Genetic Structure Analysis of the Alien Mollusk *Stenomphalia ravergiensis* (Mollusca, Gastropoda, Pulmonata) Population in Belgorod (Russia)

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Abstract—Gel electrophoretic analysis of allozymes was used to study the genetic structure of the population of the alien mollusk *Stenomphalia ravergiensis* that inhabits urbanized landscapes in the town of Belgorod (Russia). A high genetic variability level and low values of inbreeding coefficient were characteristic of most colonies. Weak genetic separation of the snail groups studied was also demonstrated. Invasive colony distribution was supposed to correspond to the island model. The calculation of effective population size and the comparison of this value to the respective parameters of native and invasive species of terrestrial mollusks revealed a high viability level for the population of the species of interest in the study area.

INTRODUCTION

Inadvertent introduction of plants and animals to various habitats on the planet is known to have unpredictable consequences sometimes, both for introduced species themselves and for the aboriginal community members. At the same time, invasive colonies exposed to novel living conditions can serve as good natural models for the validation of our concepts concerning the evolutionary mechanisms. As conventionally believed, the founder effect is manifested in such colonies (Mayr, 1954), and the combination of this effect and further inbreeding leads to allelic diversity reduction and ultimately to destabilization and extinction. This is one of the possible reasons for the unsuccessful outcomes of introduction in certain cases. Therefore, the expansion of a species beyond the native range implies a rather high level of genetic and phenotypic variability, which allows for adaptation to novel conditions and new habitat colonization (Alimov and Bogutskaya, 2004; Yeh and Trevor, 2004; Ghalambor et al., 2007; Lee and Gelembiuk, 2008). Moreover, compensatory mechanisms that alleviate the negative effect of inbreeding must be active in the genomes of such species (Snegin, 2012).

A small colony of the terrestrial mollusk *Stenomphalia ravergiensis* (Férussac, 1835) was found in the town of Belgorod in 2002 (Snegin and Prisnyi, 2008). The historical range of this species is located in the Northern Caucasus and Transcaucasia, mostly in the forest belt (Shileiko, 1978), and sometimes in open areas and on crystal-line highland massifs (Akramovskii, 1976; Kiyashko, 2007). The species was detected in different parts of Belgorod and in the environs of the town in the following years. Notably, active expansion of this species beyond the northern border of the range in Eastern Europe was observed in recent decades (Snegin and Prisnyi, 2008; Balashov et al., 2013). This type of invasive process development can possibly have a pronounced effect on the local malacofauna and the local ecosystems as a whole (Clarke et al., 1984; Murray et al., 1988; Parker et al., 1999; Coote and Loeve, 2003).

The aim of the present work was to analyze the genetic structure of invasive *S. ravergiensis* colonies using allozyme markers in order to assess the viability level of the mollusks and forecast the character of the invasive process.

MATERIALS AND METHODS

The present study continues our research on demographic and conchiometric parameters of *S. ravergiensis* colonies in Belgorod (Snegin and Adamova, 2016). Individuals from seven isolated *S. ravergiensis* groups (Fig. 1, Table 1) were collected in the town of Belgorod and its environs during the summer field season of 2015. Twenty-five sexually mature individuals were collected at each site. A sample of 68 individuals of the same species collected from a population that inhabited the native range (Noratus, Armenia) was used for comparison.

Fig. 1. *Stenomphalia ravergiensis* sampling sites: (1) Donets, (2) Michurina, (3) Kalinina, (4) Botanicheskii sad, (5) Vezelka, (6) Kar'er, (7) Vodstroi.

