

Assessment of the State of Population Gene Pools of Terrestrial Mollusks in Conditions of Influence of Ore Dressing Combines from the Example *Bradybaena Fruticum* Müll (Gastropoda, Pullmonata)

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Abstract—On the basis of analysis of the morphological and genetical variability revealed by the method of gel-electrophoresis of proteins, the state of gene pools of populations of modeling species *Bradybaena fruticum* (bush snails) in conditions of influence of ore dressing combines is investigated. The authentic decrease of the level of heterozygosity and decrease of an allelic diversification in the researched bunches is fixed in comparison with the control populations, which is caused both by natural factors and by factors of the anthropogenic parentage. The genetic-automatic processes in populations are considered, and vectors of natural selection are defined. On the basis of calculation of the effective number, a forecast of the time of existence of populations is given.

Keywords: population gene pools, terrestrial mollusks, ore dressing combines, forest—steppe landscape

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INTRODUCTION

This work is part of a comprehensive assessment of the impact of the Stoilenskii and Lebedinskii combines on the adjacent natural ecosystems. These plants are located in the south of the Central Russian Upland (Belgorod region, Russia) and enter the development system of the Kursk magnetic anomaly (Fig. 1). In the period from 2006 to 2009, the state of population gene pools of the model species *Bradybaena fruticum* Fer. (Bush snail) living in the zone of influence of the above combines was studied.

The choice of this species is not accidental. The bush snail is a rather large object (width of the shell up to 25 mm), has a strongly marked polymorphism of conchological and biochemical features, forms numerous colonies, and is widely spread on the Continent. In this regard, the species has long been used as an object of monitoring various urban areas (Matekin, Makeeva, 1977; Zeifert, 1987; Khokhutkin, 1997, Matekin et al, 2000; Makeeva, etc. 2005; Snegin, 1999, 2005a, b, 2006; Makeeva, 2008).

METHODS AND MATERIALS

In order to collect mollusks in an area of 2 × 2 m with an entomological net, mowing was carried out. In

the net samples of different ages, resting in the blades of grass, were caught. Then, at the same location mollusks that fell during mowing and mollusks that were on the ground during the collection were collected by hand from the soil. When there was a low density of mollusks, the plot size was increased twofold. In each investigated biocoenosis, we conducted three or four collections. We determined the coordinates of the samples by a Garmin 76 GPS navigator. We determined the total area occupied by the snail biotope according to a map.

As genetic markers of the population structure, we used Mendelian characters, which are presented in Table 1.

We carried out extraction of water-soluble proteins from the retractor of the mollusk leg by freezing it at –80°C followed by thawing and mechanical grinding of a teflon homogenizer in 0.05 M Tris-HCl-buffer (pH 6.7). We carried out electrophoresis of isoenzymes at 10% in polyacrylamide gel in the chamber VE-3 (Helicon). Gel Tris-HCl-buffer (stacking gel pH 6.7, running gel pH 8.9), electrode tris-glycine -buffer (pH 8.3). We carried out block staining in the substrate mixture: tris-HCl (pH 7.4), a-naftilatsetat, fast red TR.

In total for the analysis, we used 1021 unit of *B. fruticum* of 18 points. Thirteen points were located in the

