https://doi.org/10.17816/ecogen17415-26

THE ASSESSMENT OF THE GENETIC STRUCTURE OF BUSH SNAIL (*FRUTICICOLA FRUTICUM*) POPULATIONS BASED ON THE NONSPECIFIC ESTERASES LOCI

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Cite this article as: Snegin EA, Snegina EA, Artemchuk OYu. The assessment of the genetic structure of bush snail (*Fruticicola fruticum*) populations based on the nonspecific esterases loci. *Ecological genetics*. 2019;17(4):15-26. https://doi.org/10.17816/ecogen17415-26.

Received: *01.06.2019* Revised: *18.10.2019* Accepted: *17.12.2019*

❀ Using the polymorphic esterases loci, the genetic structure of the gastropod mollusk *Fruticicola* (*Bradybaena*) *fruticum* Müll., most of which lives in the south of the Central Russian Upland, was studied. For comparison, the samples were taken from the Romania, the North Caucasus, the Ural and the Vyatka regions. A total of the 1668 individuals were investigated. Of the 28 studied populations in 11 (39.3%), there was significant shortage of the heterozygotes. The level of the expected heterozygosity fluctuated in the range $H_e = 0.116-0.454$. Using the non-parametric statistics (Chao1-bc method and 1st order jackknife method), the populations with potentially high and low diversity of the multilocus genotypes were identified. The indicators of the genetic disunity between populations averaged $\Phi_{st} = 0.276$, $F_{st} = 0.292$. The principal component analysis and the Mantel correlation criterion $R_м = -0.007$ showed the absence of a reliable relationship between the geographical and genetic distance between populations, which indicates a violation of the isolation model by distance and confirms the thesis put forward by us that the urbanized forest-steppe landscape disrupts the natural migration processes, leads to the strong isolation and the genetic drift in the snail populations. At the same time, the phenomenon of increasing the degree of division of the populations against the background of reduced the allelic diversity, noted by us in many groups of bush snails, can be regarded as a shift in genetic equilibrium towards an increase in the interpopupulation diversity (according to the Wright model). The revealed absence of the effect of isolation by distance can be a consequence of the action of the stabilizing natural selection. The assumption of the dependence of the esterase alleles frequencies in the bush snail populations on the genetic (biochemical) characteristics of the food objects was proposed. The effective size, calculated using the Slatkin formula turned out to be comparable with the background, adventive and relict species of the terrestrial mollusks living in the study area ($N_e = 2.2-7.6$).

❀ **Keywords:** terrestrial mollusks; esterases; population structure; multilocus genotypes; effective size.

ОЦЕНКА ГЕНЕТИЧЕСКОЙ СТРУКТУРЫ ПОПУЛЯЦИЙ КУСТАРНИКОВОЙ УЛИТКИ (*FRUTICICOLA FRUTICUM*) НА ОСНОВЕ ЛОКУСОВ НЕСПЕЦИФИЧЕСКИХ ЭСТЕРАЗ

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Для цитирования: Снегин Э.А., Снегина Е.А., Артемчук О.Ю. Оценка генетической структуры популяций кустарниковой улитки (*Fruticicola fruticum*) на основе локусов неспецифических эстераз // Экологическая генетика. – 2019. – Т. 17. – № 4. – С. 15–26. https://doi.org/10.17816/ecogen17415-26.

Поступила: *01.06.2019* Одобрена: *18.10.2019* Принята: *17.12.2019*

❀ С использованием локусов неспецифических эстераз была исследована популяционная структура наземного моллюска *Fruticicola* (*Bradybaena*) *fruticum* Müll. в условиях Среднерусской возвышенности и других ландшафтов Восточной Европы. Из 28 исследованных популяций в 11 (39,3 %) наблюдался достоверный дефицит гетерозигот. Уровень ожидаемой гетерозиготности колебался в диапазоне $H_e = 0{,}116-0{,}454$. Используя непараметрическую статистику (метод Chao1-bc и метод «складного ножа») были выявлены популяции, обладающие потенциально высоким и низким разнообразием мультилокусных генотипов. Показатели генетической разобщенности популяций составили в среднем $\Phi_{st} = 0{,}276$, $F_{st} = 0{,}292$, при отсутствии достоверной связи географического и генетического расстояний между популяциями ($R_м = -0{,}007$). Эффективная численность, рассчитанная с помощью формулы Слаткина, оказалась сопоставимой с фоновыми, адвентивными и реликтовыми видами наземных моллюсков ($N_e = 2{,}2-7{,}6$), обитающими в районе исследования. Выдвигается предположение о зависимости частот аллелей эстераз в популяциях кустарниковой улитки от биохимических особенностей кормовых объектов.

❀ **Ключевые слова:** наземный моллюск; эстеразы; популяционная структура; мультилокусные генотипы; эффективная численность.

INTRODUCTION

Despite the development of DNA technologies in population genetics, the genetic structure of various species populations is still assessed using isoenzyme markers. The reason for this is that unlike DNA loci (for example, ISSR or SSR), the coding part of the genome, which is influenced by natural selection factors, is subjected to analysis.

Esterase-active enzymes responsible for cleavage of esters in the cell have gained significant popularity in population genetics. Unlike other enzymes, they are represented by a large number of polymorphic loci that do not require expensive reagents for detection following electrophoresis, thereby allowing for analysis of representative samples from populations.

This work aimed to assess the genetic structure of the terrestrial mollusk model *Fruticicola* (Bradybaena) *fruticum* Müll. (bush snail) in the eastern part of their contemporary habitat using non-specific esterase loci.

Our previous publications present studies on the structure of population gene pools and estimate an effective number of bush snails using polymorphic signs pertaining to shell, allozymes, and DNA markers [1–4]. Further, these studies were continued.

MATERIALS AND METHODS

The materials for this study included tissue samples of *Fr. fruticum* individuals stored in a cryobank created at the Laboratory of Population Genetics and Genotoxicology, Belgorod State University, Russia. Population samples were collected during expeditions conducted from 2004 to 2017. The bulk of samples from the Central Russian Upland were collected from 2007 to 2012. A total of 1,668 *Fr. fruticum* individuals from 28 populations were examined (see Table 1 and Fig. 1).

