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Morphogenetic Analysis of *Helix pomatia* L. (Pulmonata, Helicidae) Populations from South-Eastern and Eastern Parts of the Modern Area

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Abstract—Based on the analysis of the morphological and genetic variability detected by the method of the protein gel electrophoresis in PAAG, the gene pool state of twelve adventive *Helix pomatia L.* grape snail populations under the conditions of urbanized landscapes of south-eastern and eastern parts of the modern area. According to the data obtained, most of the studied populations of this mollusk are in a satisfactory state. This is illustrated by the large values of their efficient numbers, the high level of heterozygosity, and decreased inbreeding. The structure of the population gene pools of the grape snail in the researched region is determined by their origin, genetic-automatic processes, and microclimatic conditions of the urban environment.

Keywords: population gene pool, allozymes; ground mollusk, *Helix pomatia*, urbanized landscape

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INTRODUCTION

The grape snail (*Helix pomatia* Linnaeus, 1758) is one of the largest ground mollusks. The regions of Middle and South-Eastern Europe are the initial area and apparently the center of the origin of this species. The modern grape snail area (caused by introduction) covers the Volga region, western regions of Belarus, Ukraine, and Baltic countries (including Kaliningrad) (Starodubtseva and Dedkov, 2003; Artem'eva and Semenov, 2004; Rumyantseva, 2006). In addition, the mollusk was introduced in a number of cities, such as St. Petersburg, Kursk, Moscow, and Kharkiv (Velichkovskii V.A., 1910; Beletskii, 1918; Likharev and Rammel'meier, 1952). There are reports about the introduction of *H. pomatia* on the territory of Finland (Jarvinen et al., 1976) and the United States (Dees, 1970). Such an artificial settlement is explained by the fact that the grape snail was for a long time considered to be a valuable gastronomical object; in this respect, the attempts of the *H. pomatia's* acclimatization in order to introduce the species in the culture (frequently successful) were conducted on territories that were previously private parks, estates, farmsteads, etc.

In addition to the settlements (where this species was delivered), its populations in some regions naturalized in natural forest communities that have a nature conservation or cultural-historical status. The origin of multiple geographically isolated grape snail populations (that are good objects for the scientific research of the microevolution phenomena occurring in modern urbanized landscapes) was the result of the *H. pomatia's* introduction in foreign, albeit, as it turned out, quite suitable for the naturalization of this species of biogeocenoses. Apparently, the exact number of territorially isolated *H. pomatia* populations existing at present is unknown; however, some of them are objects for conducting ecological and genetic studies. Some works were devoted to the study of the population structure of this species based on the analysis of the morphological traits (Pollard, 1975; Khlus, L.N. and Khlus, K.M., 2001; Kramarenko and Sverlova, 2005; Sverlova, 2005; Andreev, 2006; Khlus, 2007) and allozyme markers (Vincent and Magron, 1972; Wahren and Tegelström, 1973; Tegelström et al., 1975). Previously, no such studies were conducted on the territory of the south-eastern and eastern parts of the modern area of *H. pomatia*.

The aim of the present work was to analyze the *H. pomatia's* population structure under conditions of urbanized Eastern Europe landscapes using conchiometric traits and isoenzyme markers for the study of microevolution phenomena occurring in populations of this species.

MATERIALS AND METHODS

Material Collection

The living *H. pomatia* mollusks and their empty shells were the material for the study. Three populations living in Belgorod and Kharkiv were studied in our previous works (Snegin, 2010; Artemchuk and Snegin, 2012). In the present work, the number of the

