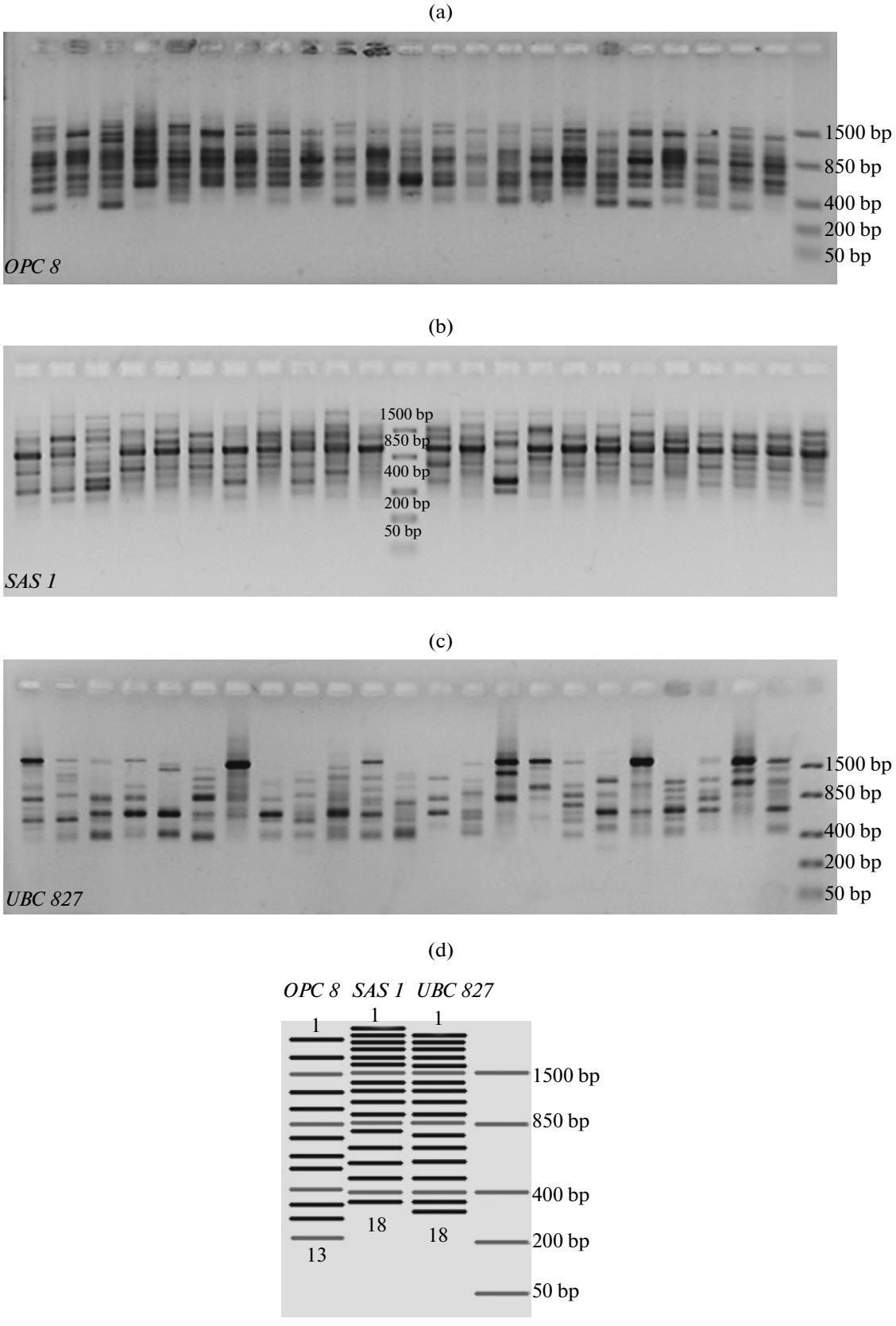

Fig. 1. (a, b, c) DNA patterns of the *Ch. tridens*. (d) Interpretation of the DNA patterns of the *Ch. tridens* (only the first and last loci are identified by numerals).