To collect mollusks from a 2 m × 2 m site, mowing was performed using an entomological net. Concurrently, different-aged individuals sitting on grass stems were

Table 1

Description of mollusk collection sites

Site No.	Site name	Biotope description	Coordinates
1	Stoylo	Oskolets floodplain, Stoylo village. Osiery, undergrowth with burdock and nettles. Territory of the Stoilensky mining and processing enterprise.	51°17'24.75'' N, 37°44'05.57'' E
2	Yamskaya step'	Yamskaya Step' Reserve. Mixed forest, overgrowth of nettles. Territory of influence for Stoilensky and Lebedinsky mining and processing enterprises.	51°11'04.66'' N, 37°39'31.97'' E
3	Dybenka	Dubenki floodplain (Belgorod region). Floodplain oak grove, undergrowth of nettles, burdock, and hops. Territory of influence for Stoilensky and Lebedinsky mining and processing enterprises.	51°03'26.75'' N, 37°50'00.50'' E
4	Olshanka	Olshanki floodplain. Osiery, undergrowth of nettles, hops, and hogweed. Territory of influence for Stoilensky and Lebedinsky mining and processing enterprises.	50°59'11.69'' N, 37°46'33.69'' E
5	Krasnyi Ostrov	Halan floodplain near the village of Krasnyi Ostrov. Osiery. Burdock, nettle, and hops.	50°56'34.06'' N, 37°46'51.71'' E
6	Dmitriyevka	Korochi floodplain near the village of Dmitriyevka. Floodplain forest of willow and maple, overgrowth of nettles. Surroundings of the "Long-living oak" nature monument.	50°30'12.47'' N, 36°59'39.62'' E
7	Lisya Gora	"Lisya Gora" natural monument near the village of Yablonovo. Oskol floodplain. Edge of an oak forest. Burdock, nettle, and hops.	50°13'24.38'' N, 38°00'34.61'' E
8	Borki	"Borki" natural monument. Kozinki floodplain, willow forest, overgrowth of burdock, nettle, and hops.	50°08'16.39'' N, 37°53'02.28'' E
9	Stenki Izgorya	Stenki Izgorya reserve area. Wetland biotope, alder overgrowth, undergrowth with burdock and nettle.	50°41'23.25'' N, 37°49'12.22'' E

Table 1 (continued)

Site No.	Site name	Biotope description	Coordinates
10	Rovenki	Rovenki natural park. Aidar floodplain, Rovenki village surroundings. Moderately moistened outdoor area. Overgrowth of burdock and hogweed mixed with nettle.	49°54'33.31'' N, 38°52'55.29'' E
11	Borisovka	Vorskla floodplain, territory of the Borisovka village, beneath the motorway bridge.	50°36'35.86'' N, 36°00'25.06'' E
12	Hotmyzhsk	Vorskla floodplain near the village of Hotmyzhsk. Burdock overgrowth with nettles.	50°35'05.99'' N, 35°52'24.83'' E
13	Yakovlevo	Vorskla floodplain in the Yakovlevo village territory. Willow forest, nettle, and hops.	50°52'05.12'' N, 36°26'49.92'' E
14	Syrtsevo	Pena floodplain in the Syrtsevo village surroundings (Ivnyansky District). Osiery and maple.	50°53'48.79'' N, 36°15'32.43'' E
15	Yasnyy Kolodets	"Yasnyy Kolodets" natural monument, Korochi floodplain, in the Korocha town surroundings. Edge of sticky alder forest.	50°49'34.23'' N, 37°12'34.24'' E
16	Koren	Koren floodplain, in the Alekseevka village surroundings (Korochansky District). Osiery.	50°45'19.01'' N, 37°01'30.91'' E
17	Seversky Donets	Seversky Donets floodplain in the Belgorod surroundings. Osiery and maple.	50°36'38.40'' N, 36°37'19.19'' E
18	Nezhegol	Nezhegol floodplain, territory of the town Shebekino. Willow forest.	50°24'32.93'' N, 36°52'38.38'' E
19	Kupyanska	Oskol floodplain near the city of Kupyansk (Kharkov region, Ukraine). Floodplain and willow forest.	49°42'37.60'' N, 37°37'26.18'' E
20	Divnogorye	"Divnogorye" natural monument (Voronezh region). Foot of rocky outlets of chalk layers. Tikhaya Sosna floodplain. Burdock, nettle, and hops.	50°57'48.99'' N, 39°17'40.3'' E
21	Galichya Gora	Galichya Gora reserve site (Lipetsk region). Don floodplain. Overgrowth of nettle, hogweed, burdock, and hops.	52°36'07.54'' N, 38°55'03.95'' E
22	Vorgol	Vorgolskoye reserve site (Lipetsk region). Rocky outlets of Devonian limestone; in the Vorgol River floodplain.	52°34'25.3'' N, 38°21'05.3'' E
23	Pluschan	Pluschan reserve site (Lipetsk region). Natural forest landmark on the right bank of the Don River. Upland birch and oak forest. Overgrowth of nettle and burdock.	52°50'00.1'' N 38°59'26.66'' E
24	Kirov	Vyatka floodplain. Territory of the city park of Kirov. Overgrowth of nettle and meadowsweet.	58°34'57.11'' N, 49°41'50.75'' E
25	Olenyi ruchyi	Olenyi Ruchyi Natural Park (Sverdlovsk Region, Nizhneserginsky District); pine and fir forest with birch and larch, meadow with overgrowth of meadowsweet and raspberry.	56°31'01.00'' N, 59°14'49.00'' E
26	Avrig	Valley of the Olt River, foothills of the Transylvanian Alps near Avrig village (Romania). Floodplain is willow and maple forest, rocky soil, with high moisture content, overgrowth of nettle, burdock, and hops.	45°43'36.87'' N, 24°20'30.12'' E
27	Kudymkar	North Perm Region. Komi-Permyak autonomous area. Kudymkar city, wasteland on the Gagarin street. Valley of the Yinwa River. Elderberry and nettle overgrowth.	59°00'59.7'' N, 54°39'58.0'' E
28	Kislovodsk	North Caucasus, Kislovodsk surroundings. Kislovodsk National Park. Floodplain of the Olkhovka River. Overgrowth of burdock and nettle.	43°53'37.1'' N, 42°43'15.6'' E

