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# 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

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**Abstract**—Studies on the gene pool structure in ten populations of the bush snail *Bradybaena fruticum* Müll. inhabiting the southern Central Russian Upland were performed with a time interval of 14 years, in 1996 and 2010. Significant changes were revealed in allele frequencies and the level of genetic diversity. Using the temporal method (Krimbas and Tsakas, 1971; Nei and Tajima, 1981), the effective population size was calculated for the studied groups of mollusks. Specific features of the metapopulation structure of *Br. fruticum* in an urbanized landscape are discussed.

*Keywords*: snail, population, gene pool dynamics, effective population size

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This study is part of long-term monitoring research on the population structure of the bush snail *Bradybaena fruticum* Müll.[1] in the territory of Eurasia. In the course of nature conservation monitoring projects, specialists estimate the effective population size of test species, i.e., the minimum population numbers sufficient for their survival. In population genetics, the effective size is defined as "the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration" (Wright, 1931). Such understanding of the effective population size allows a more objective approach to the development of nature conservation programs and is useful for studying evolutionary processes in natural populations.

It is known that the diversity of adaptations and variability of climatic factors markedly impair predictions that are based on data obtained over short periods of time. Therefore, long-term observations are the only feasible alternative for verifying proposed hypotheses and obtaining data that reflect the actual situation.

## MATERIAL AND METHODS

The structure of gene pools in *Br. fruticum* populations inhabiting the southern Central Russian Upland was studied twice, with an interval of 14 years, to calculate and compare their effective sizes. On the whole, 20 populations were studied in 1996 and 37 populations in 2010, including 10 populations studied in both years and used for comparative analysis (Table 1, figure).

Snails were sampled from 2 × 2-m plots. First, the vegetation was swept with a standard insect net, and then snails were manually collected from the surface. When their density was low, the size of sampling plots was increased twofold. Three to four samples were taken from each biocenosis. The coordinates of the plots were recorded with a Garmin 76 GPS unit. The total area of the biotope inhabited by the snails was determined using a map.

Mendelian traits used as markers of population structure were as follows: a longitudinal brown stripe on the shell (phene *S*+), which indicated snails homozygous for the corresponding recessive allele (Khokhutkin, 1979); the yellow color of the shell (phene $C_3$) in snails homozygous for the corresponding color allele (Snegin, 1999, 2005); and nonspecific esterase locus *EST2* with three codominant alleles (Matekin and Makeeva, 1977; Makeeva et al., 2005).

To extract water-soluble proteins, the snail's foot was frozen at –80°C, thawed, and homogenized with a Teflon homogenizer in 0.05 M Tris-HCl buffer, pH 6.7. Isozyme electrophoresis in 10% PAAG prepared in Tris-HCl buffer (stacking gel, pH 6.7; resolving gel, pH 8.9) was performed in a 20-cm PROTEAN II xi cell (BioRad, United States) using Tris-glycine electrode buffer, pH 8.2. Gel blocks were stained with

[1] Some authors (Kantor and Sysoev, 2005; Zeifert and Khokhutkin, 2010) assign this species to the genus *Fruticicola*.

**Table 1.** Description of sampling sites

| Site | Biotope description | Coordinates |
|---|---|---|
| 1. Stenki Izgor'ya | Specially protected area Stenki Izgor'ya. A waterlogged biotope with alder thickets and bramble and stinging nettle in the undergrowth | 50°41′23″ N, 37°49′12″ E |
| 2. Roven'ki | Roven'skii Nature Park in the Aidar River floodplain, near the village of Roven'ki. An open mesic biotope with thickets of bramble and Sosnowsky's hogweed and an admixture of stinging nettle | 49°54′33″ N, 38°52′55″ E |
| 3. Borisovka | The Vorskla River floodplain near the village of Khotmyzhsk; Thickets of bramble with an admixture of stinging nettle | 50°36′35″ N, 36°00′25″ E |
| 4. Khotmyzhsk | The Vorskla River floodplain near the village of Khotmyzhsk; Thickets of bramble with an admixture of stinging nettle | 50°35′05″ N, 35°52′24″ E |
| 5. Golovchino | The Vorskla River floodplain near the village of Golovchino, upland oak forest | 50°33′57″ N, 35°48′12″ E |
| 6. Syrtsevo | The Pena River floodplain near the village of Syrtsevo (Ivnyanskii district), willow and maple thickets | 50°53′48″ N, 36°15′32″ E |
| 7. Yasnyi Kolodets | Yasnyi Kolodets natural landmark in the Korocha River floodplain near the town of Korocha, black alder forest margin | 50°49′34″ N, 37°12′34″ E |
| 8. Koren' | The Koren' River floodplain near the village of Alekseevka (Korochanskii district), willow thickets | 50°45′19″ N, 37°01′30″ E |
| 9. Seversky Donets | The Seversky Donets River floodplain near Belgorod, willow and maple thickets | 50°36′38″ N, 36°37′19″ E |
| 10. Nezhegol | The Nezhegol River floodplain within the Shebekino city limits, willow forest | 50°24′32″ N, 36°52′38″ E |