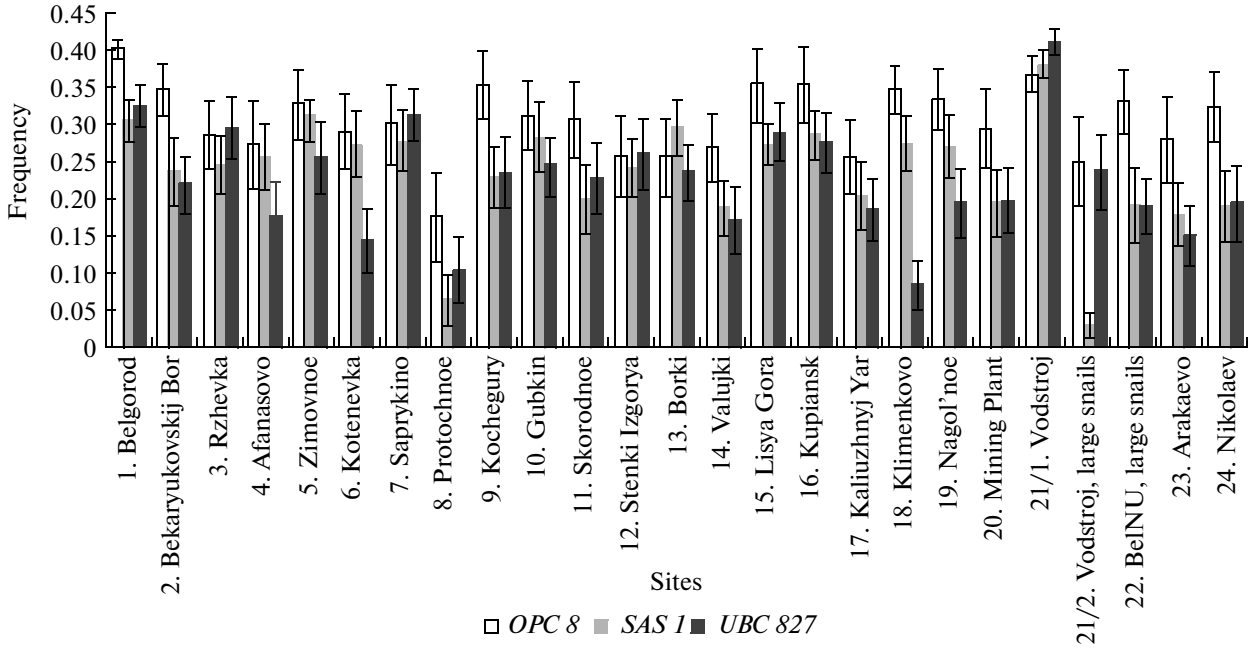

Fig. 2. Levels of heterozygosity at different DNA loci in the populations of *Ch. tridens*.

phic loci for the *OPC 8* marker were loci 12 and 13. The *SAS 1* marker showed the least variability in loci 12 and 18. Loci 1, 2, 3, and 18 were the least variable for the *UBC 827* marker. The average loci heterozygosity for the *RAPD* marker ($H_e = 0.306 \pm 0.010$) was higher than for the *ISSR* (for *SAS 1*, $H_e = 0.235 \pm 0.010$; and for *UBC 827*, $H_e = 0.225 \pm 0.011$).

The calculated levels of heterozygosity and the graphic polygons for the studied populations, constructed based on the analysis with different primers, are shown in Figures 2 and 3, and Table 4.

Our results indicate that the studied *Ch. tridens* populations differ in the degree of variations at the different DNA loci (Fig. 2). For example, for the locus *UBC 827*, the lowest values for heterozygosity were detected at the Klimenkova (no. 18) and Protochnoe (no. 8) sites. The latest site also registered the lowest level of heterozygosity for the *OPC 8* and *SAS 1* markers among the natural populations of the forest-steppes. The adventitious colony 21/2 showed a low level of heterozygosity for the *SAS 1* loci. Moreover, the obtained heterozygosity indexes were significantly higher for all loci in the aboriginal snail populations, inhabiting the same biotope (21/1), compared to the alien group in the same site (21/2). The two cohabiting colonies (21/1 and 21/2) also fell into different clusters based on the analysis of the allelic frequencies (Fig. 4). These findings, give an indirect confirmation to our hypothesis, proposed earlier, which postulated the absence of interbreeding between the two forms of snails (Snegin, 2011 b).