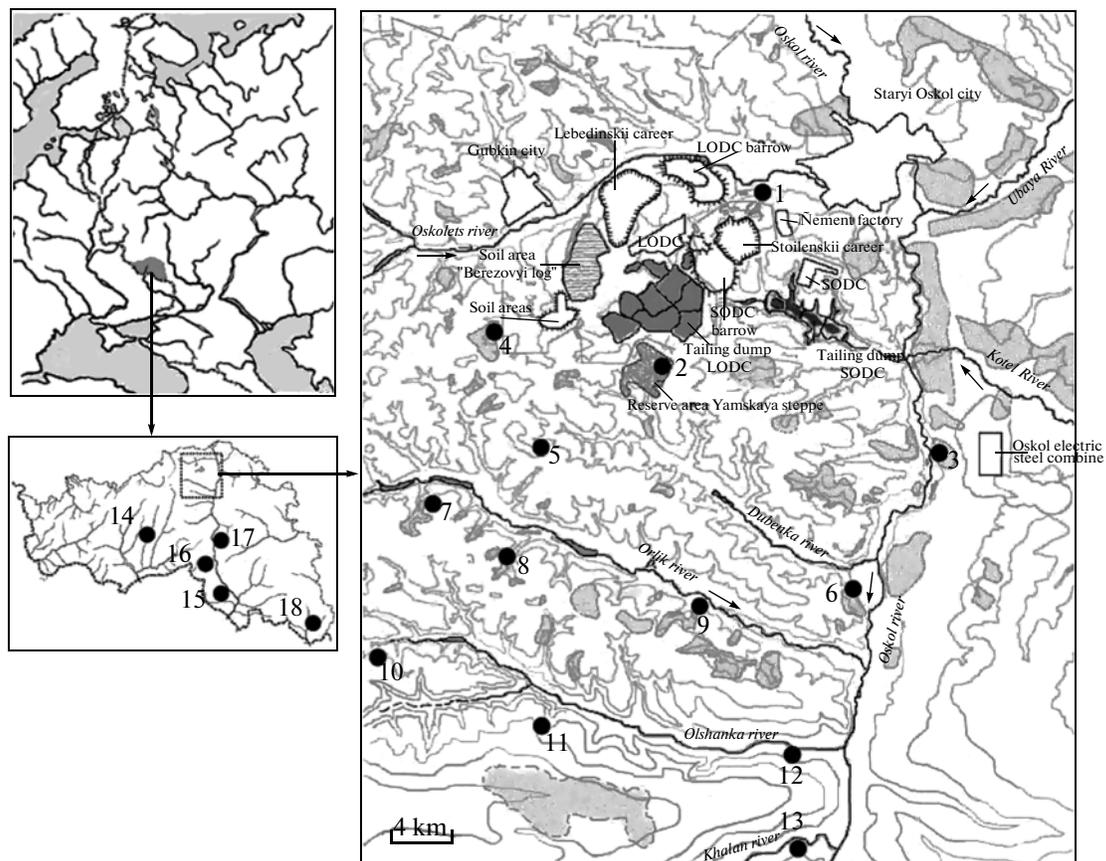


Fig. 1. Collection points of mollusks in the exploration area (abbreviations: SODC and LODC are the Stoilenski and Lebedinski ore dressing combines, respectively, OESC is the Oskol electric steel combine).

area of influence of the ore dressing combines (ODCs) at different distances from the plant (Fig. 1, Table 2). For comparison, in the capacity of control variants, we used populations in areas remote from industries: no. 14 is the floodplain of the Korocha River near the rural area of Dmitrievka (Korochanski area); no. 15 is a specially protected natural boundary Lisya Gora (near the rural settlement Yablonovo, Valuiskii area); no. 16 is the Borki tract (floodplain of the Kozinka River, Valuiskii area); no. 17 is the reserve area Stenki Izgorya

(Novooskolski area); no. 18 is the Rovenskii Natural Park (floodplain of the Aidar, Rovenskoi area).

Data processing was carried out using the program GenAlEx (Peakall R., Smouse, 2001).

RESULTS AND DISCUSSION

In our assessment of the population status of the studied region, we proceeded from the view according to which the criterion of the existence of a stable pop-

Table 1. The markers used to analyze the genetic structure of populations of *B. fruticum*

Marker	Designation	Type of inheritance	Source
Nonspecific esterases	Est 2 Est 3 Est 4 Est 5	Codominant: with three alleles, with five alleles ¹	Matekin, Makeeva 1977; Makeeva et al. 2005
Presence of brown longitudinal stripes on the shell	P+	Homozygotic phenotype according to recessive allele	Khokhutkin, 1979
Yellow coloration of the shell	Tz ₃	Homozygotic phenotype according to yellow coloration	Snegin, 1999, 2005

Footnote 1. In the study of these populations we did not find the alleles Est 4–5 and Est 5–5.