a substrate mixture of α-naphthylacetate and fast red TR in Tris-HCl buffer, pH 7.4.

To date, a fairly large amount of data on the state of gene pools in *Br. fruticum* populations have been obtained using both allozyme and DNA markers (Snegin, 2011a, 2011b). However, the genetic structure of these populations in 1996 was analyzed with regard to shell phenes *S*+ and $C_3$ and esterase locus *EST2*, only these three markers were used for comparative analysis.

The effective population size was calculated using the so-called temporal method proposed by Krimbas and Tsakas (1971) and developed by Nei and Tajima (1981). It is based on the comparison of allele frequencies at the same locus (or at several loci simultaneously) in two samples taken from the same locality with a time interval expressed in the number of generations. Hence, information on the average generation time is necessary.

The effective population size is calculated by the formula

$$Ne = \frac{t}{2\left[Fk - \frac{1}{2N_0} - \frac{1}{2N_t}\right]},$$

where $t$ is the time (number of generations) between sampling, $N_0$, $N_t$ are sample sizes at times 0 and $t$, and $Fk$ is the standardized variance of allele frequencies:

$$Fk = \frac{1}{A}\sum\left[\frac{(p_i^0 - p_i^t)^2}{\frac{(p_i^0 + p_i^t)}{2} - \left(p_i^0 \times p_i^t\right)}\right],$$

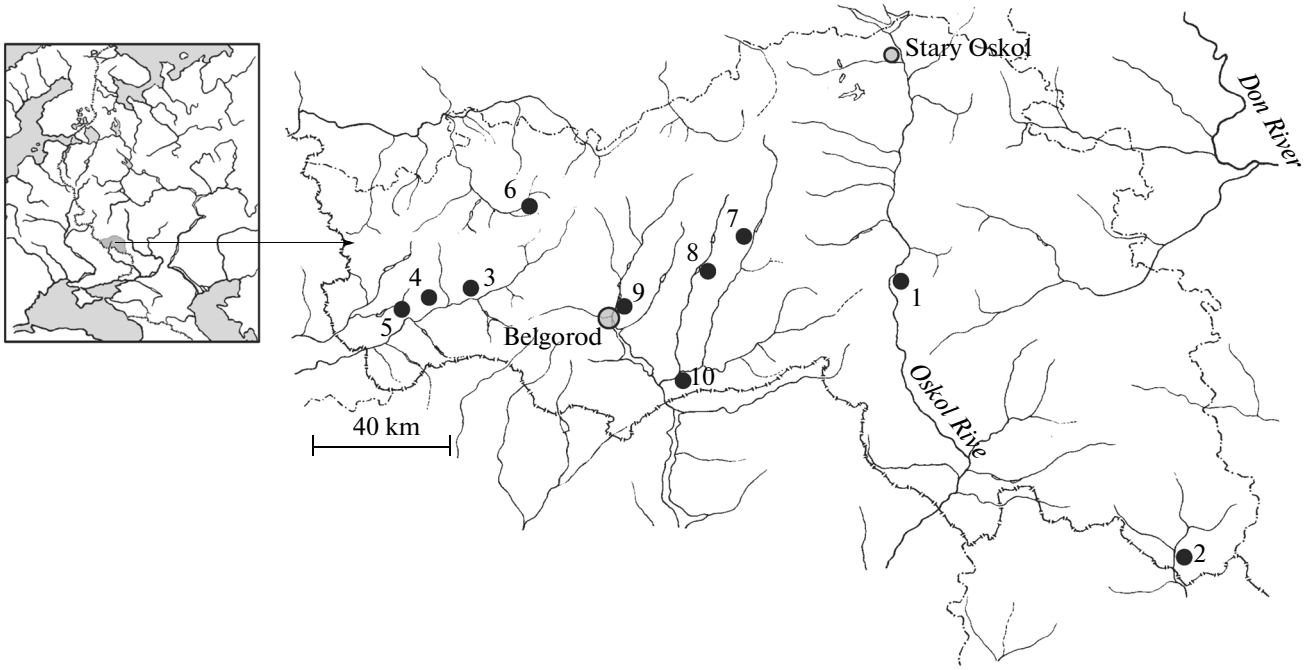
where $A$ is the number of alleles at a given locus, and $p_i^0$ and $p_i^t$ are frequencies of the $i$th allele at times 0 and $t$.