Isoenzymes were extracted from the mollusk foot retractor by freezing the tissue at −80°C with subsequent thawing and mechanical homogenization by a Teflon pestle in 0.05 M Tris-HCl buffer (pH 6.7). Polyacrylamide gel electrophoresis was performed in a VE-20 chamber (Helicon, Russia). Nonspecific esterases were fractionated in a 10% gel, whereas superoxide dismutases and malate dehydrogenases were fractionated in a 7.5% gel. Tris-HCl gel buffer was used for the electrophoresis (stacking gel buffer, pH 6.7;

Table 1. *Stenomphalia ravergiensis* sampling sites

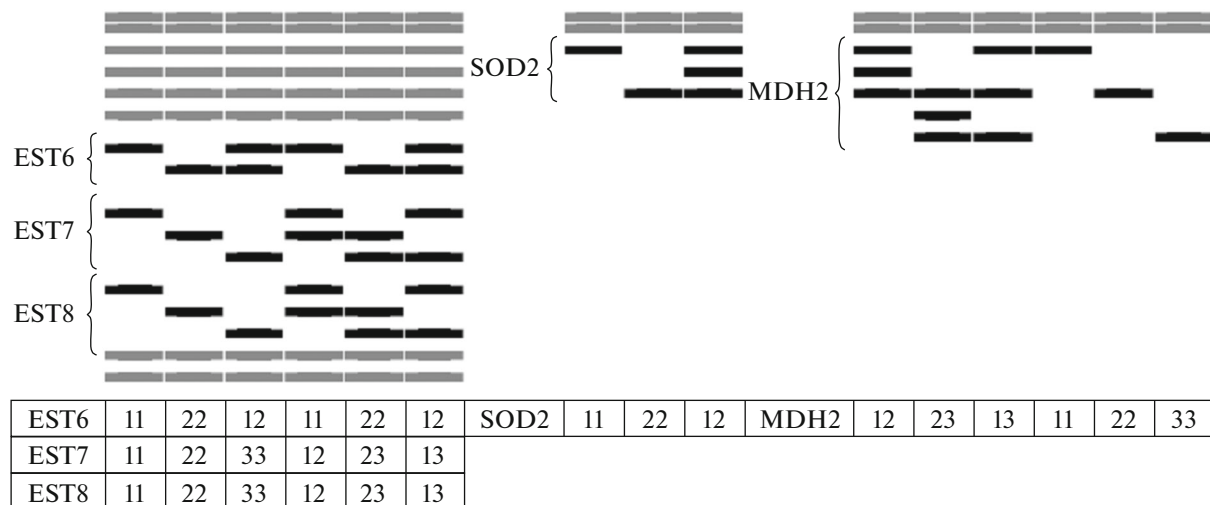| No.	Site	Description	Coordinates
1	Donets	Floodplain of Severskii Donets River near the railway station. Poplar thicket	50°35′52.13″ N 36°36′55.03″ E
2	Michurina	Wasteland with ruderal vegetation near Belenergomash production plant	50°37′0.71″ N 36°33′33.40″ E
3	Kalinina	Territory of Belgorod Construction Material Combine (BCMC) near a chalky slope	50°36′34.62″ N 36°36′33.79″ E
4	Botanicheskii sad	Maple thicket near the botanical garden of Belgorod State University	50°35′39.32″ N 36°33′17.07″ E
5	Vezelka	Floodplain of Vezelka River near an area with private houses. Moderately humid area with maple, poplar, and willow thicket	50°35′38.70″ N 36°33′52.36″ E
6	Kar'er	An open area with steppe vegetation between an area with private houses and a chalk pit	50°37′35.90″ N 36°31′1.33″ E
7	Vodstroi	Shaded site in the floodplain of Vezelka River along railway tracks. Maple thicket. Burdock, nettle, and bennet in the herb tier	50°35′25.00″ N 36°34′3.54″ E
8	Noratus (Armenia)	Pine forest edge with steppe vegetation near Lake Sevan. 1982 m above sea level	40°38′48.6″ N 45°19′47.6″ E

EST6	11	22	12	11	22	12	SOD2	11	22	12	MDH2	12	23	13	11	22	33
EST7	11	22	33	12	23	13											
EST8	11	22	33	12	23	13											

Fig. 2. Allozyme loci and the respective *Stenomphalia ravergiensis* genotypes (monomorphic loci are shown in gray).

