ISSN 2079-0597, Russian Journal of Genetics: Applied Research, 2015, Vol. 5, No. 5, pp. 469–478. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © S.S. Kramarenko, E.A. Snegin, 2014, published in Ecologicheskaya Genetika, 2014, Vol. 12, No. 2, pp. 23–34.

Genetic Structure of the Continuous and Ephemeral Populations of the Land Snail *Brephulopsis Cylindrica* (Gastropoda; Pulmonata; Enidae)

S. S. Kramarenko[a] and E. A. Snegin[b]

[a] *Mykolayiv National Agrarian University, 54020 Ukraine*
e-mail: kss0108@mail.ru
[b] *Belgorod State National Research University, Belgorod, 308015 Russia*
e-mail: snegin@bsu.edu.ru

Received February 27, 2014; in final form, July 1, 2014

Abstract—We analyzed the genetic structure of continuous and ephemeral populations of the land snails *B. cylindrica*. Based on the obtained results, we conclude that small, isolated animal populations (including, urban) tend to reduce the level of genetic diversity, which arises due to the manifestation of genetic and stochastic processes (the genetic drift or founder effect). An important consequence of the latter is the relatively high rate of random changes in genotypic profiles in small populations, which leads to a significant increase in the level of genetic differentiation between them.

Keywords: genetic structure; urban populations; land snail; *B. cylindrica*
DOI: 10.1134/S2079059715050068

INTRODUCTION

Brephulopsis cylindrica (Menke, 1828) is a type of land snail endemic to Crimea, which also has a mosaic and limited spread in the southern regions of continental Ukraine (Kramarenko, 1995; Vychalkovskaya, 2008). However, repeated cases of the introduction of this species in the urbanized habitats of the cities of Lviv (Sverlova, 1998), Kiev (Vychalkovskaya and Kramarenko, 2008), Donetsk (Vychalkovskaya, 2008), and Ordzhonikidze (Balashov et al., 2013) have been recorded in recent years.

In urban areas, *B. cylindrica* was found to inhabit a variety of habitats: parks, lawns, cemeteries, etc. The populations formed in these habitats must have originated from just a few individuals of Crimean or non-Crimean dissent. We hypothesized that groups of *B. cylindrica* dwelling in small, isolated, ephemeral populations will manifest a decline in the level of intrapopulation genetic polymorphism (in particular, in terms of allelic diversity and heterozygosity).

Therefore, the aim of our study was to carry out a comparative analysis of the genetic structure of the snail *B. cylindrica* in the context of continuous and ephemeral populations based on the variability of the allozyme loci. The obtained results were further compared with the previously published data for ecologically similar species to determine whether the identified patterns are common to a variety of animal species.

MATERIALS AND METHODS

For our analysis, we selected six populations of *B. cylindrica*, which dwell in different urban habitats in the city of Nikolaev in southern Ukraine. Based on the area occupied by the population and number of individuals, we have selected three continuous populations (Dubki, Neftebasa and Park Pobedy) and three ephemeral (Kosmos, Morechodnaya and Mira) (Fig. 1). Ephemeral populations occupied areas of not more than 10 m^2. The areas of continuous populations stretched for a few hundred m^2 and the number of individuals approached 10^4–10^5.

We collected two sampling sets spaced about 50 m apart from each of the continuous populations, whereas only a single sample set was collected at one site for each ephemeral group. Each sample set consisted of 25 mature live individuals of *B. cylindrica* collected within an area of 4 m^2. In total, we analyzed 225 individuals in this study.

To investigate the genetic structure of the land snail populations, we applied the methodology of protein electrophoresis in a polyacrylamide gel (isotachophoresis) described in more detail by E. Gaal and coauthors (1982).

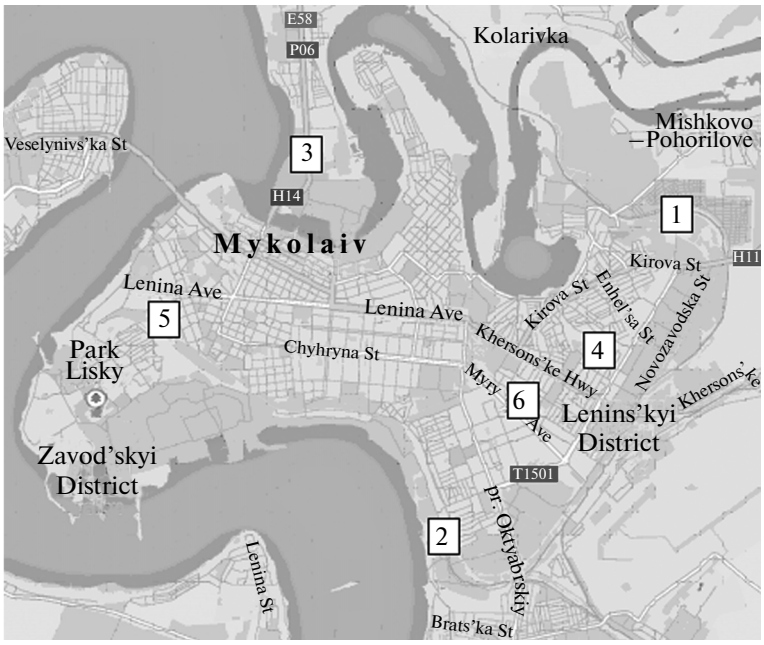
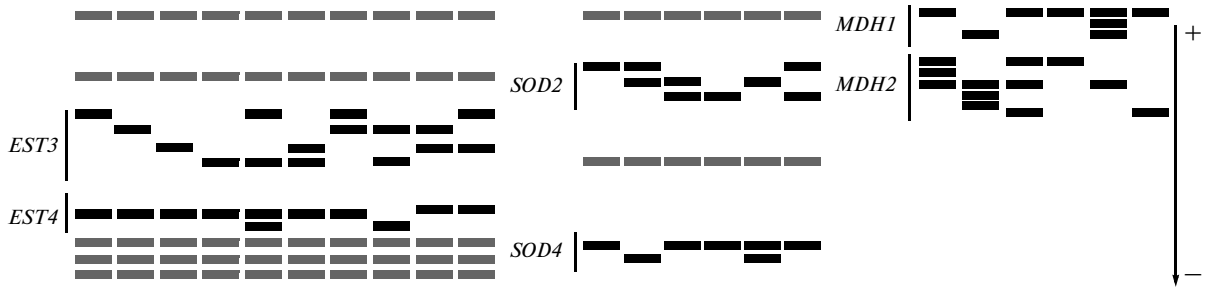