For the total of all examined DNA loci, most of the heterogeneous natural populations were the ones dwelling within the city of Belgorod (sites 1 and 21/1, Table 4). Slightly lower indices were obtained for the Kupyansk (no. 16), Lisya Gora (no. 15), Saprykino (no. 7), and Bekariukovskij Bor (no. 2) groups. The most monomorphic is the previously mentioned Protochnoe population (no. 8), which belongs to a group of populations living in the area of influence of the Stojlensky and Lebedinsky Mining and Processing Plants, where we observed an extreme fragmentation of the snail populations, presumably affected by the active landscaping process: the creation of shafts, roads, and overpasses. However, we did not detect a significant reduction in the levels of variability in other populations in the same area (sites 6, 7, 9, 10, and 20). This is particularly clear for site 20 (Mining Plant), where snails live in an isolated biotope, formed relatively recently (30 years ago) on the dumps of the Mining and Processing Plants. The ratio of allelic frequencies and the levels of genetic variability at this site (especially for the *RAPD* loci) were comparable to the values obtained for other populations, including aboriginal groups from the relic especially protected areas (12, 13, 17, 18, 19).

We suggest that this genetic similarity between the populations of *Ch. tridens* is not due to the migrations of individuals between the populations, but is determined by the fact that in the conditions of isolation, the frequency of homozygous combinations for the same alleles is increased, as we have shown previously, using the isozyme markers (Snegin, 2011a). This phenomenon is probably governed by the similarities in the vectors of natural selection in the forest-steppe