resolving gel buffer, pH 8.9), and Tris-glycine buffer, pH 8.3, was used as the running buffer. Gel block staining for nonspecific esterase detection was performed in a substrate mix of the following composition: Tris-HCl (pH 7.4), α-naphthyl acetate, and Fast Red TR. The substrate mix for superoxide dismutase detection contained potassium phosphate buffer (pH 7.8), NTS, and PMS, and that for malate dehydrogenase detection contained 0.1 M Tris-HCl (pH 8.4), sodium malate, NTS, PMS, and NAD.

Ten nonspecific esterase activity zones were detected in *S. ravergiensis*, and the three monomeric loci were *EST6* (with two alleles), *EST7*, and *EST8* (with three alleles each). We also identified one polymorphic locus of a dimeric superoxide dismutase *SOD2* with two alleles and a dimeric malate dehydrogenase locus *MDH2* with three alleles (Fig. 2).

All loci mentioned above had a codominant type of inheritance. Locus boundaries were identified by comparing the empirical genotype distribution to the theoretically expected Hardy–Weinberg distribution; Pearson's χ^2 test was used for the comparison (Table 2). GenALEx 6.501 software (Peakall and Smouse, 2012) was used for genetic data processing.

The samples used for the analysis of *S. ravergiensis* population viability were of a limited size and thus contained only a small part of the population allele pool. Therefore, we performed multilocus variability analysis. Multilocus combinations for each of the 175 individuals were calculated at the first step, and the total number of multilocus genotypes (N_{MLG}) and the number of unique multilocus genotypes (N_{MLG-1}), that is, combinations found in a single sample, were calculated at the following step. Multilocus genotype frequency distributions for each population were subsequently used to assess the potential genetic diversity expected upon an increase of sample size to infinity

(*Nmax*). The two nonparametric methods used for the analysis were the Chao1-bc (bias-corrected form for Chao1) (Chao, 2005) and the first-order jackknife method (Burnham and Overton, 1978). Multilocus genotype analysis was performed in the SPADE program (Chao and Shen, 2009).

Genetic distance calculation according to Nei and Li (1976), maximum-likelihood (ML) sample clustering, and bootstrap assessment (1000 iterations) were performed using TREECON software ver. 1.3b (Van de Peer and De Wachter, 1994).

RESULTS AND DISCUSSION

Allele frequencies and genetic variability parameters for the loci used are shown in Tables 3 and 4. The data obtained demonstrated a high level of allozyme locus variability in the groups studied. All the loci used were polymorphic ($P = 100\%$) in two invasive groups (Botanicheskii sad and Vezelka) and in the population from the native range (Noratus).

Table 2. Comparison of genotype distribution to Hardy–Weinberg equilibrium in *Stenomphalia ravergiensis* colonies

Locus	Population							
	1	2	3	4	5	6	7	8
Est6	Ns	Mono	Ns	Ns	Ns	Mono	Ns	D*
Est7	Ns	Ns	D***	D*	D**	D***	D*	Ns
Est8	Mono	Mono	Mono	D**	Ns	Mono	Mono	Ns
SOD2	Ns	Mono	Ns	Ns	Ns	Mono	Ns	Ns
MDH2	Ns	Ns	Ns	D*	Ns	Ns	Ns	Ns

Mono—monomorphic locus; D—significant difference between the actual and theoretically predicted allele frequency distribution; *—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$; ns—no significant difference between the allele frequency distributions, $p > 0.05$.