Fig. 1. Map of the collected sample populations of *B. cylindrica* with regional and/or street identifiers: 1—Dubki (Tuberculosis Hospital), 2—Neftebasa (oil refinery); 3—Park Peremoha (Ukr.), Park Pobedy (Rus.); 4—Kosmos; 5—Morechodnaya; 6—Myru (Ukr.) Ave, Mira (Rus.) Ave.



EST3	*11*	*22*	*33*	*44*	*14*	*34*	*12*	*24*	*23*	*13*	*SOD2*	*11*	*12*	*23*	*33*	*22*	*13*	*MDH1*	*11*	*22*	*11*	*11*	*12*	*11*
EST4	*11*	*11*	*11*	*11*	*12*	*11*	*11*	*22*	*11*	*11*	*SOD4*	*11*	*22*	*11*	*11*	*12*	*11*	*MDH2*	*12*	*23*	*13*	*11*	*22*	*33*

Fig. 2. Isozyme loci of *B. cylindrica* and the corresponding genotypes (monomorphic loci are shown in gray).

Water-soluble proteins were extracted from a mollusk's foot, first by freezing at –80°C, followed by thawing and mechanical grinding with a Teflon homogenizer in a 0.05 M Tris–HCl buffer (pH 6.7). Electrophoresis of the isoenzymes was carried out in a 10% polyacrylamide gel in a PROTEAN II xiCell 20 chamber (BioRad, United States). The gels were made in a Tris-HCl buffer: a stacking gel at pH 6.7 and a separating gel at pH 8.9. The electrode buffer was Tris-Glycine at pH 8.3. Gel staining for detecting nonspecific esterases was conducted in a substrate mixture with Tris–HCl pH 7.4, α-naphthyl acetate, and Fast Red TR (Sigma, United States); for the identification of superoxide dismutases, a potassium phosphate buffer (pH 7.8) with NTS and FMS was used; and to identify malate dehydrogenases, Tris–HCl 0.1 M, pH 8.4 with sodium malate, FMS, NTS, and NAD molecules were used.

We have identified five loci of nonspecific esterases in *B. cylindrica*. Two out of them turned out to be polymorphic monomeric loci that have been used previously as genetic markers (Fig. 2): *EST3* (four alleles) and *EST4* (two alleles). We isolated four loci of superoxydimutase *SOD*, two of which were polymorphic: *SOD2* (monomer with three alleles) and *SOD4* (monomer with two alleles). We identified two polymorphic loci of malate dehydrogenase: *MDH1* (dimer with two alleles) and *MDH2* (dimer with three alleles). All identified alleles are inherited as codominant.

We calculated the following indices for each examined snail populations: the mean number of alleles per locus (*Na*); the mean effective number of alleles per

locus (*Ae*); and the observed (*Ho*), as well as expected (*He*), heterozygosity for each locus and for each sample set.

The hypothesis of the conformity of the distribution of genotypes in each sample to the Hardy–Weinberg equilibrium was tested using the χ-square Pearson test.

We carried out the hierarchical analysis of molecular variance (AMOVA) in order to decompose the overall manifested variance into different components: variance between groups of populations (continual and ephemeral); between populations within each population type; and within populations (Excoffier et al., 1992). Furthermore, we calculated the index of genetic differentiation (Φst) between each pair of the investigated sample sets. The determination of the deviations of the obtained estimates compared to zero was carried out using a permutation algorithm with 999 permutations (Shitikov et al., 2008).

All the calculations were performed using the GenAIEx v.6.0 software (Peakall and Smouse, 2006).

In addition to the separate analysis of each locus, we also carried out a multilocus analysis of allozyme variation. We constructed a multilocus genotype for each of the 225 individuals and further analyzed their geographic distribution. For each population, we calculated the total number of identified variants of multilocus genotypes (N_{MLG}) and the number of unique variants (N_{MLG-1}), i.e., variants that were encountered in a single sample set.