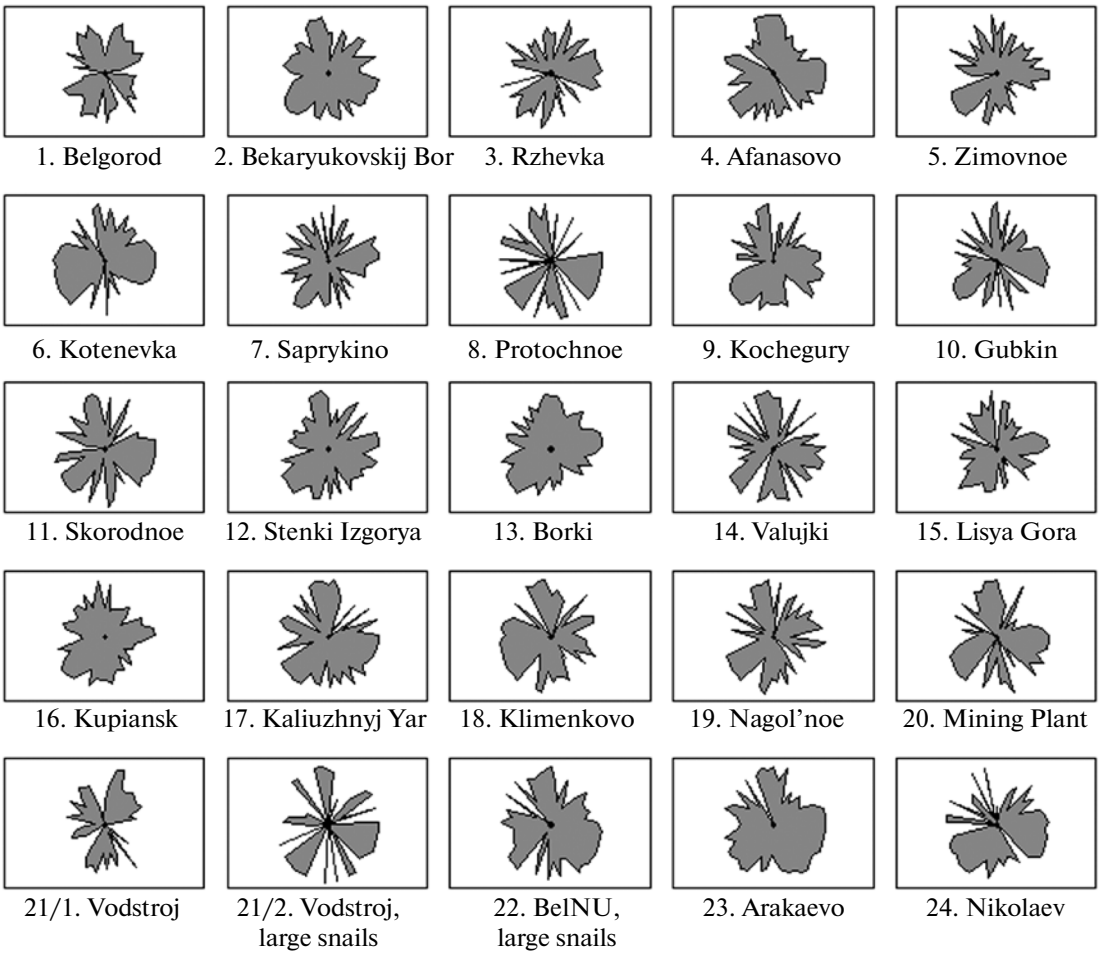

Fig. 3. Debets polygons, constructed based on the overall frequencies for the *q* allele in 49 DNA loci in the populations of *Ch. tridens*.

conditions, the genetic drift, and the processes of genetic revolution in isolated groups (Mayr, 1968).

We note that in the south of the Mid-Russian Upland, the area of suitable biotopes for the *Ch. tridens* is considerably larger (up to 20% of the total area) than for other mollusks, for example the *Bradybaena fruticum* (9% of the area). This is due to the fact that the three-toothed snail *Ch. tridens* is a xero-mesophile and can live in a diverse range of conditions, from chalky preterrace slopes, to forests, floodplains, ravines, and gullies. Such eurytopicity promotes a lesser degree of fragmentation between the populations of this species compared with the shrub snail. However, due to its relatively low mobility, the *Ch. tridens* is more likely to rely on external factors for their resettlement in the studied area, such as the water flow, wind, animals and, in particular in the last centuries, people. On the other hand, the extensive plowing of the land and destruction of the natural chalk communities violate the natural migration processes and lead to a higher degree of isolation between the groups, which is reflected in the calculated indices of subdivisions and the average gene flow.

Our assessment of the degree of differentiation in the populations of *Ch. tridens*, based on the model developed by M. Nei (Nei, 1975), uncovered a mild dissociation between the studied groups in the forest-steppes ($G_{st} = 0.177$, Table 5). The average gene flow was determined to be larger than unity ($Nm = 2.33$)[2].

The average value of G_{st} corresponds to the level of genetic differentiation during a selection-neutral process. Therefore, the loci with large values of G_{st} are presumed to be under the effect of disruptive selection, and the loci with a low index of subdivision are likely affected by the stabilizing selection (Dinamika..., 2004). Based on our data (Table 5), the largest differentiation between the populations was identified for the loci *OPC 8–11*, *SAS 1–17*, and *UBC 827–4*, and

[2] Based on the "Shifting balance theory of evolution" (Wright, 1970), maintenance of genetic equilibrium in a metapopulation requires a genetic drift of 1–2 individuals per generation.

Table 4. Parameters of genetic diversity in the populations of *Ch. tridens* averaged over the set of the studied DNA loci