For calculations based on several loci, the weighted average value of $Fk$ is used:

$$Fk(ML) = \frac{\sum(Aj - 1) \times Fk(j)}{\sum(Aj - 1)},$$

where $Fk(ML)$ is the standardized variance of allele frequencies at several loci, $Fk(j)$ is the same at the $j$th locus, and $Aj$ is the number of alleles at this locus.

The results were processed statistically using the GenAlEx 6.5 program (Peakall and Smouse, 2012). The significance of differences between allele frequencies in the samples of 1996 and 2010 was estimated with Fisher's (1958) exact test.




Map of sampling sites.

## RESULTS AND DISCUSSION

The age of maturity for *Br. fruticum* in the Russian Plain varies between 3.5 and 4 years (Khokhutkin, 1997); therefore, the period between 1996 and 2010 is equivalent to three complete generation times. The samples used for phenetic and biochemical analysis differed in size, and, to obtain more reliable results, calculations of effective population size were made in two variants, based on the sum of shell phenes and on the isozyme locus.

Data on the allele frequencies in the populations studied and the results of calculating their effective size are shown in Tables 2 and 3. It can be seen that the values of effective size calculated by the esterase locus and shell phenes markedly differ from each other. This may be evidence for different vectors and intensities of selection for different systems. The only exceptions are site 1 (Stenki Izgor'ya), where the values of effective size calculated in either way are high, and site 10 (Nezhegol), where these values calculated from phene and allozyme frequencies differ by a factor of no more than two.

Significant differences in the frequency of allele *qS+* between samples taken in different years were revealed in populations nos. 2, 3, 4, and 10, and in the frequencies of alleles *qS+* and $qC_3$, in populations nos. 6 and 9 (Table 2), which is reflected in low values of their effective size.

In the Seversky Donets group (no. 9), allele frequencies at the esterase locus in samples of different years proved to be closely similar, and the effective population size in the temporal model approached infinity. To obtain a more precise value, the sample of about 2000 ind. should be taken from this population, which may be harmful for it.

Thus, the results obtained using this model allowed us to reveal groups with more stable gene pools and high values of effective size and, on the other hand, groups with decreased viability (low effective size). To obtain a more detailed picture, it is necessary to include in analysis a greater number of both coding and noncoding DNA loci, which is planned for the near future.

Table 4 shows indices characterizing genetic heterogeneity of *Br. fruticum* populations that were calculated from allele frequencies at the esterase locus. Significant changes in these indices occurred in five out of ten populations (nos. 2, 5, 7, 8, 10), especially in that from Yasnyi Kolodets (no. 7). Judging from our data, this population in 1996 was completely homozygous for allele *EST2-2*, which may be explained as follows. The population lives in the Korocha River floodplain, at the margin of black alder forest growing at the base of a steep chalk slope. This biotope is periodically flooded in spring, and part of snails die, while others attempt to escape by moving upslope, where die in summer from overheating (as follows from the presence of numerous empty shells in the lower part of the slope). The density of snails in 1996 was 0.5 ind./$m^2$, and the total size of the adult population was about 30–40 ind. Taking into account almost 100% homozygosity and low density of this population, it had a high probability of extinction within a short time. Thus, its effective size according to the demo-