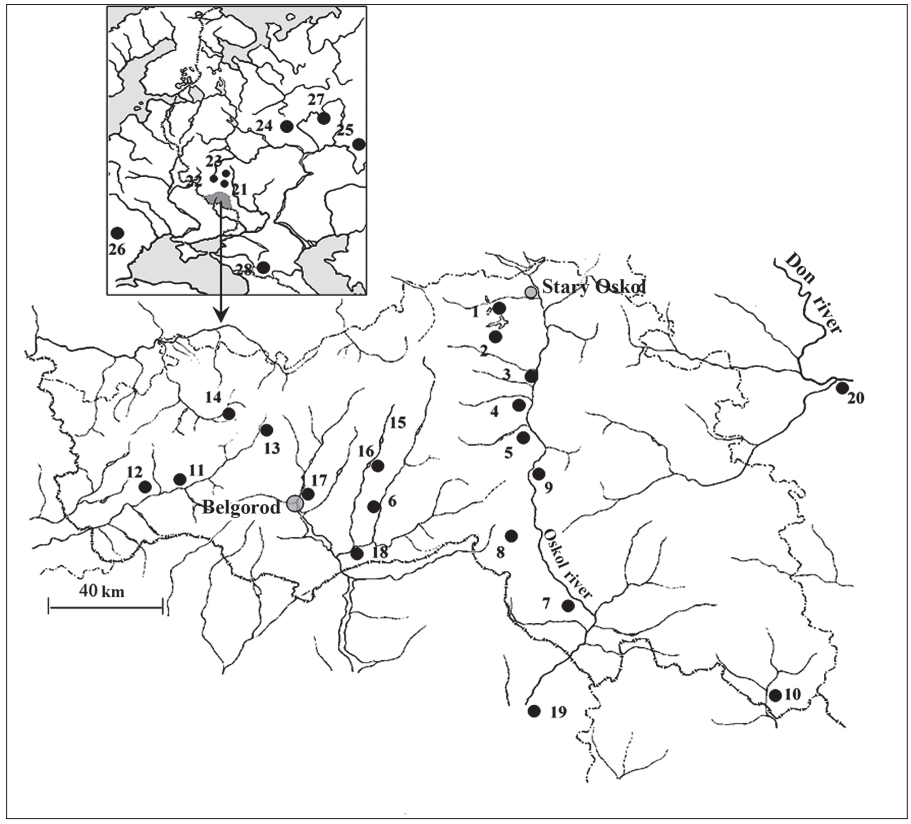
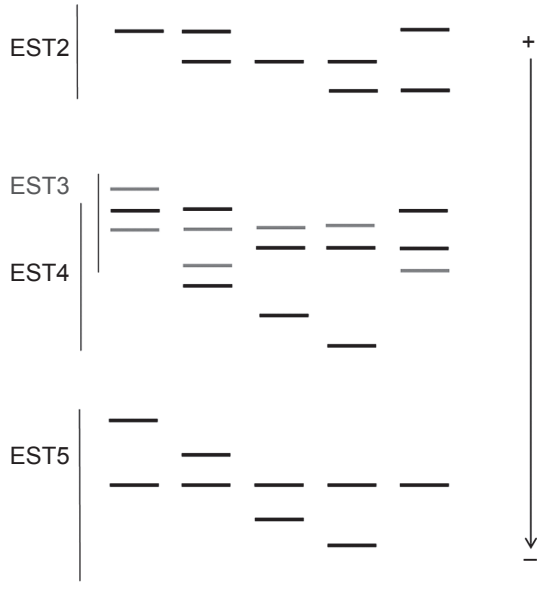


Fig. 1. Collection points of the *Fr. fruticum*



EST2	11	12	22	23	13
EST3	12	23	22	22	33
EST4	11	13	24	25	12
EST5	13	23	34	35	33

Fig. 2. Graphic image of the studied loci and allele esterases combinations of the *Fr. fruticum*

caught in the net. Then, in the same area, mollusks that had dropped during mowing were manually collected from soil, in addition to those that were located near grass-roots during collection. Because of a low density of mollusks, the size of the plot was increased twice. For each biocenosis studied, three or four samples were created. Sample coordinates were noted using the Garmin 76 GPS navigator.

Since it does not contain mucus (which impedes analysis), water-soluble proteins were extracted from mollusks' leg retractor by freezing at −80 °C, followed by thawing and mechanical grinding with a Teflon homogenizer in a 0.05 M TrisH buffer (pH 6.7). Electrophoresis of isoenzymes was performed in a 10% polyacrylamide gel in a VE-3 chamber (Helicon). For this process, a TrisHCl gel buffer (concentration gel pH 6.7, separating gel pH 8.9), and a tris-glycine electrode buffer (pH 8.3) was used. The blocks were stained in a substrate mixture of TrisHCl (pH 7.4), α-naphthyl acetate, and fast red TR.

Four loci of non-specific monomeric esterases were used for analysis, namely, EST2 (with three alleles), EST3 (with three alleles), EST4 (with four alleles), and EST5 (with five alleles) (Fig. 2) [5, 6].

The data obtained were processed using the GenAlEx v.6.5 software package [7].

RESULTS

The allele frequencies of esterase-active enzymes in populations are presented in Table 2, whereas Table 3 presents averaged indicators of genetic heterogeneity.

Since we assessed the condition of population gene pools among *Fr. fruticum*, a limited number of samples were used containing only a small part of the population allele pool. In each group, the total number of multilocus genotypes (N_{MLG}) and the number of unique multilocus genotypes (N_{MLG-1}) were estimated, i. e., combinations were noted in one single population. In the future, based on the frequency distribution of multilocus genotypes, potential genetic diversity can be calculated for each population via an increase in sample size to infinity (N_{max}). The analysis was performed using two nonparametric methods, the Chao1-bc (bias-corrected form for the Chao1) method [8] and the first-order jackknife method [9]. All calculations were performed using the SPAdes software [10]. Table 4 presents the subsequent results.

The degree of differentiation in populations under study within the territory examined was estimated using gene diversity characteristics proposed by Wright [11] and by analyzing molecular variance (AMOVA) [12]. The results are presented in Tables 5 and 6, respectively.

To clarify the degree of difference between individual populations, we calculated the values of pairwise indicators