Site	*N*	*P* %	*A*	*Ae*	I_{sh}	*He*
1. Belgorod	54	100.00	2.0 ± 0.0	1.55 ± 0.04	0.514 ± 0.019	0.339 ± 0.016
2. Bekaryukovskij Bor	189	95.92	1.96 ± 0.03	1.43 ± 0.05	0.402 ± 0.033	0.260 ± 0.024
3. Rzhevka	18	87.76	1.88 ± 0.05	1.45 ± 0.05	0.421 ± 0.032	0.275 ± 0.024
4. Afanasovo	16	67.35	1.67 ± 0.07	1.40 ± 0.05	0.348 ± 0.04	0.232 ± 0.029
5. Zimovnoe	31	85.71	1.86 ± 0.05	1.50 ± 0.05	0.442 ± 0.034	0.295 ± 0.025
6. Kotenevka	32	73.47	1.73 ± 0.06	1.39 ± 0.05	0.35 ± 0.038	0.230 ± 0.027
7. Saprykino	18	93.88	1.94 ± 0.03	1.50 ± 0.05	0.452 ± 0.03	0.297 ± 0.023
8. Protochnoe	12	28.57	1.29 ± 0.06	1.19 ± 0.05	0.160 ± 0.038	0.109 ± 0.026
9. Kochegury	38	89.80	1.90 ± 0.04	1.44 ± 0.05	0.403 ± 0.035	0.264 ± 0.026
10. Gubkin	31	85.71	1.86 ± 0.05	1.47 ± 0.05	0.419 ± 0.035	0.276 ± 0.025
11. Skorodnoe	60	89.80	1.90 ± 0.04	1.40 ± 0.05	0.363 ± 0.038	0.238 ± 0.028
12. Stenki Izgorya	37	83.67	1.84 ± 0.05	1.42 ± 0.05	0.386 ± 0.036	0.253 ± 0.026
13. Borki	58	87.76	1.88 ± 0.05	1.43 ± 0.05	0.405 ± 0.033	0.263 ± 0.024
14. Valujki	94	89.80	1.90 ± 0.04	1.32 ± 0.05	0.325 ± 0.034	0.205 ± 0.024
15. Lisya Gora	35	93.88	1.94 ± 0.03	1.50 ± 0.04	0.459 ± 0.028	0.301 ± 0.022
16. Kupiansk	86	93.88	1.94 ± 0.03	1.50 ± 0.05	0.455 ± 0.031	0.300 ± 0.023
17. Kaliuzhnyj Yar	43	83.67	1.84 ± 0.05	1.34 ± 0.05	0.329 ± 0.036	0.211 ± 0.026
18. Klimenkovo	50	79.59	1.80 ± 0.06	1.36 ± 0.05	0.348 ± 0.036	0.224 ± 0.026
19. Nagol'noe	31	85.71	1.86 ± 0.05	1.43 ± 0.05	0.395 ± 0.035	0.258 ± 0.026
20. Mining Plant	53	79.59	1.80 ± 0.06	1.37 ± 0.05	0.341 ± 0.038	0.222 ± 0.027
21/1. Vodstroj	20	100.00	2.0 ± 0.0	1.66 ± 0.03	0.574 ± 0.013	0.389 ± 0.011
Average for the Central Russian Upland		84.5 ± 3.3	1.85 ± 0.04	1.43 ± 0.05	0.395 ± 0.033	0.259 ± 0.024
21/2. Vodstroj, large snails	42	55.10	1.55 ± 0.07	1.29 ± 0.05	0.250 ± 0.040	0.166 ± 0.028
22. BelNU, large snails	50	75.51	1.75 ± 0.06	1.38 ± 0.05	0.349 ± 0.038	0.228 ± 0.027
23. Arakaevo	22	67.35	1.67 ± 0.07	1.32 ± 0.05	0.300 ± 0.039	0.195 ± 0.027
24. Nikolaev	25	67.35	1.67 ± 0.07	1.39 ± 0.05	0.339 ± 0.041	0.226 ± 0.029
Overall mean		81.6 ± 3.2	1.82 ± 0.05	1.42 ± 0.05	0.381 ± 0.034	0.250 ± 0.025

N—number of analyzed individuals, *P%*—percentage of polymorphic loci, *A*—average number of alleles per locus, *Ae*—effective number of alleles, Ish—Shannon index, *He*—Expected heterozygosity.