Distribution frequencies for multilocus genotypes for each population were used further to calculate the potential genetic diversity (*Nmax*) as the maximally possible number of variants, identified with $n \to \infty$ (i.e., with the sample size approaching infinity). This was performed using two nonparametric methods: Chao1 and the first-order Jackknife estimator.

All calculations were performed using the PaSt v.2.14 (Hummer et al., 2001) and SPADE (Chao, 2005) softwares.

RESULTS

Table 1 presents the obtained frequencies of the alleles. Table 2 shows the results of testing the hypothesis that the distribution of genotypes for different allozyme loci in the studied populations of *B. cylindrica* conforms to the Hardy–Weinberg equilibrium.

Most of the examined population was in a state of genetic equilibrium for all loci, except the *SOD2* locus (Table 2). Continuous populations were not significantly different from ephemeral populations in terms of the frequencies of the occurrence of a particular monomorphic state of the investigated loci (Fisher's exact test: $p_F = 0.351$). We also did not detect any cases of excess deviation from the Hardy-Weinberg equilibrium in the ephemeral populations compared to the continuous ones (Fisher's exact test: $p_F = 0.674$).

Overall, we detected the average level of allozyme variations in the studied populations of *B.cylindrica* (between one to four alleles in the studied loci) except for the *SOD4* locus, which was identified as monomorphic in six populations (Table 2).

In the cases when we detected a significant deviation from the Hardy-Weinberg equilibrium for individual loci, it was associated primarily with a deficit of heterozygotes in a particular population (Table 2).

The examined continuous and ephemeral populations did not show a significant difference in the level of genetic diversity (Table 3).

Table 4 shows the results of the hierarchical analysis of the molecular variance for two groups of populations of the land snail *B. cylindrical* for all six of the studied allozyme loci. As one can see, the differences between populations (within both the continuous and ephemeral groups) are much higher ($\Phi_{PG} = 0.245$; $p = 0.001$) than between the two groups (continuous and ephemeral) as a whole ($\Phi_{GT} = 0.090$; $p = 0.001$). However, in the latter case, intergroup differences were also characterized by a high level of significance.

Overall, the genetic structure of the populations differed significantly in 89% of the pairwise comparisons (32 of 36 comparisons) with a 5% significance level after Bonferroni correction (Table 5). Only two samples, from the Neftebasa and Dubki-1 populations provided exceptions. Surprisingly, significant differences in relation to the genetic structure were observed for two samples from the Dubki population (Dubki-1 and Dubki- 2), which were collected only 50 m apart.

In total we registered 92 variants of multilocus genotypes for 6 loci of allozymes among the 225 genotyped individuals of *B. cylindrica*. However, their distribution, as well as the distribution of unique variants of multilocus genotypes in different populations, was not similar. The maximum number of variants of multilocus genotypes was observed in the Park Pobedy-2 (24), Neftebasa-1 (21), and Neftebasa-2 (20) populations, which belong to the group of continuous populations. In turn, the minimal number of variations (8) was observed in the two ephemeral populations (Kosmos and Morechodnaya) and one continuous population, the Dubki-2 population (6 variants). Moreover, in the last three cases, we did not detect a unique variants of a multilocus genotype (Table 6).

We found that estimates of the potential genetic diversity calculated based on the empirical distribution of the variants of multilocus genotypes were dependent on the applied evaluation method (Table 7). However, these assessments did not exceed the number of individuals genotyped for four populations, all individuals in ephemeral groups, and those in the

Table 1. Frequencies of alleles of allozyme loci in *B. cylindrica* populations

Population	Allele	Locus					
		EST3	*EST4*	*MDH1*	*MDH2*	*SOD2*	*SOD4*
Dubki-1 (D-1)	1	0.120	0.980	0.960	0.080	0.000	1.000
	2	0.600	0.020	0.040	0.700	1.000	0.000
	3	0.180			0.220	0.000	
	4	0.100					
Dubki-2 (D-2)	1	0.000	1.000	0.980	0.000	0.000	0.960
	2	0.980	0.000	0.020	0.900	1.000	0.040
	3	0.020			0.100	0.000	
	4	0.000					
Neftebasa-1 (N-1)	1	0.000	0.980	0.920	0.000	0.000	0.860
	2	0.540	0.020	0.080	0.780	0.960	0.140
	3	0.400			0.220	0.040	
	4	0.060					
Neftebasa-2 (N-2)	1	0.020	1.000	0.840	0.060	0.060	0.960
	2	0.620	0.000	0.160	0.680	0.820	0.040
	3	0.340			0.260	0.120	
	4	0.020					
Park Podedy-1 (PP-1)	1	0.240	0.860	0.820	0.040	0.000	1.000
	2	0.280	0.140	0.180	0.960	1.000	0.000
	3	0.380			0.000	0.000	
	4	0.100					
Park Podedy-2 (PP-2)	1	0.140	0.760	0.740	0.020	0.000	1.000
	2	0.340	0.240	0.260	0.880	1.000	0.000
	3	0.360			0.100	0.000	
	4	0.160					
Kosmos (Kos)	1	0.000	1.000	0.960	0.260	0.480	1.000
	2	1.000	0.000	0.040	0.620	0.500	0.000
	3	0.000			0.120	0.020	
	4	0.000					
Morechodnaya (Mor)	1	0.000	0.920	1.000	0.120	0.040	1.000
	2	1.000	0.080	0.000	0.460	0.940	0.000
	3	0.000			0.420	0.020	
	4	0.000					
Mira (Mir)	1	0.480	0.960	0.980	0.020	0.240	1.000
	2	0.120	0.040	0.020	0.660	0.600	0.000
	3	0.140			0.320	0.160	
	4	0.260					

Dubki-2 population; the latter were of a continuous type.