Table 2. Heterozygosity frequency of esterase loci in populations of bush snails

Locus	Allel	1. Stolilo. Floodplain forest	2. Yamskaya steppe. Preserve area	3. Oskolsky electrometallurgical works. Floodplain forest	4. Sergeevka. Oak forest	5. Dubravka. Small oak forest in steppe ravines	6. Dubenka. Floodplain oak forest in steppe ravines	7. Melavoe. Small oak forest in steppe ravines	8. Dalnyaya Ivenka. Small oak forest in steppe ravines	9. Orlik. Willow—shrub	10. Konyshino. Small oak forest in steppe ravines	11. Kochehy. Small oak forest in steppe ravines	12. Olsanka. Willow—shrub	13. Krasnyi ostrov. Floodplain forest	14. Dmitrievka. Floodplain forest	15. Lisyá gora. Oak forest	16. Borki. Willow—shrub	17. Stenki izgora. Alder forest	18. Rovenskii. Floodplain forest
Est2	1	0.000	0.177	0.167	0.000	0.200	0.199	0.353	0.313	0.250	0.066	0.788	0.081	0.009	0.144	0.342	0.319	0.283	0.000
	2	0.000	0.685	0.833	1.000	0.688	0.801	0.206	0.448	0.750	0.926	0.212	0.887	0.982	0.850	0.658	0.534	0.678	0.797
	3	0.000	0.138	0.000	0.000	0.113	0.000	0.441	0.240	0.000	0.007	0.000	0.032	0.009	0.006	0.000	0.147	0.039	0.203
Est3	H ₀	0.000	0.354	0.333	0.000	0.350	0.297	0.412	0.563	0.125	0.147	0.303	0.161	0.036	0.250	0.450	0.500	0.566	0.243
	H _e	0.000	0.480	0.278	0.000	0.475	0.319	0.638	0.644	0.375	0.137	0.334	0.206	0.035	0.257	0.450	0.591	0.459	0.323
	F	—	0.262	-0.200	—	0.263	0.068	0.355	0.127	0.667	-0.072	0.093	0.215	-0.014	0.026	0.000	0.154	-0.232	0.247
Est4	1	0.025	0.063	0.106	0.423	0.013	0.044	0.029	0.000	0.156	0.096	0.000	0.161	0.161	0.369	0.008	0.009	0.053	0.189
	2	0.975	0.870	0.803	0.385	0.988	0.953	0.941	0.958	0.813	0.882	1.000	0.806	0.59	0.613	0.792	0.802	0.947	0.811
	3	0.000	0.067	0.091	0.192	0.000	0.003	0.029	0.042	0.031	0.022	0.000	0.032	0.080	0.019	0.200	0.190	0.000	0.000
Est5	H ₀	0.050	0.205	0.212	0.231	0.025	0.082	0.118	0.083	0.375	0.235	0.000	0.290	0.429	0.750	0.333	0.224	0.079	0.324
	H _e	0.049	0.235	0.336	0.636	0.025	0.091	0.112	0.080	0.314	0.212	0.000	0.323	0.392	0.489	0.333	0.321	0.100	0.307
	F	-0.026	0.127	0.368	0.637	-0.013	0.093	-0.046	-0.043	-0.193	-0.111	—	0.100	-0.094	-0.535	0.000	0.302	0.208	-0.057
N	1	0.050	0.051	0.030	0.000	0.375	0.193	0.044	0.010	0.000	0.140	0.000	0.194	0.232	0.294	0.008	0.103	0.329	0.365
	2	0.950	0.740	0.545	0.635	0.600	0.807	0.765	0.750	0.750	0.860	0.970	0.742	0.625	0.613	0.908	0.897	0.664	0.635
	3	0.000	0.209	0.424	0.365	0.025	0.000	0.191	0.240	0.000	0.000	0.030	0.048	0.107	0.094	0.075	0.000	0.000	0.000
N	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.016	0.036	0.000	0.008	0.000	0.007	0.000
	H ₀	0.050	0.402	0.303	0.731	0.450	0.222	0.471	0.500	0.500	0.221	0.061	0.226	0.536	0.575	0.150	0.207	0.395	0.243
	H _e	0.095	0.406	0.522	0.464	0.499	0.312	0.377	0.380	0.375	0.240	0.059	0.409	0.543	0.530	0.169	0.185	0.450	0.463
N	F	0.474	0.011	0.419	0.576	0.098	0.289	-0.249	-0.316	-0.333	0.082	-0.031	0.449	0.013	-0.085	0.113	-0.115	0.123	0.475
	1	0.000	0.000	0.000	0.000	0.000	0.019	0.015	0.042	0.000	0.000	0.091	0.032	0.000	0.031	0.000	0.000	0.013	0.000
	2	1.000	0.992	1.000	0.000	0.988	0.946	0.956	0.917	1.000	1.000	0.909	0.968	0.982	0.956	0.992	1.000	0.987	1.000
N	3	0.000	0.008	0.000	0.000	0.013	0.035	0.029	0.042	0.000	0.000	0.000	0.000	0.018	0.006	0.008	0.000	0.000	0.000
	4	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	0.006	0.000	0.000	0.000	0.000
	H ₀	0.000	0.016	0.000	0.000	0.025	0.095	0.088	0.083	0.000	0.000	0.182	0.065	0.036	0.088	0.017	0.000	0.026	0.000
N	H _e	0.000	0.016	0.000	0.000	0.025	0.103	0.085	0.156	0.000	0.000	0.165	0.062	0.035	0.085	0.017	0.000	0.026	0.000
	F	—	0.008	—	—	-0.013	0.079	-0.036	0.467	—	—	-0.100	-0.033	-0.018	-0.035	-0.008	—	-0.013	—
	N	40	127	33	26	40	158	34	48	16	68	33	31	56	80	60	58	76	37

Table 3. Indicators of genetic variability in the studied bush snail populations

Sample	<i>P</i> , %	<i>A_e</i>	<i>H₀</i>	<i>H_e</i>	μ	<i>h_μ</i>	<i>I</i>
(1) Stoilo	50	1.04 ± 0.024	0.025 ± 0.024	0.036 ± 0.028	1.19 ± 0.5	0.52 ± 0.15	0.08
(2) Yamskaya step	100	1.48 ± 0.065	0.244 ± 0.037	0.284 ± 0.039	2.07 ± 0.27	0.38 ± 0.08	0.47
(3) Oskolsky electro-metallurgical works	75	1.49 ± 0.112	0.212 ± 0.07	0.284 ± 0.077	1.88 ± 0.55	0.47 ± 0.16	0.47
(4) Sergeevka	50	1.65 ± 0.142	0.240 ± 0.082	0.275 ± 0.086	1.72 ± 0.54	0.49 ± 0.15	0.43
(5) Dubravka	100	1.49 ± 0.102	0.213 ± 0.063	0.256 ± 0.067	1.86 ± 0.49	0.46 ± 0.14	0.43
(6) Dubenka	100	1.28 ± 0.043	0.174 ± 0.029	0.206 ± 0.031	1.7 ± 0.27	0.5 ± 0.08	0.36
(7) Melavoe	100	1.65 ± 0.141	0.272 ± 0.074	0.303 ± 0.077	2.14 ± 0.49	0.37 ± 0.14	0.55
(8) Dalnyaya livenka	100	1.67 ± 0.136	0.307 ± 0.065	0.315 ± 0.066	2.1 ± 0.41	0.39 ± 0.11	0.55
(9) Orlik	75	1.41 ± 0.189	0.25 ± 0.106	0.266 ± 0.108	1.72 ± 0.8	0.48 ± 0.23	0.42
(10) Konyshino	75	1.19 ± 0.059	0.151 ± 0.042	0.147 ± 0.042	1.6 ± 0.4	0.53 ± 0.11	0.277
(11) Kochegry	75	1.19 ± 0.078	0.136 ± 0.058	0.140 ± 0.059	1.43 ± 0.57	0.58 ± 0.16	0.239
(12) Olshanka	100	1.37 ± 0.103	0.185 ± 0.068	0.25 ± 0.076	2.06 ± 0.57	0.4 ± 0.16	0.473
(13) Krasnyi ostrov	100	1.48 ± 0.104	0.259 ± 0.057	0.251 ± 0.056	2.07 ± 0.4	0.4 ± 0.12	0.472
(14) Dmitrievka	100	1.63 ± 0.158	0.416 ± 0.054	0.340 ± 0.051	2.1 ± 0.36	0.39 ± 0.1	0.572
(15) Lisya gora	100	1.38 ± 0.093	0.238 ± 0.054	0.242 ± 0.054	1.78 ± 0.42	0.47 ± 0.12	0.4
(16) Borki	75	1.53 ± 0.092	0.233 ± 0.054	0.274 ± 0.057	1.86 ± 0.37	0.43 ± 0.1	0.462
(17) Stenki izgorya	100	1.45 ± 0.092	0.266 ± 0.049	0.259 ± 0.049	1.81 ± 0.37	0.47 ± 0.1	0.424
(18) Rovenskii	75	1.45 ± 0.102	0.203 ± 0.065	0.273 ± 0.071	1.64 ± 0.54	0.52 ± 0.16	0.411
Average	86.11	1.42 ± 0.102	0.223 ± 0.058	0.245 ± 0.061	1.82 ± 0.44	0.46 ± 0.13	0.416