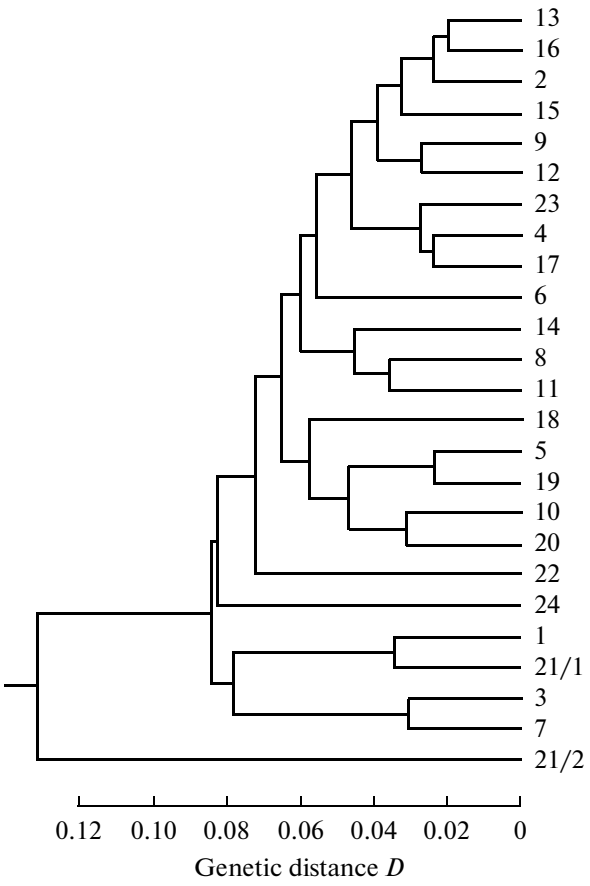

Fig. 4. Dendrogram of the Nei's genetic distances between the populations of *Ch. tridens*, calculated using UPGMA software.

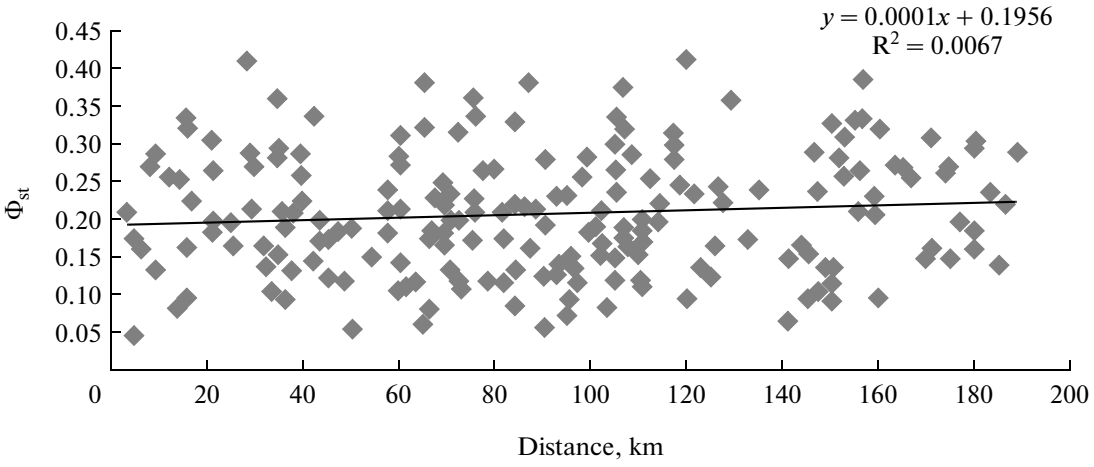

Fig. 5. Analysis of dependence of the differentiation index Φ_{st} between pairs of *Ch. tridens* populations on the geographical distances between them.

the smallest, for the loci *OPC 8–3*, *SAS 1–8*, and *UBC 827–7*.

Analysis of the molecular variance (AMOVA, Excoffier et al., 1992) (Table 6) confirmed a significant similarity between the populations of *Ch. tridens*. Only 19% of variability accounted for the interpopulation differences, with $\Phi_{st} = 0.185$ and $Nm = 0.954$.

The obtained evidence indicates that similar genetic processes take place in geographically remote populations within the same landscape structure.

Table 5. Indexes of genetic differentiation (Nei's distances) for the *Ch. tridens* populations