Overall, the values obtained for the average potential genetic diversity were: 17.2 variants (SD = 11.5) for the ephemeral populations and 93.1 (SD = 141.1) for the continuous ones. The removal of Dubki-2 population from the set of continuous populations resulted in a slight increase in the estimate value to 110.1 variants (SD = 149.4).

The permutation criterion confirms the trend of a significant reduction in the genetic diversity in the ephemeral populations compared to the continuous

Table 2. Results of testing the hypothesis that the distribution of genotypes in *B. cylindrica* populations conforms to the Hardy–Weinberg equilibrium

Population	Locus					
	EST3	*EST4*	*MDH1*	*MDH2*	*SOD2*	*SOD4*
Dubki-1 (D-1)	Ns	Ns	Ns	Ns	Mono	Mono
Dubki-2 (D-2)	Ns	Mono	Ns	Ns	Mono	Ns
Neftebasa -1 (N-1)	Ns	Ns	Ns	D*	D***	Ns
Neftebasa -2 (N-2)	Ns	Mono	Ns	Ns	D***	Ns
Park Podedy-1 (PP-1)	D*	Ns	Ns	Ns	Mono	Mono
Park Podedy-2 (PP-2)	Ns	Ns	Ns	Ns	Mono	Mono
Kosmos (Kos)	Mono	Mono	Ns	Ns	Ns	Mono
Morechodnaya (Mor)	Mono	D*	Mono	Ns	D***	Mono
Mira (Mir)	Ns	Ns	Ns	Ns	D***	Mono

Mono—monomorphic locus; D—significant deficit of heterozygotes is noted; *—$p < 0.05$; ***—$p < 0.001$; ns—not significant.

Table 3. Assessments of genetic diversity in *B. cylindrica* populations

Population	Mean number of alleles per locus (*Na*)	Mean effective number of alleles per locus (*Ae*)	Mean observed heterozygosity per locus (*Ho*)
Dubki-1	2.17 ± 0.48	1.39 ± 0.24	0.192 ± 0.105
Dubki-2	1.67 ± 0.21	1.06 ± 0.03	0.056 ± 0.027
Neftebasa-1	2.17 ± 0.17	1.39 ± 0.18	0.232 ± 0.077
Neftebasa-2	2.50 ± 0.43	1.46 ± 0.17	0.270 ± 0.082
Park Pobedy-1	2.00 ± 0.45	1.54 ± 0.39	0.220 ± 0.110
Park Ponedy-2	2.17 ± 0.48	1.65 ± 0.38	0.279 ± 0.110
Kosmos	1.83 ± 0.40	1.39 ± 0.23	0.188 ± 0.108
Morechodnaya	1.83 ± 0.40	1.30 ± 0.24	0.143 ± 0.095
Mira	2.50 ± 0.43	1.71 ± 0.34	0.300 ± 0.120

ones. At the same time, removal of the Dubki-2 population from the continuous group makes this trend significant (Table 7).

DISCUSSION

Overall, we did not detect significant differences in the levels of genetic polymorphism of the allozyme loci between the continuous and ephemeral populations of the land snail *B. cylindrica*. The frequency of the monomorphic loci and incidence of deviations from the Hardy-Weinberg equilibrium were also similar in the continuous and ephemeral populations (Table 2). Moreover, the ephemeral populations did not display lower estimates of the observed heterozygosity or the mean number of alleles per locus (Table 3).

However, our analysis of the multilocus genotypes for the studied allozyme loci revealed a fundamentally different result. We found that in overall, the ephemeral populations differed from the continuous in terms

Table 4. Results of the hierarchical analysis of the molecular variance for two groups of population of the *B. cylindrica* mollusk for the six studied allozyme loci

Source of variation	*df*	*SS*	*MS*	*E* (*MS*)	Φst; *p*
Among groups	1	33.313	33.313	0.197	$\Phi_{GT} = 0.090$; $p = 0.001$
Among populations within group	7	95.420	13.631	0.486	$\Phi_{PG} = 0.245$; $p = 0.001$
Among individuals within populations	216	322.520	1.493	1.493	$\Phi_{PT} = 0.314$; $p = 0.001$
Total	224	451.253	48.438	2.175	

Table 5. Pairwise estimates of genetic differentiation (Φst) between populations of *B. cylindrica* for six allozyme loci

Population	D-1	D-2	N-1	N-2	PP-1	PP-2	Kos	Mor	Mir
D-1		0.001	0.062	0.067	0.001	0.001	0.001	0.001	0.001
D-2	**0.216**		0.001	0.001	0.001	0.001	0.001	0.001	0.001
N-1	0.044	**0.273**		0.272	0.001	0.001	0.001	0.001	0.001
N-2	0.039	**0.245**	0.012		0.001	0.001	0.001	0.001	0.001
PP-1	**0.173**	**0.466**	**0.137**	**0.173**		0.420	0.001	0.001	0.001
PP-2	**0.144**	**0.392**	**0.111**	**0.130**	0.000		0.001	0.001	0.001
Kos	**0.327**	**0.392**	**0.366**	**0.254**	**0.489**	**0.433**		0.001	0.001
Mor	**0.191**	**0.303**	**0.286**	**0.201**	**0.488**	**0.398**	**0.272**		0.001
Mir	**0.244**	**0.513**	**0.269**	**0.228**	**0.246**	**0.239**	**0.390**	**0.431**	

Values bellow the diagonal present estimates for Φst, and above the diagonal, the levels of significance. Φst estimates deemed significant after the Bonferroni correction are marked in bold.