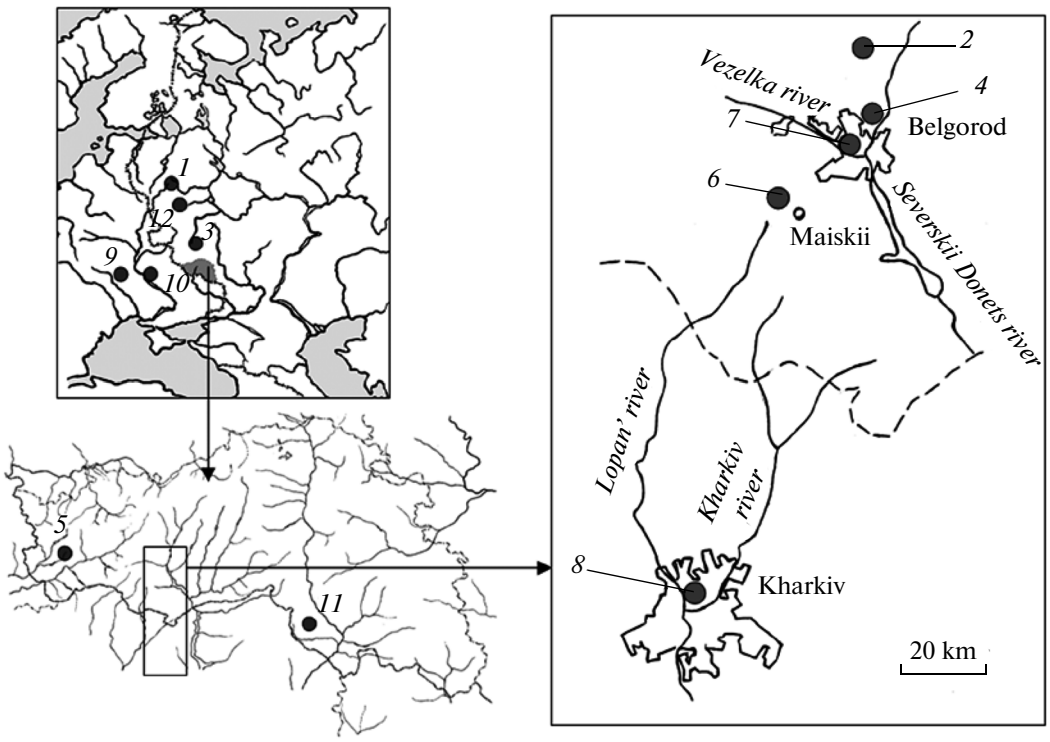

Fig. 1. Points of *Helix pomatia* collection.

studied populations was increased to twelve (Fig. 1, Table 1). Taking into account the direct dependence between the activity of the *H. pomatia* individuals and the environmental humidity, the most massive collections of freely creeping individuals were usually conducted some time after the rain. In addition to the manual collection of openly creeping individuals, specimens that are in the state of temporary anabiosis (with the mouth covered by the epiphragm) in different shelters on the soil surface, as well as in its friable surface layer and the forest litter, were sought. In addition, the collection of empty snail shell was conducted in a number of cases for morphometric studies.

Morphometric Analysis

The shell was carried measured according to the standard scheme (Shileiko, 1978): large shell diameter (*LSD*), small shell diameter (*SSD*), shell height (*SH*), aperture height (*AH*), aperture width (*AW*), spire height (*SpH*), and spire width (*SpW*). The area of the aperture ($S = (3.14 \cdot AH \cdot AW)/4$) and the shell volume ($V = (LSD^2 \cdot SH/2)$, as well as the *SH*/*LSD*, *AH*/*AW*, *SpH*/*SH*, *SpH*/*SpW*, and *V*/*S* indices, were calculated. Only shells that had completed their growth and generated a lapel on the mouth were used for the analysis.

Electrophoretic Analysis of the Enzymes

Pieces of the leg tissues taken from living mollusks were used for the electrophoretic analysis. Taking into account the small volume of the material selected for the study and the high regeneration capacity of snails, the killing of animals was avoided. The extraction of water-soluble proteins was conducted by means of a Tris-HCl buffer (pH 6.7) with subsequent mechanical homogenization, a freezing–defrosting cycle, and sample centrifugation for 20 min in an Eppendorf 5424 centrifuge at 10000 revolutions per minute. Electrophoresis was conducted in a VE-3 chamber (Helicon) in 10% polyacrylamide gel (separating gel pH 8.9 and concentrating gel pH 6.7; electrode Tris-glycine buffer pH 8.3).

In order to detect nonspecific esterases, the gels were incubated in a mixture, including a Tris-HCl buffer (pH 7.4), α-naphthyl acetate, and fast red TR. Before staining, the gel was incubated in a cold boric acid solution (3%). The incubation mixture, including the potassium–phosphate buffer (pH 7.8), NTS, FMS, and $MgCl_2$, was used for detecting superoxide dismutases; and the Tris-HCl buffer (pH 8.4), NTS, FMS, NAD, and sodium L-malate was used to detect malate dehydrogenases.

We determined the borders of two loci of nonspecific *EST3* and *EST4*[1] esterases (monomers with three alleles), one locus of the *SOD2* superoxide dismutase (dimer with two alleles), and one locus of the *MDH1* malate dehydrogenase (dimer with two alleles) on electrophotegrams of the *H. pomatia* enzymes (Fig. 2).

[1] Heterozygous genotype 13 has a manifestation in the form of three bands, which is atypical for monomeric proteins.