Table 3. Allele frequencies for allozyme loci in *Stenomphalia ravergiensis* populations

Locus	Allele	Populations							
		1	2	3	4	5	6	7	8
EST6	1	0.100	0.000	0.080	0.480	0.360	0.000	0.420	0.309
	2	0.900	1.000	0.920	0.520	0.640	1.000	0.580	0.691
EST7	1	0.260	0.000	0.560	0.360	0.340	0.560	0.440	0.022
	2	0.280	0.200	0.100	0.160	0.160	0.100	0.000	0.478
	3	0.460	0.800	0.340	0.480	0.500	0.340	0.560	0.500
EST8	1	0.000	0.000	0.000	0.120	0.020	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.140	0.060	0.000	0.000	0.184
	3	1.000	1.000	1.000	0.740	0.920	1.000	1.000	0.816
SOD2	1	0.860	1.000	0.820	0.480	0.760	1.000	0.800	0.978
	2	0.140	0.000	0.180	0.520	0.240	0.000	0.200	0.022
MDH2	1	0.540	0.640	0.500	0.580	0.740	0.620	0.440	0.360
	2	0.320	0.360	0.500	0.320	0.260	0.380	0.560	0.574
	3	0.140	0.000	0.000	0.100	0.000	0.000	0.000	0.066
N		25	25	25	25	25	25	25	68

Moreover, the level of actual and expected heterozygosity in the majority of invasive colonies was comparable to that in the aboriginal population. The group that lived near the chalk pit (Kar'er) and the colony found on the side of the road leading to the chalk pit (Michurina site) formed an exception.

The values of the inbreeding coefficient were rather low in all groups investigated. The average actual heterozygosity for all loci studied in all samples showed significant similarity to the theoretically expected value (Kruskal–Wallis test: $p > 0.05$ for all groups). An excess of heterozygotes was observed for some loci in individual colonies, but this excess proved statistically insignificant. For instance, a slight excess of individuals heterozygous for *EST6*, *SOD2*, and *MDH2* loci was identified in the Donets group, and a slight excess of heterozygotes for *EST7* and *MDH2* loci was identified in the Michurina group, and therefore the inbreeding coefficient value for these groups was negative. All these data are indicative of high viability and genetic plasticity of *S. ravergiensis*, as expected for an invasive species (Barrett and Richardson, 1986).

The results of molecular analysis of variance (AMOVA) for the Belgorod colonies are shown in Table 5 (the Caucasian population was not included in this analysis). The data demonstrate the predomination of intrapopulational variance over interpopulational variance in the sample studied, this being indicative of weak genetic differentiation of the colonies analyzed (Wrait, 1940).

Importantly, genetic variability at the individual level predominated in the invasive groups. This was evident from the inbreeding coefficient for the individual relative to the large population (*Fit* = 0.216, p =

0.001), being almost two times higher than the inbreeding coefficients for the individual relative to the subpopulation (*Fis* = 0.111, p = 0.001) and for the subpopulation relative to the large population (*Fst* = 0.117, p = 0.001).

Analysis of pairwise matrices for gene flow values (*Nm*) and geographical distances between the snail groups investigated (*Dg*) did not reveal a significant correlation between these parameters, as is evident from a rather low value of the Mantel correlation coefficient (0.375, p = 0.084) (Mantel, 1967; José et al., 2013). Thus, the absence of a correlation between the spatial structure of the population and its genetic structure is likely (Fig. 3).

Deviation from the model of isolation by distance is observed in this case. The structure of expansion of the invasive species under investigation is more likely to correspond to the island model (Wrait, 1943), which implies migrant exchange between multiple daughter populations and a parent population (at an equal exchange rate).

As evident from Table 6, the genetic distance (*D*) inferred from allozyme loci frequencies as described by Nei (1975) is rather low for the populations studied, and this corroborates poor genetic differentiation of the populations.

This assumption is partially confirmed by maximum-likelihood (ML) cluster analysis (Fig. 4). Samples from the Vezelka and Botanicheskii sad sites belong to the same cluster, and the Donets group is close to them. The Michurina and Kar'er groups belong to a separate cluster, and the Kalinina group is the closest to this cluster.