Table 6. Sample estimates of the overall genetic diversity for the six allozyme loci in the studied *B. cylindrica* populations

Indicies	Population								
	D-1	D-2	N-1	N-2	PP-1	PP-2	Kos	Mor	Mir
N_{MLG}	18	6	21	20	18	24	8	8	16
N_{MLG-1}	7	0	10	10	8	14	0	0	8

N_{MLG}—the total number of identified variants of multilocus genotypes; N_{MLG-1}—the number of unique variants of multilocus genotypes.

of distribution of the frequencies of different variants of multilocus genotypes. Only one population presented an exception to this pattern (the Dubki-2 population) (Fig. 3).

The low levels of genetic diversity, calculated based on multilocus genotypes, may be a result of the introduction of single individuals with the subsequent formation of the ephemeral population. The fact that a similar pattern was observed for one continuous population (Dubki) indicates that processes of local extinction and recolonization also take place in continuous populations of the land snail.

On the other hand, the level of interpopulation genetic diversity between the ephemeral populations

Table 7. Estimates of the potential genetic diversity for the studied *B. cylindrica* populations obtained by different methods

Population	Method			
	Chao1		First-order Jackknife	
	Nmax ± SE	95% CI	*Nmax* ± SE	95% CI
D-1	78.0 ± 34.8	34.7–234.2	33.4 ± 5.5	25.8–48.3
D-2	9.0 ± 4.4	6.4–30.6	8.9 ± 2.4	6.7–17.8
N-1	106.5 ± 59.5	46.0–314.0	39.2 ± 6.0	30.8–55.1
N-2	50.0 ± 21.0	28.7–123.2	34.5 ± 5.5	27.8–50.3
PP-1	48.3 ± 22.8	26.2–130.4	41.4 ± 5.1	24.5–45.7
PP-2	150.5 ± 83.9	62.7–437.1	46.1 ± 6.6	36.5–63.1
Kos	8.2 ± 0.6	8.0–12.1	9.9 ± 1.9	8.4–17.9
Mor	9.0 ± 1.8	8.1–18.7	10.9 ± 2.4	8.7–19.8
Mir	25.0 ± 7.6	18.1–54.0	25.6 ± 4.3	20.1–38.3
p_{perm}	0.085* (0.029)	–	0.172 (0.008)	–

*—The values given in brackets represent evaluations of the criterion after removal of the Dubki-2 population from the analysis.