Locus	Locus number	Ht	Hs	Gst	Nm
OPC 8	1	0.260	0.233	0.104	4.30
	2	0.264	0.233	0.120	3.65
	3	0.436	0.403	0.077	6.04
	4	0.500	0.377	0.245	1.54
	5	0.330	0.283	0.141	3.04
	6	0.499	0.431	0.136	3.18
	7	0.495	0.380	0.233	1.65
	8	0.453	0.410	0.095	4.75
	9	0.476	0.395	0.171	2.43
	10	0.496	0.408	0.176	2.34
	11	0.320	0.223	0.304	1.14
	12	0.174	0.146	0.160	2.62
	13	0.095	0.079	0.175	2.37
SAS 1	1	0.111	0.097	0.125	3.51
	2	0.037	0.036	0.039	12.28
	3	0.141	0.122	0.138	3.13
	4	0.149	0.141	0.056	8.40
	5	0.230	0.207	0.097	4.64
	6	0.207	0.187	0.098	4.59
	7	0.480	0.385	0.198	2.03
	8	0.217	0.204	0.061	7.71
	9	0.491	0.404	0.177	2.32
	10	0.370	0.321	0.133	3.26
	11	0.404	0.345	0.146	2.92
	12	0.394	0.354	0.100	4.48
	13	0.458	0.398	0.132	3.28
	14	0.419	0.360	0.140	3.08
	15	0.298	0.249	0.164	2.56
	16	0.489	0.394	0.193	2.09
	17	0.362	0.289	0.203	1.96
	18	0.059	0.049	0.182	2.25
UBC 827	1	0.049	0.040	0.183	2.23
	2	0.089	0.071	0.197	2.04
	3	0.092	0.080	0.128	3.40
	4	0.442	0.270	0.390	0.78
	5	0.146	0.131	0.109	4.10
	6	0.434	0.377	0.131	3.32
	7	0.309	0.284	0.081	5.64
	8	0.400	0.302	0.244	1.55
	9	0.380	0.261	0.315	1.09
	10	0.247	0.215	0.131	3.31
	11	0.494	0.359	0.272	1.34
	12	0.359	0.308	0.143	3.00
	13	0.470	0.383	0.184	2.22
	14	0.455	0.364	0.201	1.99
	15	0.428	0.297	0.306	1.13
	16	0.229	0.183	0.201	1.99
	17	0.209	0.171	0.182	2.24
	18	0.073	0.060	0.181	2.26
Mean		0.315 ± 0.023	0.259 ± 0.015	0.177	2.33

Gst—share of interpopulation genetic diversity in the overall diversity of the species, Ht—expected proportion of heterozygous genotypes in the general population, Hs—average for all sub-populations of intra-population diversity value, Nm—average genetic drift per generation.

Table 6. Values for the molecular variance (AMOVA) of the DNA loci, calculated for the *C. tridens* populations (for 21 populations)[3]

Origin of diversity	Number of dimensions (df)	Sum of squares (SS)	Square mean (MS)	Dispersion (V)	%	Φ_{st}	*P*	*Nm*
Between populations	20	1688.784	84.439	1.662	19%	0.185	0.010	0.954
Within populations	985	7207.496	7.317	7.317	81%			
Overall	1005	8896.280	91.756	8.979				

The cluster analysis of the DNA loci (the results are shown in Fig. 4) showed the absence of any geographical reference for most of the defined clusters, except for the two urban populations at sites 1 and 21/1. Thus, a relatively large genetic distance was detected both between geographically closely spaced and remote groups. This is due, as mentioned before, to violations in the naturally formed channels of gene migration between the populations in the context of the urbanized landscape. This conclusion is confirmed by the fact that pairwise estimates of the Φ_{st} index are not correlated to the geographical distances between the populations (Fig. 5, $R = 0.082 \pm 0.069$).

Among the snail groups taken for outgroup comparisons, the Ural population (no. 23) was genetically closer to that of forest-steppe populations than the Black Sea shore sample (no. 24). The later was significantly distant from the majority of the studied populations in the forest-steppe region.

CONCLUSIONS

The obtained results give an idea of the structure of resettlements and the state of the population's gene pool of the *Ch. tridens* in the context of the urbanized forest-steppe landscape in the Mid-Russian Upland. The results indicate that the populations of this mollusk show a considerable level of genetic polymorphism despite the pressure from human activities. This is likely due to their habitation in a wide range of conditions, including the chalk slopes, which are considered rather extreme for mollusks, with their sharp daily fluctuations in temperature and humidity. However, the processes of insularization, taking place in the studied territory, result in violations of the historically developed routes of migration, which lead to a significant isolation of the populations and to a gradual loss of the allelic diversity. The presented results can be considered as a starting point for further monitoring of this model organism in order to clarify the features of the evolutionary processes in its populations.

3 The adventitious colonies, 21/2 and 22, and the populations from the Urals (23) and the Black Sea shore (24), were excluded from this analysis.

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