**Table 2.** Allele frequencies ($q \pm m_q$) in groups of *Br. fruticum* and their effective size calculated by the temporal method on the basis of data on shell phenes *S*+ and $C_3$

| Sampling site | Year | *N* | Allele frequencies | | *Fk* | *Ne* |
|---|---|---|---|---|---|---|
| | | | $qC_3$ | *qS*+ | | |
| 1. Stenki Izgor'ya | 1996 | 119 | 0.344 ± 0.044 | 0.400 ± 0.045 | 0.019 | 76.3 |
| | 2010 | 94 | 0.371 ± 0.050 | 0.462 ± 0.052 | | |
| 2. Roven'ki | 1996 | 140 | 0.643 ± 0.041 | 0.615 ± 0.041 | 0.386 | 3.8 |
| | 2010 | 65 | 0.723 ± 0.056 | 0.303 ± 0.057 | | |
| 3. Borisovka | 1996 | 199 | 0.283 ± 0.032 | 0.381 ± 0.035 | 0.314 | 4.7 |
| | 2010 | 67 | 0.322 ± 0.058 | 0.669 ± 0.058 | | |
| 4. Khotmyzhsk | 1996 | 80 | 0.224 ± 0.047 | 0.000 | 0.511 | 2.9 |
| | 2010 | 47 | 0.253 ± 0.064 | 0.253 ± 0.064 | | |
| 5. Golovchino | 1996 | 120 | 0.259 ± 0.040 | 0.570 ± 0.045 | 0.078 | 17.4 |
| | 2010 | 42 | 0.155 ± 0.057 | 0.512 ± 0.078 | | |
| 6. Syrtsevo | 1996 | 75 | 0.200 ± 0.046 | 0.529 ± 0.058 | 1.458 | 1.0 |
| | 2010 | 39 | 0.000 | 0.000 | | |
| 7. Yasnyi Kolodets | 1996 | 113 | 0.297 ± 0.043 | 0.297 ± 0.043 | 0.030 | 44.3 |
| | 2010 | 63 | 0.378 ± 0.062 | 0.281 ± 0.057 | | |
| 8. Koren' | 1996 | 87 | 0.442 ± 0.054 | 0.263 ± 0.047 | 0.171 | 8.7 |
| | 2010 | 57 | 0.265 ± 0.059 | 0.459 ± 0.067 | | |
| 9. Seversky Donets | 1996 | 179 | 0.374 ± 0.036 | 0.290 ± 0.034 | 0.851 | 1.7 |
| | 2010 | 42 | 0.000 | 0.155 ± 0.057 | | |
| 10. Nezhegol | 1996 | 132 | 0.288 ± 0.040 | 0.261 ± 0.038 | 0.583 | 2.5 |
| | 2010 | 36 | 0.409 ± 0.083 | 0.000 | | |

graphic model was only 2.5–3.3 ind., and life expectancy was 18–25 years.[2]

The situation observed 14 years later, in 2010, was different. Two other alleles, *EST2-1*and *EST2-3*, appeared with high frequency in the population gene pool, with the effective number of alleles increasing from 1.0 to 2.72; Shannon's index increased from zero to 1.044; the level of observed heterozygosity increased significantly, reaching 0.381; and the coefficient of inbreeding (arbitrary units) decreased from unity to 0.398. The density of adult snails was fairly high, up to 10 ind./m$^2$. It may well be that some snails managed to survive spring floods on small elevations and thereby provide for population reproduction.

It was impossible to recover the allelic potential due to mutations during such a short period, and the enrichment of population gene pool due to migration by land also was hardly probable, because this population inhabits an isolated biotope surrounded by waterlogged floodplain areas. However, the dispersal of snails (especially adults) may take place during spring floods: they withdraw into the shell and can be transferred downstream for short distances (Snegin, 2004). In 1996, we performed genetic analysis of the neighboring population living in a floodplain maple forest 1.5 km upstream of Yasnyi Kolodets (50°50′42″ N, 37°12′58″ E). This population was characterized by increased heterozygosity ($H_o = 0.381$), the presence of all three *EST-2* alleles (*EST-2-1*, 0.026; (*EST-2-2*, 0.553; (*EST-2-3*, 0.421; $N = 38$). It may well be that the gene pool of Yasnyi Kolodets population was enriched from that area, with the average gene flow (*Nm*) between the populations increasing from 0.683 to 1.859 ind. per generation. Note that, according to the shifting balance theory of evolution (Wright, 1970), a gene flow of 1–2 ind. per generation is required to maintain panmixia in a divided population. It may be concluded from these data that direct observations on the time course of changes in the structure of population gene pools provide a more reliable picture than that drawn from computational models.