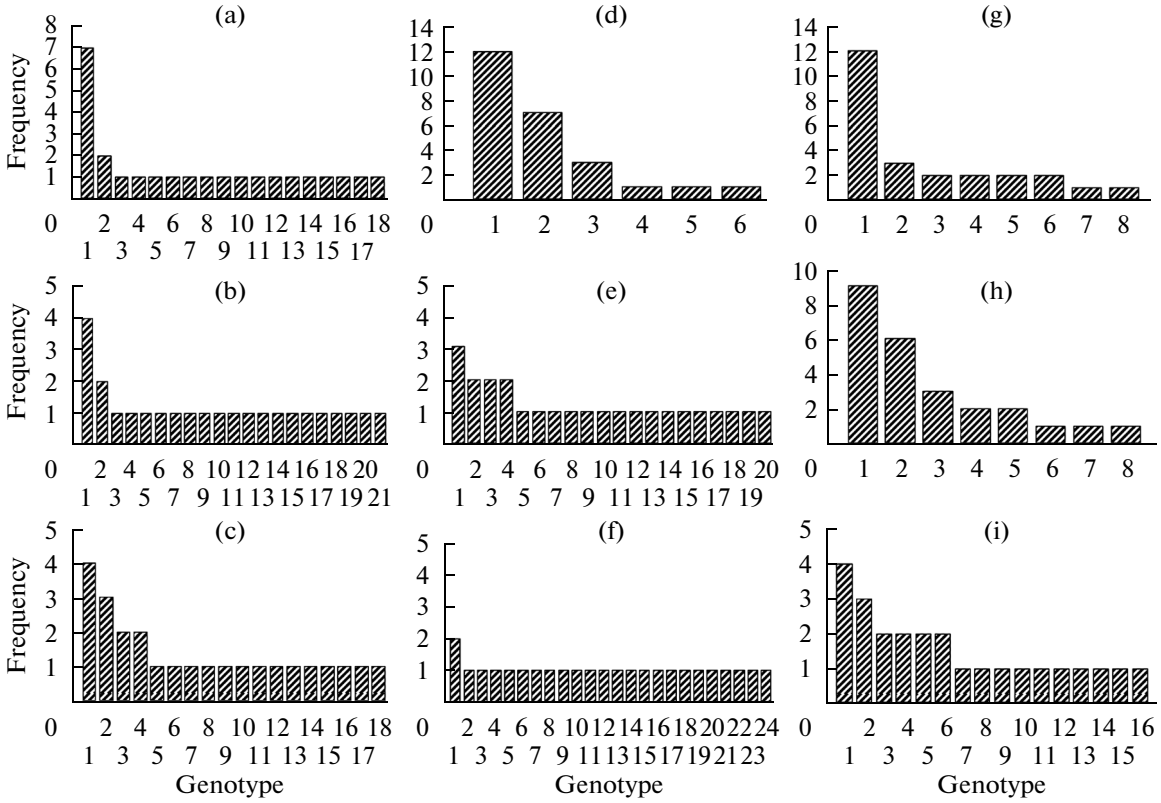


Fig. 3. Distribution of *B. cylindrica* individuals, which possess different variants of multilocus genotypes, in different populations: a—Dubki-1; b—Neftebasa-1; c—Park Pobedy-1; d—Dubki-2; e—Neftebasa-2; f—Park Pobedy-2; g—Kosmos; h—Morechodnaya; i—Mira.

of *B. cylindrica* snails turned out to be significantly higher than between the continuous populations. Overall, the level of genetic differentiation between all nine investigated populations (without consideration for subgroups) was calculated as $\Phi st = 0.281$ ($p = 0.001$). However, we found that interpopulation differentiation was much lower among continuous populations ($\Phi st = 0.167$, $p = 0.001$) than among ephemeral ones ($\Phi st = 0.378$, $p = 0.001$). Thus, based on our analysis of the genetic structure (pool of genotypic profiles) of *B. cylindrica*, individuals in the ephemeral population are more variable than in the continuous populaiton.

In order to determine the extent to which the defined patterns may be applicable to other animal taxa, we analyzed the published literature that presented estimates of genetic diversity (in particular, in relation to the mean number of alleles and observed heterozygosity) and the levels of genetic differentiation for species dwelling in populations under strong anthropogenic pressure (from the urbanized, fragmented, and/or ephemeral populations) (Table 8). This analysis included only organisms with low migration capabilities (soil invertebrates and small nonflying vertebrates, 30 species in total).

We tested two hypotheses. First, genetic diversity is lower in isolated populations composed of a small number of individuals than in continuous ones that consist of a large number of animals. Second, the level of genetic differentiation between isolated a small number of (sub)populations will be higher than among numerous, continuous groups.

Our analysis showed that the first hypothesis was confirmed only in 19 cases and was rejected in 10 other cases. Thus, the first hypothesis can not be unambiguously accepted based on the empirical data (sign test: $0.1 < p < 0.05$). However, the overall downward trend in the level of genetic diversity in small, isolated populations is noticeable (Table 8).

The second hypothesis was confirmed by the empirical data in 18 cases and rejected only in two instances (sign test: $p < 0.01$).

Thus, based on the obtained data, we can conclude that small, isolated (including urbanized) populations tend to reduce the level of genetic diversity, manifested due to the genetic and stochastic processes (genetic drift or founder effect). In addition, an important consequence of the latter is the unpredictable changes in the genotypic profiles of such populations, which lead

Table 8. Analysis of the impact of urbanization and fragmentation on the estimates of genetic diversity and estimates of the level of genetic differentiation for different animal species

Species	Country	Marker*	Genetic variation (*Ho*, *Ae*)	Genetic differentiation (*F*st, Φst, ect.)	Reference
Beetle *Pterostichus madidus* (Fabricius, 1775)	Belgium, Great Britain	Allozymes	Higher in smaller urban populations	–	Desender et al., 2005
Beetle *Abax ater* (Villers, 1789)	Belgium, Great Britain	Allozymes	Lower in smaller, fragmented urban populations	–	Desender et al., 2005
Beetle *Carabus violaceus* L., 1758	Switzerland	MS	Lower in smaller, fragmented populations	Higher among fragmented populations	Keller, Largiader, 2003
Bedbug *Triatoma infestans* (Klug, 1834)	Bolivia	cyt b	Does not differ in urban and rural populations	–	Giordano et al., 2005
Land snail *Brephulopsis cylindrica*	Ukraine	Allozymes	Lower in smaller ephemeral populations	Higher between small ephemeroid populations	Our own data
Land snail *Fruticicola fruticum* (Mueller, 1774)	Russian Federation	Allozymes	Lower in urban populations	–	Makeeva et al., 2005
Land snail *Cepaea vindobonensis* (Ferussac, 1821)	Russian Federation, Ukraine	Allozymes	Does not differ in urban and rural populations	–	Snegin, 2011
Frog *Rana arvalis* Nilsson, 1842	Netherland	MS	Lower in urban fragmented populations	Higher between urban fragmented populations	Arens et al., 2007
Frog *Rana dalmatina* Fitzinger, 1839	France	Allozymes	Lowe in fragmented populations	Higher among fragmented populations	Lesbarreres et al., 2006
Frog *Rana temporaria* L., 1758	Great Britain	Allozymes	Lower in urban populations	Higher between urban populations	Hitchings, Beebee, 1997
Frog *R. temporaria*	Finland	MS	Does not differ in urban and rural populations	–	Saarikivi et al., 2013
Frog *R. temporaria*	Great Britain	MS	Does not differ in urban and rural populations	–	Zeisset, Beebee, 2010
Frog *Hyla arborea* (L., 1758)	Denmark	MS	Lowe in fragmented populations	–	Andersen et al., 2004
Frog *H. arborea*	Switzerland	MS	Lower in urban populations	Does not depend on the level of urbanization	Dubey et al., 2009
Frog *Pelophylax ridibundus* (Pallas, 1771)	Slovakia	MS	Lower in urban fragmented populations	Higher between urban fragmented populations	Mikulíček, Pišút, 2013
Toad *Bufo bufo* (L., 1758)	Great Britain	Allozymes	Lower in urban populations	Higher between urban populations	Hitchings, Beebee, 1998

Table 8. (Contd.)