Table 2

Allele frequencies of esterase loci in *Fr. fruticum* populations

Locus	EST2			EST3			EST4					EST5				
Population No.	Allele															
	1	2	3	1	2	3	1	2	3	4	5	1	2	3	4	5
1	0.053	0.932	0.015	0.015	0.894	0.091	0.053	0.939	0	0.008	0	0	0.008	0.985	0.008	0
2	0.303	0.685	0.012	0.063	0.87	0.067	0.051	0.74	0.209	0	0	0	0	0.992	0.008	0
3	0.199	0.801	0	0.044	0.953	0.003	0.193	0.807	0	0	0	0	0.019	0.946	0.035	0
4	0.081	0.887	0.032	0.161	0.806	0.032	0.194	0.742	0.048	0.016	0	0	0.032	0.968	0	0
5	0.009	0.982	0.009	0.161	0.759	0.08	0.232	0.625	0.107	0.036	0	0	0	0.982	0.018	0
6	0.144	0.85	0.006	0.369	0.613	0.019	0.294	0.613	0.094	0	0	0.056	0.025	0.9	0.013	0.006
7	0.342	0.658	0	0.008	0.792	0.2	0.008	0.908	0.075	0.008	0	0	0	0.992	0.008	0
8	0.319	0.534	0.147	0.009	0.802	0.19	0.103	0.897	0	0	0	0	0	1	0	0
9	0.266	0.628	0.106	0.043	0.957	0	0.314	0.681	0	0.005	0	0	0.011	0.989	0	0
10	0	0.797	0.203	0.189	0.811	0	0.365	0.635	0	0	0	0	0	1	0	0
11	0.073	0.903	0.024	0.048	0.935	0.016	0.081	0.911	0.008	0	0	0	0	1	0	0
12	0.25	0.675	0.075	0.525	0.25	0.225	0.825	0.075	0.1	0	0	0	0	1	0	0
13	0.316	0.566	0.118	0.105	0.882	0.013	0.066	0.921	0.013	0	0	0	0	1	0	0
14	0.103	0.795	0.103	0	1	0	0.256	0.718	0	0.026	0	0	0	1	0	0
15	0.444	0.19	0.365	0	0.071	0.929	0	0.762	0.238	0	0	0	0	1	0	0
16	0.395	0.272	0.333	0.079	0.895	0.026	0.211	0.746	0	0.044	0	0	0	1	0	0
17	0.131	0.821	0.048	0.024	0.524	0.452	0.143	0.702	0.143	0.012	0	0.214	0	0.762	0.024	0
18	0.403	0.597	0	0.236	0.764	0	0.375	0.597	0	0.028	0	0	0	1	0	0
19	0.194	0.793	0.013	0.25	0.75	0	0.017	0.953	0.017	0.013	0	0	0.013	0.961	0.026	0
20	0.189	0.703	0.108	0.135	0.838	0.027	0.514	0.257	0.122	0.081	0.027	0.014	0.041	0.946	0	0
21	0	1	0	0.4	0.6	0	0.213	0.763	0.025	0	0	0	0.025	0.975	0	0
22	0	1	0	0.05	0.95	0	0.3	0.7	0	0	0	0.475	0	0.525	0	0
23	0.15	0.725	0.125	0.125	0.85	0.025	0.35	0.65	0	0	0	0	0.2	0.8	0	0
24	0.027	0.689	0.284	0.378	0.622	0	0	1	0	0	0	0.054	0.014	0.811	0.122	0
25	0.403	0.21	0.386	0.205	0.795	0	0.182	0.818	0	0	0	0.04	0.96	0	0	0
26	0.145	0.66	0.195	0.285	0.71	0.005	0.99	0.005	0	0.005	0	0.02	0.005	0.885	0.09	0
27	0.019	0.915	0.066	0.226	0.774	0	0.16	0.84	0	0	0	0	0.009	0.972	0.019	0
28	0.106	0.864	0.03	0.455	0.515	0.03	0.409	0.5	0.091	0	0	0	0.621	0.379	0	0

Table 3

Genetic diversity indicators in *Fr. fruticum* populations, obtained from the analysis of esterase loci

Population No.	*N*	*P*, %	*A*	A_e	*I*	H_o	H_e	*F*
1	66	100	3.00	1.137	0.252	0.064	0.116	0.362*
2	127	100	2.75	1.447	0.474	0.244	0.274	0.081
3	158	100	2.50	1.284	0.359	0.174	0.206	0.132*
4	31	100	3.00	1.374	0.473	0.185	0.250	0.183
5	56	100	3.00	1.476	0.472	0.259	0.251	−0.028
6	80	100	3.50	1.664	0.627	0.441	0.365	−0.151*
7	60	100	2.75	1.384	0.400	0.238	0.242	0.026
8	58	75	2.25	1.537	0.462	0.233	0.274	0.114
9	94	100	2.50	1.498	0.443	0.269	0.266	0.041
10	37	75	1.75	1.446	0.411	0.203	0.273	0.222*
11	62	75	2.50	1.138	0.244	0.121	0.116	−0.032
12	20	75	2.50	1.729	0.602	0.288	0.348	0.196
13	38	75	2.50	1.436	0.414	0.145	0.231	0.301*
14	39	50	2.00	1.313	0.333	0.090	0.191	0.533*
15	63	75	2.00	1.612	0.463	0.163	0.282	0.425*
16	57	75	2.50	1.708	0.542	0.281	0.312	0.054
17	42	100	3.25	1.748	0.711	0.262	0.416	0.401*
18	36	75	2.00	1.625	0.499	0.278	0.336	0.132
19	116	100	3.00	1.321	0.388	0.205	0.219	0.036
20	37	100	3.50	1.798	0.702	0.250	0.372	0.277*
21	40	75	2.00	1.392	0.355	0.331	0.225	−0.322*
22	20	75	1.75	1.456	0.375	0.313	0.253	−0.145
23	20	100	2.50	1.608	0.604	0.450	0.368	−0.213
24	37	75	2.50	1.542	0.504	0.284	0.310	0.082
25	88	100	2.25	1.699	0.552	0.278	0.336	0.126
26	100	100	3.25	1.503	0.498	0.175	0.287	0.266*
27	53	100	2.50	1.288	0.364	0.146	0.208	0.249*
28	33	100	2.75	1.918	0.718	0.394	0.454	0.108
$M \pm m$		88.4 ± 2.7	2.58 ± 0.38	1.503 ± 0.220	0.473 ± 0.018	0.242 ± 0.094	0.278 ± 0.097	0.123 ± 0.037

Note. *N* is the number of individuals in the sample; *P* is the percentage of polymorphic loci; *A* is the average number of alleles; A_e is effective number of alleles; *I* is Shannon index; H_o is observed heterozygosity; H_e is expected heterozygosity; and *F* is fixation index (inbreeding coefficient). *Cases of significant differences between the expected and observed heterozygosity ($p \le 0.05$) are explained in the text.

for genetic distance (according to Ney) between the studied bush snail groups (see Table 7). The genetic relationships of populations were further investigated using principal component analysis (PCA) (Fig. 3). Accordingly, the first principal component (PC1) reflected 34.06% of the registered variability of populations and the second (PC2) reflected 29.1% of the total variance.

In the final stage, we evaluated the effective population size of *Fr. fruticum* using a model based on a linear function coefficient between pairwise estimates of gene flow (N_m) and geographical distance between populations (D_g): $\log N_m = a + b \cdot \log D_g$. The effective population size (for all studied populations as a whole) was calculated as $N_e = 10^a$, where a is the coefficient obtained in the equation [13]. The equation used is presented in Fig. 4, and the results are shown in Table 8. For comparison, similar data are given here for other terrestrial mollusk species, studied by us at an earlier stage.