Likewise, an increase in the level of heterozygosity was revealed in small floodplain populations such as Koren', Nezhegol, and Roven'ki, but allele frequencies remained unchanged in large floodplain popula-

[2] Life expectancy was determined by the equation $t = 1.5Ne$, where $t$ is the number of generations (Soule, 1985); $Ne = (4N - 2)/(V + 2)$, where $N$ is the number of adult individuals in the population and $V$ is the variance of individual fecundity (Crow and Kimura, 1970). This issue is discussed in detail in previous publication (Snegin, 2011b).

**Table 3.** Allele frequencies ($q \pm m_q$) in groups of *Br. fruticum* and their effective size calculated by the temporal method on the basis of allele frequencies at esterase locus *EST*-2

| Sampling site | Year | *N* | *EST*-2 allele frequencies | | | *Fk* | *Ne* |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | | |
| 1. Stenki Izgor'ya | 1996 | 100 | 0.305 ± 0.032 | 0.635 ± 0.034 | 0.060 ± 0.017 | 0.012 | 1045.3 |
| | 2010 | 94 | 0.266 ± 0.023 | 0.628 ± 0.025 | 0.106 ± 0.016 | | |
| 2. Roven'ki | 1996 | 112 | 0.005 ± 0.005 | 0.937 ± 0.016 | 0.058 ± 0.016 | 0.117 | 15.2 |
| | 2010 | 37 | 0.000 | 0.797 ± 0.033 | 0.203 ± 0.033 | | |
| 3. Borisovka | 1996 | 118 | 0.030 ± 0.011 | 0.949 ± 0.014 | 0.021 ± 0.009 | 0.023 | 142.2 |
| | 2010 | 62 | 0.073 ± 0.016 | 0.903 ± 0.019 | 0.024 ± 0.010 | | |
| 4. Khotmyzhsk | 1996 | 65 | 0.138 ± 0.030 | 0.669 ± 0.041 | 0.192 ± 0.034 | 0.065 | 47.0 |
| | 2010 | 20 | 0.250 ± 0.048 | 0.675 ± 0.052 | 0.075 ± 0.029 | | |
| 5. Golovchino | 1996 | 66 | 0.265 ± 0.038 | 0.614 ± 0.042 | 0.121 ± 0.028 | 0.136 | 13.2 |
| | 2010 | 34 | 0.147 ± 0.030 | 0.824 ± 0.033 | 0.029 ± 0.014 | | |
| 6. Syrtsevo | 1996 | 71 | 0.028 ± 0.014 | 0.781 ± 0.035 | 0.190 ± 0.031 | 0.05 | 49.4 |
| | 2010 | 39 | 0.103 ± 0.024 | 0.795 ± 0.032 | 0.103 ± 0.024 | | |
| 7. Yasnyi Kolodets | 1996 | 57 | 0.000 | 1.000 | 0.000 | 1.079 | 1.4 |
| | 2010 | 63 | 0.444 ± 0.031 | 0.190 ± 0.025 | 0.365 ± 0.030 | | |
| 8. Koren' | 1996 | 54 | 0.138 ± 0.033 | 0.713 ± 0.043 | 0.149 ± 0.034 | 0.380 | 4.1 |
| | 2010 | 57 | 0.395 ± 0.032 | 0.272 ± 0.029 | 0.333 ± 0.031 | | |
| 9. Seversky Donets | 1996 | 171 | 0.120 ± 0.017 | 0.833 ± 0.020 | 0.046 ± 0.011 | 0.0007 | $\to \infty$ |
| | 2010 | 42 | 0.131 ± 0.026 | 0.821 ± 0.029 | 0.048 ± 0.016 | | |
| 10. Nezhegol | 1996 | 66 | 0.083 ± 0.024 | 0.901 ± 0.026 | 0.015 ± 0.011 | 0.319 | 5.0 |
| | 2010 | 36 | 0.403 ± 0.041 | 0.597 ± 0.041 | 0.000 | | |