Species	Country	Marker*	Genetic variation (*Ho*, *Ae*)	Genetic differentiation (*F*st, Φst, ect.)	Reference
Salamander *Plethodon cinereus* (Green, 1818)	USA	RAPD	Does not differ in fragmented and continuous populations	Higher among fragmented populations	Gibbs, 1998
Salamander *P. cinereus*	Canada	MS	Lower in urban populations	Higher between urban populations	Noël et al., 2007
Lizard *Uta stansburiana* (Baird & Girard, 1852)	USA	MS	Lower in more isolated populations	Higher among fragmented populations	Delaney et al., 2010
Lizard *Plestiodon skiltonianus* (Baird & Girard, 1852)	USA	MS	Lower in more isolated populations	Higher among fragmented populations	Delaney et al., 2010
Lizard *Sceloporus occidentalis* Baird & Girard, 1852	USA	MS	Higher among fragmented populations	Higher among fragmented populations	Delaney et al., 2010
Lizard *Podarcis muralis* (Laurenti, 1768)	Germany	MS	Lower in marginal (newly formed) populations	Higher between marginal (newly formed) populations	Schulte et al., 2013
Lizard *Gnypetoscincus queen-slandiae* (De Vis, 1890)	Australia	MS	Does not differ in fragmented and continuous populations	Does not differ between fragmented and continual habitats	Sumner et al., 2004
Gecko *Oedura reticulata* Bustard, 1969	Australia	MS	Lowe in fragmented populations	Higher among fragmented populations	Hoehn et al., 2007
Gecko *Gehyra variegata* (Dumeril & Bibron, 1836)	Australia	MS	Lowe in fragmented populations	Higher among fragmented populations	Hoehn et al., 2007
Turtle *Emydoidea blandingii* (Holbrook, 1838)	USA	RAPD	Does not differ in urban and natural populations	–	Rubin et al., 2001
Hamster *Calomys musculinus* (Thomas, 1913)	Argentina	MS	Does not differ in urban and natural populations	Higher between urban subpopulations	Chiappero et al., 2011
Mouse *Apodemus agrarius* (Pallas, 1771)	Poland	MS	Does not differ in urban and natural populations	Higher among fragmented populations	Gortat et al., 2013
Mouse *Apodemus speciosus* (Temminck, 1844)	Japan	mtDNA	–	Higher between populations isolated by urbanized areas	Hirota et al., 2004
Prairie Dog *Cynomys ludovicianus*	USA	MS	Lower in smaller, more isolated colonies	–	Magle et al., 2010

* MS—microsatellites; cyt b—cytochrome b; RAPD—Random Amplified Polymorphic DNA; mtDNA—mitochondrial DNA.

to a significant increase in the level of genetic differentiation between them.

It is important to note that our findings are in agreement with the provisions of the "Shifting Balance Theory of Evolution" (Wright, 1970), according to which subdivisions in a metapopulation result in increased volatility in a background of reduced heterogeneity in the subpopulations. In turn, variability in the subpopulations leads to differentiation between populations, which is reflected in the increase in the Fst index.

REFERENCES

Andersen, L.W., Fog, K., and Damgaard, C., *Proc. Roy. Soc. B: Biol. Sci.,* 2004, vol. 271, no. 1545, pp. 1293–1302.

Arens, P.F.P., van der Sluis, T., van't Westende, W.P.C., et al., *Landscape Ecol.*, 2007, vol. 27122, pp. 1489–1500.

Balashov, I.A., Kramarenko, S.S., Zhukov, A.V., et al., *Malacologica Bohemoslovaca*, 2013, vol. 12, pp. 62–69.

Chao, A. Species richness estimation, in *Encyclopedia of Statistical Science*, Balakrishnan, N., Read, C.B., and Vidakovic, B., Eds., New York: Wiley, 2005, pp. 7907–7916.

Chiappero, M.B., Panzetta-Dutari, G.M., Gomez, D., et al., *Mammal. Biol.,* 2011, vol. 76, no. 1, pp. 41–50.

Delaney, K.S., Riley, S.P.D., and Fisher, R.N., *PLoS ONE*, 2010, vol. 5, no. 9, p. e12767.

Desender, K., Small, E., Gaublomme, E., and Verdyck, P., *Conserv. Genet.*, 2004, vol. 6, no. 1, pp. 51–62.

Dubey, S., Ursenbacher, S., Pellet, J., and Fumagalli, L., *Amphibia–Reptilia,* 2009, vol. 30, pp. 127–133.