Footnote 1. *P* is the percentage of polymorphic loci; *A_e* is the average effective number of alleles per locus; *H₀* is the average observed heterozygosity; *H_e* is the average expected heterozygosity; μ is the average number of phenotypes; *h_μ* is the proportion of rare morphs; *I* is the Shannon index (indices μ and *h_μ* are listed according to Zhivotovskii, 1991.)

ulation was the level of its allelic and genotypic diversity. It is known that if in an isolated numerically insignificant population there is no exchange of genetic information with other intraspecific groups for long time, the allelic diversity of the population is reduced due to inbreeding. This effect of hermaphroditic species of mollusks can be enhanced by self-fertilization, which sometimes takes place in isolated groups (Baur, Klemm, 1989). In addition, due to increase in frequency of consanguineous mating in such a population, the probability of segregation of recessive mutations increases. This, in turn, significantly reduces the viability of this group, because a certain level of genetic variability, as the “mobilization reserve,” ensures the stability of the population as a system.

It has been experimentally proved, for example, that an increase in homozygosity in the populations of some species by 10% reduces *their* reproductive capacity by not less than 25% (Frankel, Soule, 1981).

Previous studies of the population structure of *B. fruticum* within the Southern Upland steppe showed that in most colonies of the snails elevated homozygote phenotypes and allelic diversity decline compared with populations from other regions are observed. In addition, in a series of papers we have demonstrated that in the zones of influence of the industrial centers in the

southern part of the forest steppe, this process becomes catastrophic (Snegin, 1999; Snegin, 2005a, b). This phenomenon is probably due to the superposition of three factors. First, there is a natural fragmentation of the population in the conditions of the forest–steppe landscape. Secondly, there is artificial separation of populations by suddenly created isolating man-induced obstacles as a result of road construction and plowing areas. Thirdly, in such disparate groups, where genetic drift causes an imbalance in the ratio of gene frequencies, additional external pressure that in the industrial zones is necessarily present in one form or another (release of pollutants, climate change, habitat destruction, etc.) causes further genetic fluctuations.

It is worth noting that, of course, the transition of an allele in the homozygous state for the species and populations can be physiologically optimal, but for some narrow environmental conditions. A shift of these conditions can become a disaster because of reducing fitness. This is a useful and, simultaneously, hazardous feature of homozygotization. That is what threatens unbalanced, sudden-onset “anthropogenic” populations.

Thus, estimating the degree of heterozygosity, which reflects the general properties of the genotype and the full range of interactions of the unit with the

Table 4. The values of the half locus coefficients of inbreeding and the χ^2 test for heterogeneity of allelic frequencies in the studied samples of bush snail

Locus	Fit	Fis	Fst	X2	d. f.	Prob
Est2	0.348	0.146	0.237	164.1	36	0.000
Est3	0.204	0.071	0.143	95.7	36	0.000
Est4	0.160	0.037	0.128	102.5	54	0.000
Est5	0.102	0.071	0.034	39.48	54	0.930
Average	0.204	0.081	0.135	401.78	180	0.000

Footnote 2. Fit is inbreeding coefficient of species relative to a large population; Fis is inbreeding coefficient of species relative to subpopulations; Fst is the subpopulation inbreeding coefficient of a relatively large population; Prob is the probability of significance test χ^2 ; d. f. are degrees of freedom.

environment, we can estimate the degree of stability of populations at each given point in time. And as our studied species is inextricably *linked* not only with the physical and geographical conditions, but also with other species with which it coexists, then its subsequent evolutionary fate to a large extent reflects the fate of the entire biological community.