**Table 4.** Indices of genetic heterogeneity of *Br. fruticum* populations in 1996 and 2010

| Population | $A_a$ | | $A_e$ | | $I_{sh}$ | | $H_o$ | | $H_e$ | | *F* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1996 | 2010 | 1996 | 2010 | 1996 | 2010 | 1996 | 2010 | 1996 | 2010 | 1996 | 2010 |
| 1. Stenki Izgor'ya | 3.0 | 3.0 | 2.00 | 2.10 | 0.819 | 0.883 | 0.550 | 0.574 | 0.500 | 0.524 | –0.100 | –0.096 |
| 2. Roven'ki | 3.0 | 2.0 | 1.13 | 1.48 | 0.250 | 0.504 | 0.089 | 0.243 | 0.118 | 0.323 | 0.241 | 0.247 |
| 3. Borisovka | 3.0 | 3.0 | 1.11 | 1.22 | 0.236 | 0.372 | 0.059 | 0.194 | 0.098 | 0.178 | 0.393 | –0.085 |
| 4. Khotmyzhsk | 3.0 | 3.0 | 1.98 | 1.91 | 0.860 | 0.806 | 0.385 | 0.250 | 0.496 | 0.476 | 0.225 | 0.475 |
| 5. Golovchino | 3.0 | 3.0 | 2.17 | 1.43 | 0.907 | 0.546 | 0.591 | 0.206 | 0.538 | 0.299 | –0.097 | 0.312 |
| 6. Syrtsevo | 3.0 | 3.0 | 1.54 | 1.53 | 0.609 | 0.650 | 0.380 | 0.154 | 0.352 | 0.347 | –0.080 | 0.557 |
| 7. Yasnyi Kolodets | 1.0 | 3.0 | 1.00 | 2.72 | 0.000 | 1.044 | 0.000 | 0.381 | 0.000 | 0.633 | – | 0.398 |
| 8. Koren' | 3.0 | 3.0 | 1.82 | 2.93 | 0.798 | 1.087 | 0.130 | 0.456 | 0.450 | 0.659 | 0.712 | 0.308 |
| 9. Seversky Donets | 3.0 | 3.0 | 1.41 | 1.44 | 0.549 | 0.573 | 0.199 | 0.071 | 0.289 | 0.306 | 0.312 | 0.766 |
| 10. Nezhegol | 3.0 | 2.0 | 1.22 | 1.93 | 0.364 | 0.674 | 0.076 | 0.250 | 0.180 | 0.481 | 0.579 | 0.480 |

Designations: $A_a$, average number of alleles per locus; $A_e$, effective number of alleles; $I_{Sh}$, Shannon's index; $H_o$, observed heterozygosity; $H_e$, expected heterozygosity, *F*, coefficient of inbreeding (fixation index).

tions with a low degree of isolation (Stenki Izgor'ya, Borisovka, Syrtsevo, and Seversky Donets).

Thus, it appears that the dynamics of *Br. fruticum* in the south of forest–steppe zone are adequately described by a metapopulation model. Small groups inhabiting floodplain biotopes periodically die off but are replenished by snails from large forest populations. Apparently, natural dispersal of juveniles (by crawling) is insufficient for this, because landscape in the south of forest–steppe zone is fragmented and small areas

with suitable conditions for the snails (away from floodplains) are separated by plowed fields, roads, and steppificated wasteland. Apart from spring floods, the main role in population replenishment is most probably played by anthropogenic factor (the transport of agricultural produce) and, in addition, dispersal by domestic animals and wild birds.

On the whole, comparison of data on the genetic structure of the total sets of *Br. fruticum* populations studied in 1996 and 2010 provides evidence for stabilization of their gene pools in the south of the forest–steppe zone (Table 5). It is only the frequency of phene $C_3$ that proved to be increased in 2010, while all other parameters with calculated confidence interval (at 95% significance level) remained statistically unchanged. These data confirm the concept that species population structuring based on polymorphism at genomic loci provides for the survival of the species in space and time (Chetverikov, 1926; Altukhov, 1995; etc.).