Table 4

Number of marked multilocus genotypes and estimates of potential genetic diversity obtained by different methods for the studied *Fr. fruticum* populations

Population No.	N_{MLG}	N_{MLG-1}	Method			
			Chao1-bc		First-order jackknife	
			$N_{max} \pm SE$	95 % CI	$N_{max} \pm SE$	95 % CI
1	12	1	15.0 ± 3.4	12.5–29.7	17.9 ± 3.4	14.1–29.0
2	14	3	16.0 ± 2.9	14.2–29.9	18.0 ± 2.8	15.1–27.9
3	10	1	11.0 ± 2.3	11.1–23.9	12.0 ± 2.0	10.4–20.2
4	11	2	24.5 ± 12.8	13.8–76.0	18.7 ± 3.9	14.0–30.7
5	12	3	15.3 ± 4.1	12.5–33.7	16.9 ± 3.1	13.6–27.4
6	25	3	37.8 ± 9.2	28.7–70.1	38.8 ± 5.2	31.7–53.4
7	11	3	15.9 ± 6.0	11.8–42.6	15.9 ± 3.1	12.6–26.4
8	7	0	7.0 ± 0.5	7.0–7.0	8.0 ± 1.4	7.1–14.7
9	17	3	44.7 ± 21.1	24.4–121.4	24.9 ± 4.0	20.1–30.0
10	6	1	6.0 ± 0.2	6.0–6.0	7.0 ± 1.4	6.1–13.7
11	13	0	22.2 ± 8.7	14.9–57.3	20.9 ± 4.0	16.1–32.9
12	14	7	35.9 ± 16.6	20.3–97.6	26.4 ± 4.7	20.2–39.8
13	12	0	14.4 ± 3.1	12.4–28.6	16.9 ± 3.1	13.6–27.3
14	10	0	10.5 ± 2.5	9.1–23.7	11.9 ± 2.4	9.7–20.9
15	23	2	30.7 ± 6.2	24.9–54.0	33.8 ± 4.6	27.8–47.2
16	22	2	23.1 ± 2.5	21.3–34.2	26.9 ± 3.4	23.1–37.9
17	26	13	67.7 ± 27.5	38.9–161.3	44.5 ± 6.1	35.9–60.6
18	18	2	23.0 ± 4.5	19.1–40.9	26.8 ± 4.2	21.6–39.2
19	21	1	26.9 ± 5.3	22.3–47.9	29.9 ± 4.2	24.7–42.5
20	20	5	32.8 ± 10.0	23.3–69.3	31.7 ± 4.8	25.4–45.3
21	7	1	12.9 ± 6.9	7.9–43.5	10.9 ± 2.8	8.1–20.7
22	5	0	5.0 ± 0.2	5.0–5.0	6.0 ± 1.4	5.1–12.5
23	13	4	24.4 ± 10.3	15.5–64.7	21.6 ± 4.1	16.5–33.8
24	17	5	21.1 ± 4.2	17.8–38.8	23.8 ± 3.7	19.5–35.3
25	32	14	51.2 ± 12.5	38.0–93.6	48.8 ± 5.8	40.7–64.4
26	31	14	68.9 ± 25.4	42.5–156.0	48.8 ± 6.0	40.4–64.7
27	14	1	27.7 ± 13.0	16.9–79.9	21.8 ± 3.9	17.1–33.9
28	23	10	49.4 ± 17.7	31.0–109.9	39.5 ± 5.7	31.5–54.8

Note: N_{max} is sample size to infinity; N_{MLG}, total number of multilocus genotypes; and N_{MLG-1}, the number of unique multilocus genotypes.

Table 5

Values of locus values of inbreeding coefficients and the level of gene flow in *Fr. fruticum* populations under study

Locus	F_{is}	F_{it}	F_{st}	N_m
EST2	0.281	0.409	0.177	1.159
EST3	0.015	0.261	0.250	0.752
EST4	0.176	0.383	0.251	0.747
EST5	−0.183	0.395	0.489	0.261
$M \pm m$	0.072 ± 0.101	0.362 ± 0.034	0.292 ± 0.068	0.730 ± 0.184

Note: F_{is} is an inbreeding coefficient of an individual relative to the subpopulation; F_{it}, an inbreeding coefficient of an individual relative to a large population; F_{st}, an inbreeding coefficient of a subpopulation relative to a large population; and N_m, an average indicator of the intensity of gene exchange between populations.

Table 6

Values of the analysis of molecular variance (AMOVA) by esterases in *Fr. fruticum* populations

Source of variability	*df*	*SS*	*MS*	*V*	%	Φ_{st}	*P*	N_m
Interpopulation	27	668.73	24.768	0.205	28	0.276	0.001	0.654
Intrapopulation	3308	1773.98	1.074	0.537	72			
Total	3335	2442.71		0.742	100			