According to findings (Tables 2, 3), in the locus of esterases in some populations of *B. fruticum* dwelling in the zone of influence of the ore dressing combine, the amount of realized genetic combinations is reduced, against the background of the transition of the remaining alleles in homozygous phenotypes. A particularly unfavorable situation is noted in the Stoilo colony (no. 1), which is on the territory of the industrial zone. Here we record the lowest value of the average heterozygosity (0.025 ± 0.024) and observe the fixation of two alleles Est 2–2 Est 5–2.¹ In addition, in this group we noted the lowest value of the Shannon index (0.08) and the smallest effective number of alleles (1.04 ± 0.024). In the colonies Kochegury (no. 11) and Konshino (no. 12), we also showed a lower content of heterozygous phenotypes, indicating the depressed state of these groups. The latter two colonies are far from the industrial area (= 30 km), but judging by the chemical analysis of shells of studied snails, conducted earlier (Snegin, 2009), there is a subsidence zone of emissions from the chimneys from manufactures of hot iron pressing, which are located on the territory of ore dressing combines. In addition, the colonies Dubenko (no. 6) and Olshanka (no. 12) is among the groups with low average heterozygosity, but judging by the evaluation of the expected heterozygosity and number of implemented combinations, these colonies have not yet moved into a state of depression. In the colony Sergeevka (no. 4), as well as in the colony Stoilo, there is absolute homozygosity for the allele of esterases Est 2–2 and Est 5–2. However, the overall level of heterozygosity is raised by two other loci, especially Est 4 ($H_0 = 0.731$). This phenomenon is probably due to the various selective significance of the stud-

ied loci of esterases. In general, according to Table 2, in 55% of cases the expected heterozygosity exceeds the actual level, and in 14% of the cases, we marked fixation of an allele, indicating a deficit of heterozygotes in most of the groups studied.

It is worth noting that according to available data, as in the world practice, and from our own experience the situation, the reduction of allelic diversity can be characteristic of newly emerging or introduced colonies, where a “founder effect” is manifested. However, in this case, it is a relic form that exists in the area of the Quaternary period. This is precisely why the part of the colonies dwelling in an industrial area, but due to topography and forest-steppe landscape *landing* under less influence of man, has preserved as part of the gene pools the allelic potential that can be considered the starting point for other groups. Such colonies are, for example, the colonies Yamskaya steppe, Dubravka, Melavoe, Dalnyaya livenka, and Krasnyi ostrov. According to the balance of the allelic frequency, the heterozygosity level and variety of implemented combinations of these groups do not differ from control populations of specially protected natural areas.

It is worthwhile to highlight a colony living in the protected area Yamskaya steppe that is in the area immediately adjacent to the ore dressing combines. Despite the proximity of the industrial area, according to the allele balance, this population is in a greater metastable state than, for example, the neighboring group Stoilo. This can partly be explained by the conservation status of this site, so the additional negative effects such as burning of flame cultivation and overgrazing are absent here. On the other hand, terrain features can play a very important role here, because this population is located in a wooded area at the foot of the Cretaceous slope, which helps to prevent pollutants entering from the ore dressing combines.

The action of very important factor of isolation in the studied area illustrates the use of the F-statistics of Wright (Table 4). The index of the spatial differential dissociation of F_{st} is quite high (0.135), and the largest contribution to the interpopulation differences is made by the locus Est 2 ($F_{st} = 0.235$)². The index F_{It} ,

¹ Locus Est 5 in seven studied populations is monomorphic (Table 2).

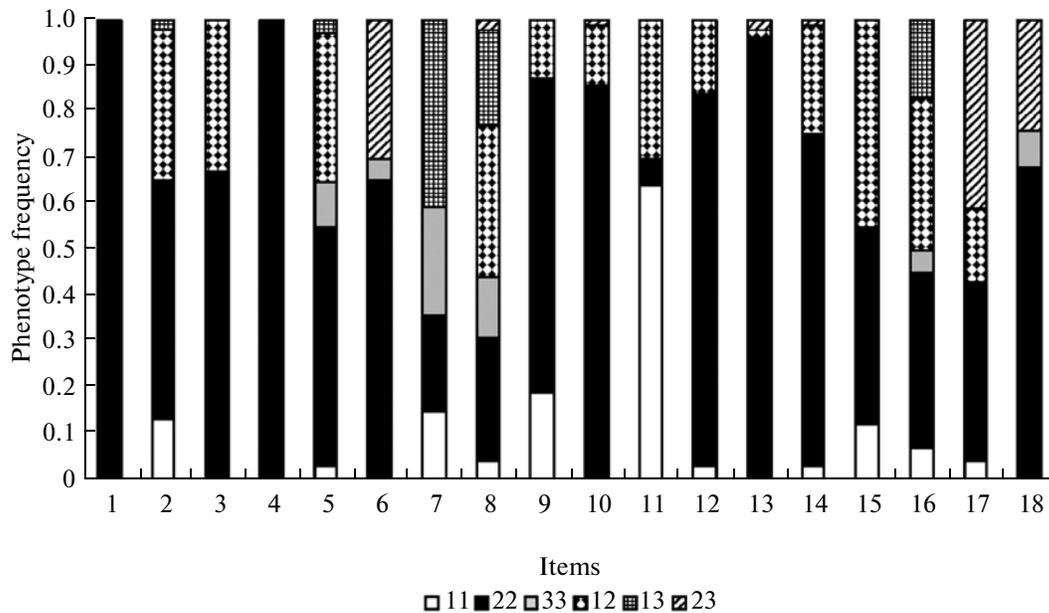


Fig. 2. The frequency of phenotype distribution of locus Est 2 in the studied populations.