Table 4. Genetic diversity parameters in *Stenomphalia ravergiensis* populations ($M \pm m$)

Population	Locus	Aa	Ae	I	Ho	He	F	P %
1. Donets	EST6	2.000	1.220	0.325	0.200	0.180	–0.111	80 ± 20
	EST7	3.000	2.796	1.064	0.480	0.642	0.253	
	EST8	1.000	1.000	0.000	0.000	0.000	–	
	SOD2	2.000	1.317	0.405	0.280	0.241	–0.163	
	MDH2	3.000	2.418	0.973	0.760	0.586	–0.296	
	Average	2.200 ± 0.374	1.750 ± 0.359	0.553 ± 0.202	0.344 ± 0.129	0.330 ± 0.123	–0.079 ± 0.105	
2. Michurina	EST6	1.000	1.000	0.000	0.000	0000	–	40 ± 24
	EST7	2.000	1.471	0.500	0.400	0.320	–0.250	
	EST8	1.000	1.000	0.000	0.000	0.000	–	
	SOD2	1.000	1.000	0.000	0.000	0.000	–	
	MDH2	2.000	1.855	0.653	0.560	0.461	–0.215	
	Average	1.400 ± 0.245	1.265 ± 0.173	0.231 ± 0.143	0.192 ± 0.120	0.156 ± 0.098	–0.233 ± 0.011	
3. Kalinina	EST6	2.000	1.173	0.279	0.160	0.147	–0.087	80 ± 20
	EST7	3.000	2.277	0.922	0.280	0.561	0.501	
	EST8	1.000	1.000	0.000	0.000	0.000	–	
	SOD2	2.000	1.419	0.471	0.280	0.295	0.051	
	MDH2	2.000	2.000	0.693	0.520	0.500	–0.040	
	Average	2.000 ± 0.316	1.574 ± 0.244	0.473 ± 0.160	0.248 ± 0.085	0.301 ± .105	0.106 ± 0.120	
4. Botanicheskii sad	EST6	2.000	1.997	0.692	0.480	0.499	0.038	100
	EST7	3.000	2.593	1.013	0.440	0.614	0.284	
	EST8	3.000	1.719	0.753	0.200	0.418	0.522	
	SOD2	2.000	1.997	0.692	0.640	0.499	–0.282	
	MDH2	3.000	2.228	0.911	0.680	0.551	–0.234	
	Average	2.600 ± 0.245	2.107 ± 0.146	0.812 ± 0.064	0.488 ± 0.085	0.516 ± 0.032	0.066 ± 0.153	

Table 4. (Contd.)

Population	Locus	Aa	Ae	I	Ho	He	F	$P\%$
5. Vezelka	EST6	2.000	1.855	0.653	0.400	0.461	0.132	
	EST7	3.000	2.556	1.007	0.320	0.609	0.474	
	EST8	3.000	1.176	0.324	0.160	0.150	−0.070	100
	SOD2	2.000	1.574	0.551	0.320	0.365	0.123	
	MDH2	2.000	1.625	0.573	0.360	0.385	0.064	
	Average	2.400 ± 0.245	1.757 ± 0.228	0.622 ± 0.111	0.312 ± 0.041	0.394 ± 0.075	0.145 ± 0.090	
6. Kar'er	EST6	1.000	1.000	0.000	0.000	0.000	–	
	EST7	3.000	2.277	0.922	0.280	0.561	0.501	
	EST8	1.000	1.000	0.000	0.000	0.000	–	40 ± 24
	SOD2	1.000	1.000	0.000	0.000	0.000	–	
	MDH2	2.000	1.891	0.664	0.440	0.471	0.066	
	Average	1.600 ± 0.400	1.434 ± 0.272	0.317 ± 0.198	0.144 ± 0.092	0.206 ± 0.127	0.283 ± 0.137	
7. Vodstroi	EST6	2.000	1.950	0.680	0.520	0.487	−0.067	
	EST7	2.000	1.972	0.686	0.240	0.493	0.513	
	EST8	1.000	1.000	0.000	0.000	0.000	–	80 ± 20
	SOD2	2.000	1.471	0.500	0.320	0.320	0.000	
	MDH2	2.000	1.972	0.686	0.560	0.493	−0.136	
	Average	1.800 ± 0.200	1.673 ± 0.194	0.511 ± 0.132	0.328 ± 0.102	0.359 ± 0.096	0.077 ± 0.132	
8. Noratus	EST6	2.000	1.745	0.618	0.324	0.427	0.242	
	EST7	3.000	2.088	0.784	0.529	0.521	−0.016	
	EST8	2.000	1.429	0.477	0.309	0.300	−0.029	100
	SOD2	2.000	1.045	0.106	0.044	0.043	−0.023	
	MDH2	3.000	2.159	0.866	0.603	0.537	−0.123	
	Average	2.400 ± 0.245	1.693 ± 0.208	0.570 ± 0.134	0.362 ± 0.098	0.366 ± 0.091	0.010 ± 0.061	
Total average		2.050 ± 0.113	1.657 ± 0.084	0.511 ± 0.055	0.302 ± 0.035	0.328 ± 0.035	0.052 ± 0.039	77.50 ± 8.81