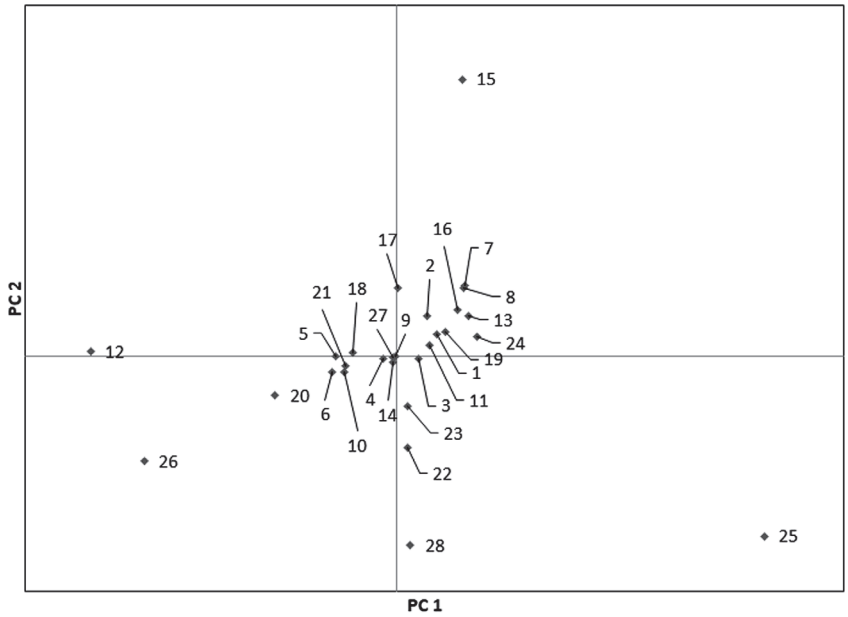


Fig. 3. The result of the PCA analysis

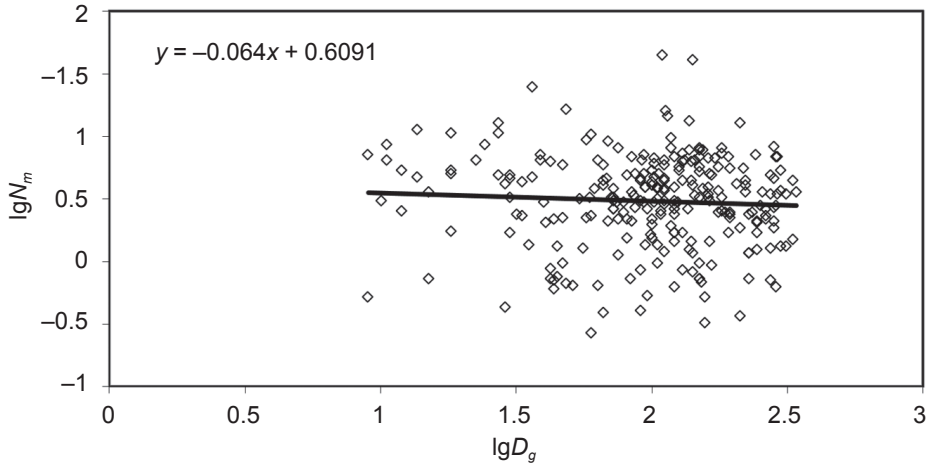


Fig. 4. Linear regression of the logarithm of the N_m gene flow between pairs of *Fr. fruticum* populations on the logarithm of the geographical distance between them D_g

DISCUSSION

The data obtained (see Table 3) indicates a significant deficiency of heterozygotes[1] in 11 studied populations (39.3%), and their significant excess was noted in two cases (no. 6, 21) ($p \le 0.05$). There were no significant differences in the remaining variants between observed and expected heterozygosity. This was also evidenced by data presented in Fig. 4, which indicated a trend toward a deficit of heterozygotes, whereas the regression coefficient resulted as 0.631 ± 0.111 ($p \le 0.001$).

[1] The significance of heterozygous deficiency was estimated by the formula $\chi^2 = F^2N(k-1)$, $df = k-1$, where F is the inbreeding coefficient, N is the sample size, and k is the number of alleles [17].

Table 7

Genetic distance calculated by Ney between *Fr. fruticum* populations

Population No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	0.028	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	0.012	0.017	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	0.012	0.026	0.011	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	0.026	0.044	0.028	0.007	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	0.056	0.057	0.046	0.019	0.019	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	0.026	0.013	0.026	0.040	0.066	0.081	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	0.038	0.023	0.030	0.048	0.080	0.091	0.009	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	0.042	0.025	0.012	0.028	0.049	0.059	0.040	0.029	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	0.042	0.056	0.030	0.015	0.019	0.028	0.080	0.068	0.027	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	0.002	0.023	0.007	0.008	0.024	0.049	0.028	0.037	0.032	0.033	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	0.420	0.363	0.355	0.285	0.244	0.176	0.418	0.396	0.296	0.223	0.404	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	0.033	0.017	0.020	0.038	0.073	0.076	0.014	0.008	0.023	0.059	0.026	0.414	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	0.020	0.027	0.006	0.013	0.026	0.053	0.044	0.040	0.009	0.017	0.013	0.332	0.032	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	0.438	0.368	0.478	0.462	0.484	0.476	0.284	0.266	0.449	0.491	0.470	0.553	0.361	0.498	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–
16	0.115	0.065	0.075	0.107	0.152	0.145	0.067	0.033	0.038	0.093	0.101	0.393	0.032	0.072	0.338	0.000	–	–	–	–	–	–	–	–	–	–	–	–
17	0.062	0.080	0.085	0.067	0.063	0.084	0.064	0.081	0.114	0.101	0.075	0.325	0.109	0.095	0.248	0.193	0.000	–	–	–	–	–	–	–	–	–	–	–
18	0.079	0.042	0.038	0.044	0.062	0.039	0.059	0.052	0.020	0.045	0.066	0.192	0.043	0.044	0.427	0.059	0.131	0.000	–	–	–	–	–	–	–	–	–	–
19	0.017	0.027	0.022	0.018	0.041	0.039	0.024	0.035	0.049	0.051	0.015	0.380	0.020	0.041	0.411	0.096	0.084	0.057	0.000	–	–	–	–	–	–	–	–	–
20	0.128	0.094	0.080	0.072	0.062	0.067	0.147	0.135	0.050	0.045	0.112	0.143	0.129	0.057	0.574	0.128	0.163	0.048	0.145	0.000	–	–	–	–	–	–	–	–
21	0.045	0.076	0.055	0.020	0.019	0.011	0.090	0.105	0.085	0.035	0.043	0.236	0.087	0.063	0.508	0.184	0.090	0.076	0.033	0.113	0.000	–	–	–	–	–	–	–
22	0.089	0.137	0.085	0.086	0.090	0.110	0.158	0.171	0.119	0.104	0.086	0.459	0.154	0.087	0.799	0.248	0.114	0.164	0.125	0.156	0.119	0.000	–	–	–	–	–	–
23	0.041	0.047	0.019	0.021	0.036	0.041	0.065	0.056	0.019	0.020	0.034	0.271	0.048	0.017	0.503	0.083	0.101	0.039	0.051	0.052	0.058	0.087	0.000	–	–	–	–	–
24	0.058	0.088	0.076	0.059	0.087	0.074	0.080	0.074	0.105	0.075	0.056	0.437	0.057	0.090	0.413	0.124	0.120	0.123	0.030	0.218	0.056	0.159	0.089	0.000	–	–	–	–
25	0.576	0.558	0.519	0.581	0.698	0.631	0.541	0.497	0.511	0.606	0.565	1.205	0.473	0.558	0.976	0.451	0.670	0.571	0.532	0.684	0.678	0.552	0.392	0.483	0.000	–	–	–
26	0.369	0.331	0.277	0.252	0.222	0.194	0.415	0.368	0.206	0.164	0.341	0.076	0.366	0.230	0.865	0.309	0.373	0.170	0.384	0.071	0.267	0.318	0.189	0.445	0.931	0.000	–	–
27	0.013	0.040	0.019	0.004	0.013	0.022	0.049	0.056	0.043	0.019	0.010	0.311	0.042	0.024	0.476	0.119	0.076	0.060	0.013	0.101	0.013	0.091	0.032	0.038	0.587	0.286	0.000	–
28	0.257	0.288	0.238	0.195	0.195	0.152	0.324	0.340	0.269	0.210	0.252	0.311	0.313	0.256	0.852	0.413	0.267	0.243	0.237	0.232	0.164	0.221	0.143	0.249	0.281	0.309	0.200	0.000

Table 8

Values of effective size calculated based on linear function coefficients between pairwise estimates of gene flow (N_e) and geographical distance between populations of various species of terrestrial mollusks

Species	N_e	95%, CI
Helix pomatia	9.8	3.9–25.1
Helicopsis striata	7.9	2.4–25.1
Chondrula tridens	3.8	2.6–5.7
Cepaea vindobonensis	1.1	0.14–8.5
Fruticicola fruticum	4.1	2.2–7.6

Note: Data on *C. vindobonensis*, *Ch. tridens*, *H. striata*, and *H. pomatia* were taken from our previous publications [14–16].