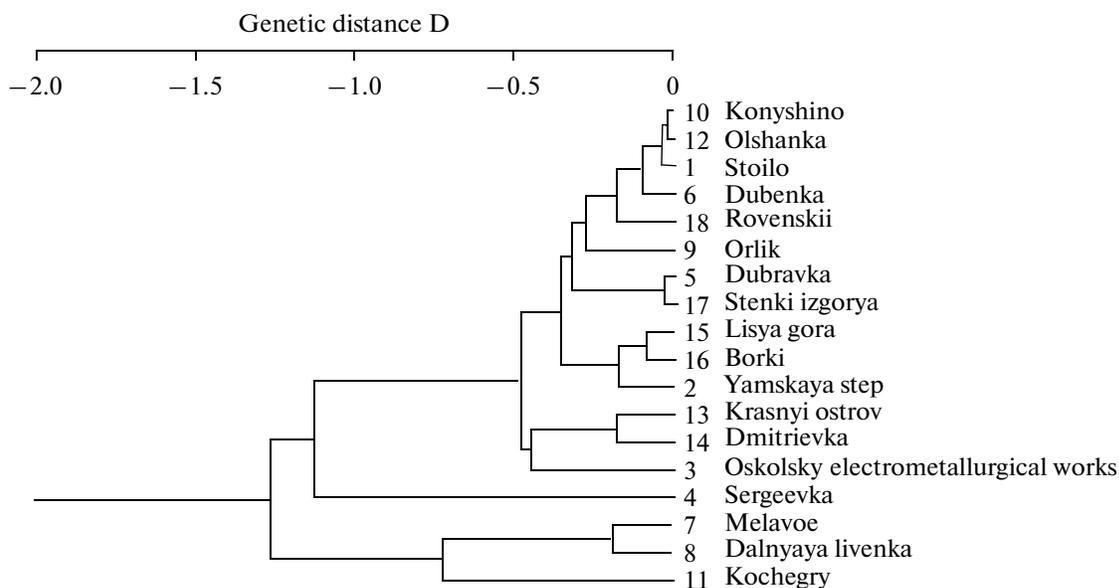


Fig. 3. Dendrogram of genetic distances according to Nei (Nei, 1972).

reflecting the degree of inbreeding relative to the species is also high (0.204), indicating the apparent deficiency of species heterozygosity in populations of the region.

Figure 2 shows the distribution of phenotypes of the locus Est 2 in the studied groups, according to which we can judge the degree of fixation of alleles and the selective sampling of certain combinations. According to the schedule, in the area adjacent to the ore dressing combines, we trace the trend to fixation of

the allele Est 2–2. In addition, we direct attention to the fact that in some populations there is a selection of rare combinations for the forest–steppe. Thus, in the population Melavoe, the bulk ratio to the level of homozygosity is contributed by the phenotype Est 2–33 (0.23), and in the colony Kochegry, it is the phenotype Est 2–11 (0.64). Data of the past years (Snegin, 2005) show that these phenotypes are very rare in natural populations of bush snail in the southern part of the Central Russian Upland forest–steppe

Table 5. Nei's genetic distance (Nei, 1972) between populations of bush snail in the south of the forest–steppe region

		Populations (no.)																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.000																		
2	0.035	0.000																	
3	0.057	0.023	0.000																
4	0.112	0.111	0.063	0.000															
5	0.049	0.034	0.065	0.182	0.000														
6	0.013	0.017	0.052	0.138	0.017	0.000													
7	0.155	0.078	0.144	0.304	0.096	0.110	0.000												
8	0.076	0.023	0.065	0.201	0.053	0.048	0.020	0.000											
9	0.032	0.022	0.054	0.111	0.052	0.023	0.118	0.059	0.000										
10	0.004	0.026	0.049	0.096	0.034	0.007	0.143	0.071	0.024	0.000									
11	0.188	0.093	0.203	0.393	0.144	0.123	0.073	0.067	0.125	0.176	0.000								
12	0.012	0.026	0.039	0.078	0.029	0.011	0.145	0.074	0.025	0.003	0.188	0.000							
13	0.025	0.044	0.035	0.060	0.042	0.028	0.194	0.107	0.042	0.015	0.260	0.007	0.000						
14	0.059	0.056	0.055	0.053	0.057	0.046	0.196	0.123	0.051	0.035	0.238	0.019	0.018	0.000					
15	0.040	0.013	0.058	0.131	0.059	0.026	0.087	0.037	0.027	0.033	0.079	0.040	0.066	0.079	0.000				
16	0.053	0.023	0.083	0.168	0.047	0.030	0.054	0.028	0.037	0.042	0.071	0.048	0.080	0.088	0.009	0.000			
17	0.044	0.024	0.063	0.170	0.004	0.010	0.094	0.048	0.037	0.028	0.112	0.025	0.042	0.049	0.041	0.034	0.000		
18	0.041	0.056	0.069	0.113	0.021	0.030	0.138	0.090	0.056	0.025	0.233	0.015	0.019	0.027	0.080	0.068	0.028	0.000	