Aa—average allele number, Ae—effective allele number, I—information index (Shannon index), Ho—observed heterozygosity, He—expected heterozygosity, F—inbreeding coefficient, $P\%$—the share of polymorphic loci.

Table 5. Results of molecular variance analysis for invasive *Stenomphalia ravergiensis* populations

Variability source	df	SS	MS	Est. Var.	%	Fst	Fis	Fit
Intrapopulational	6	38.506	6.418	0.110	12			
Interpopulational	168	154.240	0.918	0.092	10	0.117	0.111	0.216
Interindividual	175	128.500	0.734	0.734	78			
Total	349	321.246		0.936	100			

Fit—inbreeding coefficient for the individual relative to the large population, *Fis*—inbreeding coefficient for the individual relative to the subpopulation, *Fst*—inbreeding coefficient for the subpopulation relative to the large population; $p = 0.001$.

Table 6. Pairwise genetic distance (*D*) estimates for *Stenomphalia ravergiensis* populations

Populations	1	2	3	4	5	6	7	8
1	0.000							
2	0.031	0.000						
3	0.029	0.084	0.000					
4	0.118	0.190	0.122	0.000				
5	0.039	0.075	0.055	0.045	0.000			
6	0.028	0.067	0.012	0.174	0.064	0.000		
7	0.066	0.105	0.047	0.071	0.039	0.077	0.000	
8	0.060	0.068	0.111	0.168	0.097	0.117	0.094	0.000

The first *S. ravergiensis* individuals found in the town of Belgorod were collected from the Vodstroi site (Snegin and Prisnyi, 2008), and therefore one may assume that further expansion of the species started at this site and proceeded along the Vezelka River and onto the botanical garden territory. Small privately owned houses and vegetable patches occupy a major part of this area, and this may underlie the expansion of mollusks together with plant debris. Genetic remoteness of the other more monomorphic groups from a different cluster can be a consequence of gene drift evoked by the transport of a small number of *S. ravergiensis* individuals into the industrial area from the primary introduction site. All sites mentioned above are located in the industrial area and connected by roads. However, a second independent introduction of *S. ravergiensis* into Belgorod cannot be ruled out.

The results of multilocus genotype analysis are shown in Table 7. Data of multilocus variability analysis are in agreement with the results presented above. Multilocus variability was the lowest in the Kar'er and Michurina groups, this being due to the high share of monomorphic loci. The number of multilocus genotypes was the largest in colonies from the Botanicheskii sad and Vezelka sites. The nonparametric Chao1-bc method showed that the potential genetic diversity was higher in these groups than in the aboriginal population from Armenia.