The data presented in Table 3 show that the greatest genetic diversity occurred in populations 6, 12, 17, 20, 23, and 28. Moreover, the largest effective number of alleles per locus (A_e) was noted in group 28 (Kislovodsk); the largest Shannon index (I) was also observed here. The highest level of observed heterozygosity (H_o) was recorded in population 23 (Plyushchan). In groups 20 (Divnogorye) and 6 (Dmitriyevka), alleles EST4-5 and EST5-5 were revealed, respectively, which is very rare for the species (Table 2). Populations 1 (Stoylo, inhabits the industrial zone territory) and 14 (Syrtsevo) were the most monomorphic. In population 14, the highest inbreeding coefficient (F) value was observed.

The results pertaining to level of variability between populations are only partially consistent with data on multilocus genotypes (Table 4). Among populations of the Central Russian Upland, the largest number of multilocus genotypes was observed for groups 17 (Seversky Donets) and 6 (Dmitrievka). The largest number of unique combinations was also noted for 17, where calculations also revealed the highest values of potential genetic diversity. Outside the Central Russian Upland, the most genetically diverse populations were groups 25 (Olenyi ruchyi) and 26 (Avrig) living in mountainous areas. The smallest number of multilocus genotypes, both real and potential, was observed for groups 8, 10, and 22.

Assessment of the degree of differentiation among populations by allozyme loci was performed using F-statistics devised by Wright and, on average, showed a rather large disunity among the studied snail groups by esterase loci (Table 5). We observed a similar result when comparing data obtained via AMOVA (Table 6). In both cases, the average indicator of the gene exchange intensity between populations (N_m) was less than unity, which according to the theory of evolution with shifting equilibrium indicates a violation of gene exchange between populations [18]. This was also indicated by the relatively high values of the F_{it} inbreeding coefficient. In this case, the largest contribution to interpopulation diversity estimated by inbreeding coefficient F_{st} (Table 5) was for the EST5 locus. The level of gene flow between groups (N_m) resulted in higher than unity at the EST2 locus only, whereas the highest F_{it} value was noted at this locus. It is worth noting that this locus has long been used as a marker for studying the population structure of bush snails in various Eastern European landscapes [1, 5, 19].

According to PCA, it was not possible to identify any isolated aggregate groups of bush snails (Fig. 3). The populations were distributed quite randomly, whereas geographically distant populations living in different natural zones were found to be genetically similar (for example, 9, 14, and 27 or 10 and 21). Conversely, populations living close to each other in similar biotopes were found to be genetically different (for example, 1–5). Populations 12, 15, 25, 26, and 28 distanced themselves significantly from the remaining groups.

The PCA data were confirmed by a graph illustrating dependence of gene flow (N_m) level between populations, based on the geographical distances between them (D_g) (Fig. 4). The graph demonstrates the absence of a reliable relationship between these parameters (Mantel correlation coefficient $R_м = -0.007$, $p = 0.422$, 9999 permutations).

The above information indicates a violation of the migration channels between the studied groups of *Fr. fruticum* and their prolonged isolation from each other. Moreover, their population gene pools may potentially be formed under the influence of Genetic drift processes. At the same time, an increase in the degree of subdivision of populations against a decrease in allelic diversity, as observed by us in many groups of bush snails, can be regarded as a shift in genetic equilibrium toward an increase in interpopulation diversity (based on Wright's model). It is also known that if there is no effect of isolation by distance (as observed in our study), it is likely that the loci will be exposed to stabilizing selection [13]. In this regard, in our opinion, it is significant that the main feed plant for the species under study is stinging nettle (*Urtica dioica* L.), regardless of landscape, and that the frequency distribution aspects of esterase alleles in snail populations may therefore be associated with genetic (and as a result, biochemical) peculiarity among the populations of this plant. We believe that to clarify the situation in the future, it will be extremely useful to study the population structure of this feed object. Also, in some situations (for example, under conditions of high snail density or the absence of nettles), some individuals shift to other plants (burdock, hops, and meadowsweet), which can also affect the frequency ratio of esterase alleles that will be formed under natural selection.

Regarding analysis of effective snail size, this can be stated as in the range that coincides with other species of terrestrial mollusks living in conditions of the Central

Russian Upland. According to average values, the effective size occupies an intermediate position between specially protected relict species (*Helicopsis striata* and *Cepaea vindobonensis*) on the one hand and background (*Chondrula tridens*) and adventive species (*Helix pomatia*) on the other.

CONCLUSION

On the basis of the above results, we can state a continuing trend of reduction in allelic diversity and a change in the ratio of genotype frequencies in populations of bush snails in the study area, which partly reflects changes in the structure of the entire forest steppe and steppe landscapes of the Central Russian Upland as a result of anthropogenic pressure. Concurrently, the absence of clear dependence of the frequencies of alleles of esterase-active enzymes in populations of bush snails within a geographical location may indicate that the formation of population gene pools by esterase-active loci is less dependent on the zonal characteristics of various landscapes and may, to a greater extent, be determined by the action of microclimatic and microbiotopic factors.