Excoffier, L., Smouse, P.E., and Quattro, J.M., *Genetics,* 1992, vol. 131, no. 2, pp. 479–491.

Gaal, O., Medgyesi, G.A., and Vereczkey, L., *Electrophoresis in the Separation of Biological Macromolecules*, New York: John Wiley, 1980.

Gibbs, J.P., *Biol. Conservat.*, 1998, vol. 86, no. 1, pp. 77–81.

Giordano, R., Cortez, J.C.P., Paulk, S., and Stevens, L., *Memorias do Instituto Oswaldo Cruz*, 2005, vol. 100, no. 7, pp. 753–760.

Gortat, T., Rutkowski, R., Gryczynska-Siemiatkowska, A., et al., *Mammal. Biol.*, 2013, vol. 78, no. 3, pp. 171–177.

Hirota, T., Hirohata, T., Mashima, H., et al., *Mol. Ecol.*, 2004, vol. 13, no. 11, pp. 3275–3282.

Hitchings, S.P. and Beebee, T.J.C., *Heredity*, 1997, vol. 79, no. 2, pp. 117–127.

Hitchings, S.P. and Beebee, T.J.C., *J. Evol. Biol.*, 1998, vol. 11, no. 3, pp. 269–283.

Hoehn, M., Sarre, S.D., and Henle, K., *Mol. Ecol.,* 2007, vol. 16, no. 16, pp. 3299–3312.

Hummer, O., Harper, D.A.T., and Ryan, P.D., *Paleontologia Electronica,* 2001, vol. 4, pp. 1–9.

Keller, I. and Largiader, C.R., *Proc. Roy. Soc. B: Biol. Sci.,* 2003, vol. 270, pp. 417–423.

Kramarenko, S.S., Phenotypic variability of the Crimean mollusks of the genus *Brephulopsis* Lindholm, 1925 (Gastropoda; Pulmonata; Buliminidae), *Abstract of the PhD Thesis*, Kiev, 1995.

Lesbarreres, D., Primmer, C.R., Lode, T., and Merila, J., *Ecoscience*, 2006, vol. 13, pp. 531–536.

Magle, S.B., Ruell, E.W., Antolin, M.F., and Crooks, K.R., *J. Mammal.*, 2010, vol. 91, no. 2, pp. 326–335.

Makeeva, V.M., Belokon, M.M., and Malyuchenko, O.P., 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* Mull., *Russ. J. Genet.,* 2005, vol. 41, no. 11, pp. 1495–1510.

Mikulicek, P. and Pisut, P., *Eur. J. Wildlife Res.*, 2012, vol. 58, no. 5, pp. 833–845.

Noel, S., Ouellet, M., Galois, P., and Lapointe, F.-J., *Conserv. Genet.*, 2007, vol. 8, no. 3, pp. 599–606.

Peakall, R. and Smouse, P.E., *Mol. Ecol. Notes,* 2006, vol. 6, no. 1, pp. 288–295.

Rubin, C.S., Warner, R.E., Bouzat, J.L., and Paige, K.N., *Biol. Conserv.,* 2001, vol. 99, no. 3, pp. 323–330.

Saarikivi, J., Knopp, T., Granroth, A., and Merila, J., *Conserv. Genet.,* 2013, vol. 14, pp. 1057–1064.

Schulte, U., Veith, M., Mingo, V., et al., *Biol. Invasions,* 2013, vol. 15, no. 12, pp. 2639–2649.

Shitikov, V.K., Rozenberg, G.S., Kramarenko, S.S., and Yakimov, V.N., Modern approaches to the statistical analysis of experimental data, in *Problemy ekologicheskogo eksperimenta (planirovanie i analiz nablyudenii)* (Problems of Ecological Experiment (Planning and Analysis of Observations)), Rozenberg, G.S. and Gelashvili, D.B., Eds., Tolyatti: Sam-SC RAN, Kassandra, 2008, pp. 212–250.

Snegin, E.A., Estimation of the viability of populations of the especially protected species *Cepaea vindobonensis* (Mollusca, Gastropoda, Pulmonata) in the conditions of the forest–steppe south of the Central Russian Upland, *Vestnik KrasGAU,* 2011, no. 11, pp. 142–148.

Sverlova, N.V., First finding of the *Brephulopsis cylindrical* (Gastropoda, Buliminidae) in Lviv, *Vestnik Zool.*, 1998, vol. 32, nos. 5–6, p. 72.

Sumner, J., Jessop, T., Paetkau, D., and Moritz, C., *Mol. Ecol.*, 2004, vol. 13, no. 2, pp. 259–269.

Vitchalkovskaya, N.V., Distribution and intraspecific variability of the Crimean endemic snail *Brephulopsis cylindrical* (Gastropoda, Pulmonata, Buliminidae) beyond the native area of distribution, *Vestnik Zool.*, 2008, vol. 42, no. 3, pp. 229–235.

Vitchalkovskaya, N.V. and Kramarenko, S.S., First finding of the land-snail *Brephulopsis cylindrical* (Gastropoda, Pulmonata, Buliminidae) in Kyiv,

Wright, S., Berlin: Springer-Verlag, 1970, pp. 1–31.

Zeisset, I. and Beebee, T.J., *Heredity*, 2010, vol. 104, no. 5, pp. 423–430.

Translated by I. Grishina