Notably, the data obtained by us are somewhat in agreement with the regularities identified for other invasive mollusk species. For instance, genetic differentiation between invasive populations of such species

often turns out to be insignificant. This phenomenon was thoroughly characterized in aquatic mollusks. For instance, high genetic similarity between the colonies was observed in the studies of Dreissenidae invasions (Marsden and Spidle, 1996; Voroshilova, 2015). Studies of Eurasian and North American populations of two species of the genus *Dreissena* revealed high genetic diversity and the absence of a "founder effect" in the invasive populations of both species. However, the genetic structure of invasive populations changed with time, with dissimilar changes observed in different species studied (Brown and Stepien, 2010). Studies of the populations of another invasive mollusk, *Perna viridis*, did not reveal any differences between the invasive populations, but all invasive groups differed from the native ones. Moreover, the genetic diversity of invasive groups was lower, in contrast to the observa-
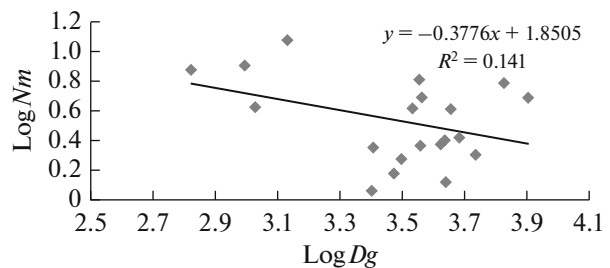

Fig. 3. Linear regression of gene flow logarithm *Nm* between pairs of *Stenomphalia ravergiensis* populations with regard to the logarithm *Dg* of the geographic distance between the populations.
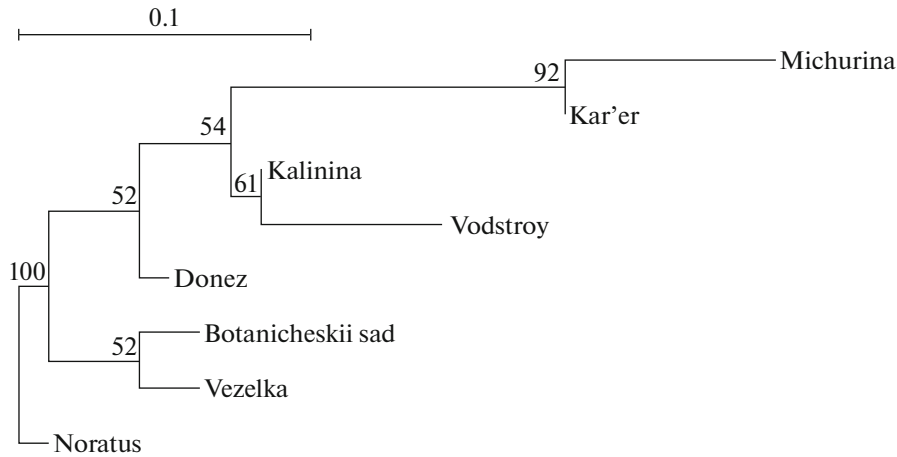
Fig. 4. Maximum-likelihood (ML) dendrogram of genetic distances between *Stenomphalia ravergiensis* groups. The share of populations grouped after the bootstrap test is shown at the nodes.

tions made in most native populations (Gilg et al., 2012). Slight differences between the inbreeding coefficients of invasive and native populations were reported for the freshwater mollusk *Physa acuta* (Bousset et al., 2004). A study of the population of the bivalve mollusk *Potamocorbula amurensi* revealed high genetic diversity in the subpopulations, whereas the population as a whole was genetically uniform (Duda, 1994). Allozyme analysis of invasive populations of the terrestrial snail *Theba pisana* in Australia revealed considerable reduction of genetic diversity (Johnson, 1988).