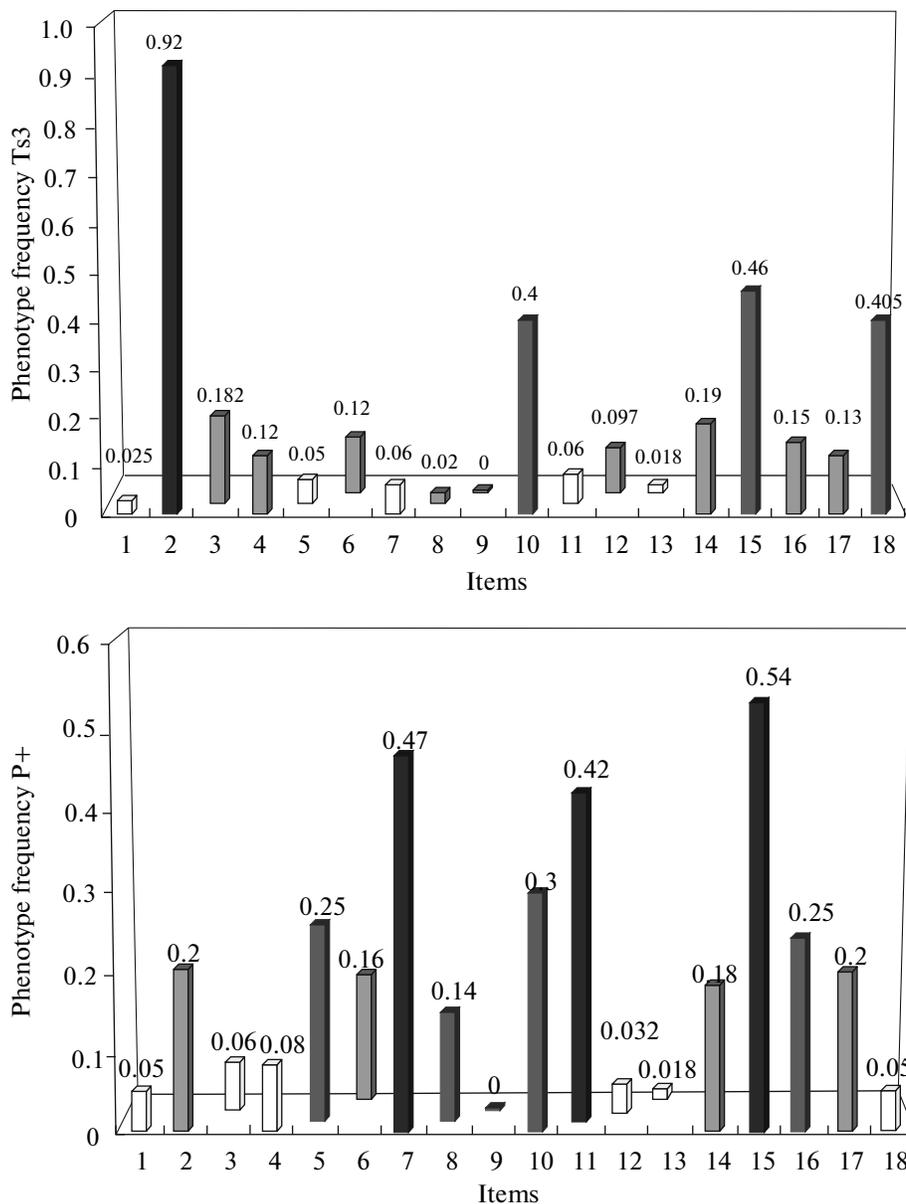


Fig. 4. Phenotypes frequency of the shell in *B. fruticum* populations in the southern steppe.

(medium frequency for the region Est 2–33 = 0.03 ± 0.02 , Est 2–11 = 0.045 ± 0.036). The apparently higher content of these phenotypes at these sites requires attention and further study of the causes. This may be due to either selective action of natural selection in these habitats or to random genetic drift caused by the insulation.

Results of cluster analysis based on genetic distances (Nei, 1978) are presented in Table 5 and Fig. 3. The resulting picture reflects the significant genetic divergence of the studied populations. Moreover, a greater genetic distance is observed among geographically closely located groups and among remote ones. This is probably due to the violation of naturally

formed channel migration of genes among populations in urbanized terrain.

Analysis of the heterogeneity of the studied populations showed reliable heterogeneity of allelic frequencies: χ^2 test on the sum of all loci $\chi^2 = 401.8$, d.f. = 68 (Table 4).

One more indicator that was involved in the analysis of gene pools of the studied mollusks populations is color variations of *B. fruticum* shells. Figure 4 shows the occurrence frequency in populations of species with yellow coloring of shell species with a longitudinal stripe on the shell.