However, the calculated coefficients of inbreeding at different levels of population hierarchy indicate that the degree of separation among the groups of bush snails has become markedly higher between 1996 and 2010. Indices $F_{st}$ and $\Phi_{st}$[3] has increased twofold over the elapsed period of time, with a proportional decrease in the average gene flow between populations (*Nm*). The integrated estimate of effective size (*Ne*) calculated from index $F_{st}$ (Wright, 1951) has decreased from 17.2 in 1996 to 9.2 in 2010.

These data are evidence for the tendency toward increasing anthropogenic insularization of natural *Br. fruticum* populations in the southern forest–steppe zone of the Central Russian Upland, with consequent formation of highly isolated groups. When small snail colonies die off, migrants from large populations sometimes have difficulties in populating vacant areas, since recolonization can be successful only when the corresponding biotopes remain unchanged. Unfortunately, increasing urbanization in the study region has resulted in degradation of many floodplain areas previously occupied by large *Br. fruticum* colonies, which have been transformed into arable fields, beaches, construction sites, and garbage dumps. The situation is aggravated by the fact that these anthropogenic biotopes with altered microclimate are invaded by some adventive and some native species that compete with brush snails, thereby promoting the extinction of their original colonies (competitive displacement).

As an example, consider the fates of three *Br. fruticum* colonies within the Belgorod city limits. The first two colonies were in the Vezelka River floodplain. One of them lived in willow forest near the dam (50°35′38.31″ N, 36°34′05.72″ E) and was destroyed during the construction of a beach, and the other occupied a ravine opening to the river (50°35′24.48″ N, 36°35′15.76″ E) and perished when an illegal garbage dump was set up at that place. The third colony (see Snegin, 2005) was isolated in a large ravine behind the Belgorod University of Consumer Cooperation (50°37′28.94″ N, 36°35′27.01″ E) and perished when storm sewer pipes were drawn to the ravine. Note that these biotopes were not destroyed completely: synusiae of stinging nettle, burdock, and hop (favorable for bush snails) survived at their margins. However, they were occupied by the gray field slug *Deroceras reticulatum* Müll. and adventive North Caucasian snail *Stenomphalia ravergieri* Fer., the species feeding on the aforementioned plants, which have spread over these synusiae and displaced *Br. fruticum*. Not a single bush snail has been found in these biotopes over the past 5 years, and only empty shells remind of their former presence.

**Table 5.** Genetic parameters of the sets *Br. fruticum* populations studied in the south of the forest–steppe zone in different years ($M \pm \Delta$, $P < 0.05$) (according to Snegin, 1999, 2012)

| Year | 1996 | 2010 |
|---|---|---|
| Number of populations | 20 | 37 |
| *S+* | 0.133 ± 0.050 | 0.229 ± 0.055 |
| $C_3$ | 0.108 ± 0.039 | 0.259 ± 0.076 |
| *EST2-1* | 0.114 ± 0.047 | 0.186 ± 0.055 |
| *EST2-2* | 0.800 ± 0.061 | 0.741 ± 0.074 |
| *EST2-3* | 0.086 ± 0.031 | 0.072 ± 0.035 |
| *Ae* | 1.53 ± 0.19 | 1.63 ± 0.18 |
| $I_{sh}$ | 0.539 ± 0.122 | 0.534 ± 0.110 |
| $H_o$ | 0.234 ± 0.072 | 0.228 ± 0.055 |
| $H_e$ | 0.301 ± 0.078 | 0.317 ± 0.068 |
| *F* | 0.269 ± 0.152 | 0.379 ± 0.119 |
| $F_{is}$ | 0.220 | 0.281 |
| $F_{it}$ | 0.301 | 0.445 |
| $F_{st}$ | 0.104 | 0.227 |
| $\Phi_{st}$ | 0.137 | 0.266 |
| *Nm* | 1.703–2.161 | 0.850–0.952 |
| *Ne* | 17.2 | 9.2 |

The results described above may be regarded as a starting point for further development of *Br. fruticum* population monitoring in the study region. Their comparison with data obtained in other regions of European Russia, including both territories under heavy anthropogenic pressure and specially protected natural areas, may provide the possibility to determine the level of response from the genetic component under different ecological conditions and develop more

[3] Index $F_{st}$ was calculated on the basis of Wright's (1943) *F* statistics, and index $\Phi_{st}$, on the basis of analysis of molecular variance (AMOVA) (Excoffier et al., 1992).

competent approaches to the protection and restoration of the living environment.

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