REFERENCES

1. Снегин Э.А. Эколого-генетические аспекты расселения *Bradybaena fruticum* (Mollusca, Gastropoda, Pullmonata) в элементах лесостепного ландшафта // Экология. – 2005. – № 1. – С. 39–47. [Snegin EA. Ecological and genetic characteristics of the distribution of *Bradybaena fruticum* (Mollusca, Gastropoda, Pullmonata) in a forest-steppe landscape. *Russian Journal of Ecology*. 2005;36(1):33-40. (In Russ.)]. https://doi.org/10.1007/s11184-005-0006-1.
2. Снегин Э.А. Оценка состояния популяционных генофондов наземных моллюсков в условиях влияния горно-обогатительных комбинатов на примере *Bradybaena fruticum* Müll. (Gastropoda, Pulmonata) // Экологическая генетика. – 2010. – Т. 8. – № 2. – С. 45–55. [Snegin EA. Assessment of the state of population gene pools of terrestrial mollusks in conditions of influence of ore dressing combines from the example of *Bradybaena fruticum* Müll. (Gastropoda, Pullmonata). *Ecological genetics*. 2010;8:45-55. (In Russ.)]. https://doi.org/10.17816/ecogen8245-55.
3. Снегин Э.А. Временная динамика генетической структуры и эффективная численность популяций *Bradybaena fruticum* Müll. (Mollusca, Gastropoda, Pullmonata) в условиях юга Среднерусской возвышенности // *Экология*. – 2015. – Т. 46. – № 3. – С. 198–205. https://doi.org/10.7868/S0367059715020110. [Snegin EA. Temporal dynamics of the genetic structure and effective size of *Bradybaena fruticum* Müll. (Mollusca, Gastropoda, Pulmonata) populations in the south of the Central Russian Upland. *Russian Journal of Ecology*. 2015;46(3):260-266. (In Russ.)]. https://doi.org/10.1134/S1067413615020113.
4. Снегин Э.А., Снегина Е.А. Оценка состояния популяционных генофондов малоподвижных видов животных на примере наземного моллюска *Bradybaena fruticum* Müll. (Gastropoda, Pulmonata) с использованием ДНК-маркеров // Экологическая генетика. – 2017. – Т. 15. – № 3. – С. 4–19. [Snegin EA, Snegina EA. Assessment of the state of population gene pools of the slightly mobile animal species on the example of the land snail *Bradybaena fruticum* Müll. (Gastropoda, Pulmonata) based on the DNA markers. *Ecological genetics*. 2017;15(3):4-19. (In Russ.)]. https://doi.org/10.17816/ecogen1534-19.
5. Матекин П.В., Макеева В.М. Полиморфная система эстераз и пространственная структура вида у кустарниковой улитки (*Bradybaena fruticum* Müll.) // Журнал общей биологии. – 1977. – Т. 38. – № 6. – С. 908–913. [Matyokin PV, Makeeva VM. Polymorphic system of esterases and spatial structure of a species in a bush snail (*Bradybaena fruticum* Müll.). *Journal of general biology*. 1977;38(6):908-913. (In Russ.)]
6. Макеева В.М., Белоконь М.М., Малюченко О.П. Оценка состояния генофонда природных популяций беспозвоночных животных в условиях фрагментарного ландшафта Москвы и Подмосковья (на примере кустарниковой улитки *Bradybaena fruticum* (Müll.)) // Генетика. – 2005. – Т. 41. – № 11. – С. 1495–1510. [Makeeva VM, Belokon' MM, Malyuchenko OP. Estimating the gene pool condition in natural populations of invertebrates in the fragmented landscape of Moscow and Moscow Region with special reference to bush snail Bradybaena fruticum Müll. *Russian Journal of Genetics*. 2005;41(11):1230-1244. (In Russ.)]
7. Peakall R, Smouse PE. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Mol Ecol Notes*. 2006;6(1):288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x.
8. Chao A. Species richness estimation. In: Balakrishnan N, Read CB, Vidakovic B, ed. Encyclopaedia of statistical science. New York: Wiley; 2005. pp. 7907-7916.
9. Burnham KP, Overton WS. Estimation of the size of a closed population when capture probabilities vary among animals. *Biometrika*. 1978;65(3):625-633. https://doi.org/10.1093/biomet/65.3.625.
10. Chao A, Shen TJ. SPADE [cited 12.03.2015]. 2009. Available from: http://chao.stat.nthu.edu.tw/wordpress/
11. Wright S. The genetical structure of populations. *Ann Eugen*. 1951;15(4):323-354.
12. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochon-

drial DNA restriction data. *Genetics*. 1992;131(2): 479-491.
13. Slatkin M. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*. 1993;47(1):264-279. https://doi.org/10.1111/j.1558-5646.1993.tb01215.x.
14. Снегин Э.А. Пространственные и временные аспекты эколого-генетической структуры популяций беспозвоночных животных (на примере наземных моллюсков и насекомых юга Среднерусской возвышенности): Дис. ... докт. биол. наук. – Белгород, 2012. – 473 с. [Snegin EA. Prostranstvennyye i vremennyye aspekty ekologo-geneticheskoy struktury populyatsiy bespozvonochnykh zhivotnykh (na primere nazemnykh mollyuskov i nasekomykh yuga Srednerusskoy vozvyshennosti). [dissertation] Belgorod; 2012. 473 p. (In Russ.)]. Доступно по: https://www.dissercat.com/content/prostranstvennye-i-vremennye-aspekty-ekologo-geneticheskoi-struktury-populyatsii-bespozvonoc. Ссылка активна на 16.07.2019.
15. Снегин Э.А., Артемчук О.Ю. Морфогенетический анализ популяций *Helix pomatia* L. (Pulmonata, Helicidae) юго-восточной и восточной части современного ареала // Экологическая генетика. – 2014. – Т. 12. – № 4. – С. 25–37. [Snegin EA, Artemchuk OYu. Morphogenetic analysis of *Helix pomatia* L. (Pulmonata, Helicidae) populations from south-eastern and eastern parts of the modern area. *Ecological genetics*. 2014;12(4):25-37. (In Russ.)]. https://doi.org/10.17816/ecogen12425-37.
16. Снегин Э.А., Снегина Е.А. Генетическая структура популяций особо охраняемого моллюска *Cepaea vindobonensis* (Mollusca, Gastropoda, Pulmonata) в условиях северо-восточной части современного ареала // Экологическая генетика. – 2016. – Т. 14. – № 3. – С. 13–27. https://doi.org/10.17816/ecogen14313-27. [Snegin EA, Snegina EA. Genetic structure of populations of specially protected mollusk *Cepaea vindobonensis* (Mollusca, Gastropoda, Pulmonata) in the north-eastern part of the modern-day range. *Ecological genetics*. 2016;14(3):13-27. (In Russ.)]. https://doi.org/10.17816/ecogen14313-27.
17. Ли Ч.Ч. Введение в популяционную генетику / пер. с англ. Е.А. Салменковой, Е.Я. Тетушкина, под ред. Ю.П. Алтухова, Л.А. Животовского. – М.: Мир, 1978. – 555 с. [Li CC. First course in population genetics. Transl. from English E.A. Salmenkova, E.Ya. Tetushkin, ed. by Yu.P. Altukhov, L.A. Zhivotovskiy. Moscow: Mir; 1978. 555 p. (In Russ.)]
18. Wright S. Random drift and shifting balance theory of evolution. Mathematical topics in population genetics. Berlin: Springer Verlag; 1970, pp. 1-31.
19. Макеева В.М. Роль естественного отбора в формировании генотипического своеобразия популяций моллюсков (на примере кустарниковой улитки *Bradybaena fruticum* (Mull.)) // Журнал общей биологии. – 1989. – Т. 50. – № 1. – С. 101–107. [Makeeva VM. The role of natural selection in the formation of the genotypic originality of the populations of mollusks (on the example of the shrub snail *Bradybaena fruticum* (Müll.)). *Journal of general biology*. 1989;50(1):101-107. (In Russ)]

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