In the area of research with respect to species with a yellow shell color, in the majority of items we

Table 6. Lifecycle prognosis of the studied populations of *B. fruticum*

Items	The total number of pubescent species	Actual number (N_e)	Lifecycle in generations	Lifecycle in years	The level of heterozygosity at the end of existence
Stoilo	120	10	15	75	0.012
Yamskaya step	168	14	21	105	0.114
Oskolsky electrometallurgical works	312	26	39	195	0.099
Sergeevka	260	22	32	162	0.115
Dubravka	130	11	16	81	0.101
Dubenka	1580	132	197	987	0.082
Melavoe	72	6	9	45	0.124
Dalnyaya livenka	108	9	13	67	0.146
Orlik	320	27	40	200	0.095
Konyshino	135	11	17	84	0.0684
Kochegry	110	9	14	68	0.061
Olshanka	875	73	109	547	0.087
Krasnyi ostrov	1354	113	169	846	0.122
Dmitrievka	3125	260	391	1953	0.196
Lisya gora	1562	130	195	976	0.112
Borki	1152	96	144	720	0.11
Stenki izgorya	1367	114	171	854	0.125
Rovenskii	1015	85	127	634	0.096

obtained a result that does not go beyond the regional exponent (0.11 ± 0.04). But in four items, Yamskaya steppe, Konshino, Lisya gora, and Rovenskii, we recorded a significant increase in the frequency of finding species of the given phenotype. Particularly strongly stands out the colony Yamskaya steppe, where the frequency of “yellow” snails was 0.92. This fact, probably, indicates increased radioactivity in these areas. Previous studies have shown that a similar situation in the southern part of the Central Russian Upland is formed in places affected by the Chernobyl radioactive accident. In particular, such a case happened ten years ago in the Rovenskii Nature Park and some other items (Snegin, 1999). However, in the soil of the reserved area Yamskaya steppe, we did not find traces of Chernobyl cesium (Cs^{137}), but, as we have found out as a part of the study, a large share in the background radiation is introduced by radium (Ra^{226}), having probably a natural origin, due to the presence of tectonic faults. In the soil of this region, its concentration amounted to 70.51 mg/kg, three times exceeding the adjacent areas (for example, in item no. 1 it is 22.41 mg/kg and in item no. 5 it is 43.23 mg/kg).

As for the species with a longitudinal stripe on the shell, we get the reverse picture. In a number of items of the industrial zone, we have noted a significantly lower frequency of this phenotype, in comparison with the four control plots in particular, and with the southern forest–steppe, in general (0.133 ± 0.05). This tendency of natural selection against the striped forms

indicates the general direction of changes in physical and geographical characteristics of the forest–steppe landscape in the zone of influence of ore dressing combines, connected with its bleaching (that is, with a reduction of the *land* of vegetation, with a decrease in the leaf area index and changes in the species composition of plants) similar to what we observe in the steppe areas (item 18). Previously expressed assumptions were that “striped” snails are better adapted to the conditions of shading as a result of apostatic selection (eating by blackbirds) and have increased oxidative capacity of enzymes (Matekin, Mackeeva, 1979; Runkova et al., 1974). This fact of “striped” species decreasing as a result of anthropogenic degradation of plant communities has previously been clearly demonstrated by the example of Moscow and Moscow region (Serbin, 2008).

To conclude the discussion of the results, we carried out a prognosis of the duration of existence of the studied populations of bush snails in the studied areas, taking into account the effective population size (N_e), which in our case was calculated on the basis of variability of species fertility for hermaphrodite animals (Crow, Kimura, 1970), where N is the number of mature species in the population, V is the variance of species fecundity, k_i is the fertility of each species, and k is the average fertility.

$$N_e = 4N - 2/2V + 2V \quad V = (\sum (k_i - k)^2) / N.$$

Calculations based on the twenty-six clutch show that the number of eggs in one clutch of *B. fruticum* on average is 23.5 ± 2 , $V = 25$ (the calculations are made according to I.M. Kokhutkina, 1997). We calculated the total number of pubescent snails in the populations taking into account the practiced methodology of collection. We carried out the timing of the existence of the studied populations using the following formula (Soule, 1985).

$$t = 1.5N_e,$$

where t is the number of generations.

The process of decline of heterozygosity for t generations, we have calculated by the formula (Crow, Kimura, 1970):

$$H_t = \left(1 \times \frac{1}{2Ne}\right)^t H_0,$$

where H_0 is the level of heterozygosity at the starting point.

Taking into account that the lifecycle of one generation is 5 years, we calculated the total life expectancy of the studied populations in years. These calculations are given in Table 6.

The results show that the time existence of a number of populations in the anthropogenic area is not more than 100 years, which is considerably less than in the controlled regions. The exceptions are the items Dubenko, Olshanka, and Krasnyi Ostrov, where many colonies of bush snails have been found, due to the surviving inundated woodlands with a rich food supply and high humidity due to the proximity of rivers. It is worth noting that the safest of all studied groups, according to our data, is the colony Dmitrievka, which is the largest of the studied groups and the most distant from major industrial centers.

Of course, this prognosis is realized only in the existence of natural populations, without human intervention. Taking into account such a course of events that is hardly possible, we can assume that the lifecycle of these populations will be reduced.

CONCLUSIONS

Thus, on the basis of these results, we can talk about the continuing tendency of decline of allelic diversity and change in the ratio of phenotype frequencies in populations of bush snail in the studied area, which partly reflects changes in the structure of the whole forest-steppe landscape of the southern part of Central Russian Upland under the influence of anthropogenic pressure. This pattern calls for the necessity of greater attention to natural communities of the region in terms of maintaining its biopotential and expanding the network of specially protected natural sites.

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