Allozyme marker-based studies of continuous and ephemeral populations of the invasive species *Brephulopsis cylindrica* in the town of Nikolaev (Ukraine) also showed that small isolated populations of this terrestrial mollusk (including urbanized populations) tended toward a decrease in genetic diversity related to the occurrence of stochastic genetic processes (gene drift or founder effect) (Kramarenko and Snegin, 2015). However, the intragroup variability decrease was accompanied by interpopulation distance increase in this species (Fst = 0.281), this being in agreement with the shifting balance theory of evolution (Wright, 1970). This theory implies an increase in subdivided metapopulation variability concomitantly to a decrease in subpopulation heterogeneity.

The concluding stage of our study involved effective size calculation for the colonies investigated. The formula used took the inbreeding level in the population into account (Li, 1976):

$$Ne = \frac{N}{1 + F},$$

where *Ne* is the effective population size, *N* is the number of individuals in the sample, and *F* is the inbreeding coefficient.

Table 7. The number of multilocus genotypes identified and estimates of potential genetic diversity for *Stenomphalia ravergiensis* population studied

Population	N_{MLG}	N_{MLG-1}	Method			
			Chao1-bc		first-order jackknife	
			Nmax ± *SE*	95% CI	*Nmax* ± *SE*	95% CI
Botanicheskii sad	23	19	90.2 ± 44.4	43.6–241.8	43.2 ± 6.3	34.1–59.6
Vezelka	22	13	113.2 ± 62.6	49.0–330.4	41.2 ± 6.1	32.4–57.4
Vodstroi	20	7	48.8 ± 20.1	28.4–119.2	27.8 ± 50.3	34.4–72.3
Donets	20	11	36.8 ± 11.7	24.9–77.6	34.4 ± 5.3	21.1–49.0
Kalinina	16	5	33.6 ± 14.5	20.3–88.2	26.6 ± 4.5	20.7–39.7
Kar'er	10	0	12.9 ± 4.0	10.4–32.2	13.8 ± 2.7	11.1–23.5
Michurina	5	0	5.0 ± 0.0	5.0	5.0 ± 0.0	5.0
Noratus	34	21	57.4 ± 14.1	41.9–103.6	53.7 ± 6.3	44.7–70.2

N_{MLG}—total number of multilocus genotype variants identified; N_{MLG-1}—number of unique variants of multilocus genotypes; *Nmax*—potential genetic variability expected upon an increase in sample size to infinity.

Table 8. Effective size of *Stenomphalia ravergiensis* colonies

Parameter	Colonies							
	1	2	3	4	5	6	7	8
Ne	25	25	22.6	23.4	21.8	19.5	23.2	24.7
Ne/N	1	1	0.904	0.936	0.872	0.780	0.928	0.988

Importantly, this formula implies that inbreeding coefficient values range from 0 to 1. Therefore, we assigned a zero value of the inbreeding coefficient *F* to some populations which were shown to have negative values of this coefficient.

We calculated the ratio between the effective sample size and total sample size in order to obtain comparable data (Table 8). The average *Ne/N* value derived from the data obtained was 0.917 ± 0.029, this being within the overall range of relative *Ne* suggested by Crow, Morton, and Kimura (Crow and Morton, 1955; Crow and Kimura, 1970). These researchers showed that the average value of relative *Ne* was 0.75 for most organisms. Moreover, the average relative *Ne* calculated for Caucasian snail was significantly higher (at $p = 0.05$) than the analogous values for the aboriginal reference species of terrestrial mollusks from the study site (the value for *Bradybaena fruticum* was 0.800 ± 0.021 (data for 40 populations) and that for *Chondrula tridens* was 0.661 ± 0.013 (data for 25 populations)) (Snegin, 2012a, 2012b) and similar to the value for the thriving invasive species *Helix pomatia* (0.870 ± 0.043; data for 12 populations) (Snegin and Artemchuk, 2016), this being an indirect indication of a high viability level of *S. ravergiensis* populations studied.

CONCLUSIONS

Thus, our results point at the high viability of invasive *S. ravergiensis* colonies manifested as gene pool richness and high values of effective population size. A high share of individual variability provides for genetic plasticity, which is known (Lee, 2002) to promote further development of the invasion process.

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