Table 1. Points of *H. pomatia* collection

Point	Point description	Coordinates
1. *Tver*	Tver, old park near the carriage-building plant	56°51′41.65″ northern latitude 35°54 11.28″ eastern longitude
2. *Shopino*	Belgorod province, Belgorod region, Shopino village, bairak forest close to the Belgorod–Moscow route	50°42′59.37″ northern latitude 36°29′29.98″ eastern longitude
3. *Kursk*	Kursk, territory of forest massive adjacent to garages and construction waste dump	51°45′24.44″ northern latitude 36°08′28.95″ eastern longitude
4. *Donets*	Severskii Donets river floodplain, Belgorod vicinity. Thickets of willow and maple	50°36′38.40″ northern latitude 36°37′19.19″ eastern longitude
5. *Khotmyzhsk*	Belgorod province, Khotmyzhsk village, Vorskla river floodplain, bairak forest close to Resurrection Church, recreation area of Krasivo sanatorium	50°35′26.00″ northern latitude 35°54′11.28″ eastern longitude
6. *Maiskii*	Belgorod province, Belgorod region, Maiskii settlement. Bairak oak forest	50°30′59.26″ northern latitude 36°27′15.98″ eastern longitude
7. *Belgorod*	Belgorod, willow forest in the Vezekla river floodplain, close to the complex of Belgorod National Research University buildings	50°35′39.17″ northern latitude 36°34′04.49″ eastern longitude
8. *Kharkiv*	Kharkiv (Ukraine), T.G. Shevchenko city park, Lopan' river floodplain	50°00′15.72″ northern latitude 36°13′31.31″ eastern longitude
9. *Zhitomir*	Zhitomir (Ukraine) park belt of the Teterev river floodplain, Yu.A. Gagarin culture and recreation park on the opposite side of the river	50°14′19.27″ northern latitude 28°40′07.79″ eastern longitude
10. *Kiev*	Kiev (Ukraine), Fomin botanical garden	50°24′52.38″ northern latitude 30°33′29.29″ eastern longitude
11. *Yablonovo*	Belgorod province, Valuiskii region, Yablonovo village,vicinity. Fox mountain natural boundary, Oskol river floodplain. Alder thickets	50°13′22.46″ northern latitude 38°00′34.51″ eastern longitude
12. *Bykovo*	Moscow province, Ramenskii region, Bykovo settlement, the territory of All Russian Research Institute of Plant Quarantine. Mixed forest with a predominance of the common pine	55°38′11.43″ northern latitude 38°06′02.81″ eastern longitude

The results obtained were analyzed using the GenAlex 6.4 (Peakall and Smouse, 2001), MEGA5 (Tamura et al., 2011), and Statistica 6.0 programs.

RESULTS AND DISCUSSION

The results of the morphometric analysis demonstrate an ambiguous picture (Table 2). On the one hand, significant differences between the studied populations (both by the absolute values of the shell measurements and by the indices) were obtained. The significant originality of the studied populations according to the metric characteristics is confirmed by the result of the single-factor analysis of the variance (Table 3), according to which the intrapopulation variances significantly exceed the interpopulation ones by all conchiometric traits ($P < 0.05$). On the other hand, the clusterization of the samples according to the conchiological traits did not detect any dependence between the average parameters of the shell and the geographical localization of the group (Fig. 3). For example, the rather distant Bykovo

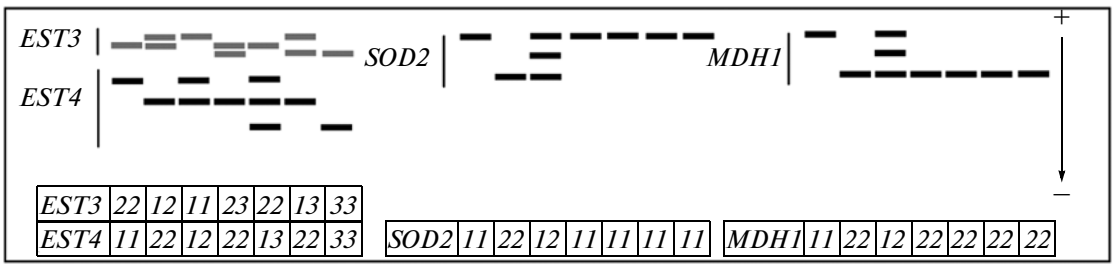

Fig. 2. Graphic image of the studied loci and allele combinations.

Table 2. Values of morphometric shell traits in *H. pomatia* colonies (mm, $M \pm \Delta$, $P = 0.05$)

Point	*N*	*SH*	*LSD*	*AH*	*AW*	*CH*	*CW*	*SpH/SpW*	*SpH/SH*	*SH/LSD*	*AH/AW*	*V*	*S*	*V/S*
1	7	37.5 ± 2.4	36.6 ± 2.4	27.8 ± 1.1	24.5 ± 2.5	10.9 ± 1.1	27.2 ± 1.8	0.401 ± 0.024	0.290 ± 0.014	1.02 ± 0.03	1.16 ± 0.12	25635.0 ± 4751.6	534.1 ± 56.3	47.5 ± 4.5
2	24	38.3 ± 0.7	39.9 ± 0.8	27.4 ± 0.6	24.7 ± 0.8	12.1 ± 0.6	29.5 ± 0.6	0.409 ± 0.018	0.314 ± 0.013	0.96 ± 0.01	1.12 ± 0.06	30698.0 ± 1702.8	532.5 ± 24.4	58.1 ± 4.0
3	14	42.8 ± 1.1	41.6 ± 1.1	30.2 ± 0.9	26.7 ± 0.9	13.4 ± 0.9	30.8 ± 0.9	0.433 ± 0.025	0.311 ± 0.015	1.03 ± 0.02	1.13 ± 0.02	37331.8 ± 2799.0	634.4 ± 37.2	58.7 ± 2.1
4	40	37.6 ± 0.6	37.5 ± 0.8	26.2 ± 0.4	23.7 ± 0.5	13.0 ± 1.4	27.5 ± 0.9	0.469 ± 0.037	0.344 ± 0.036	1.01 ± 0.02	1.11 ± 0.01	26661.9 ± 1349.9	489.8 ± 16.4	54.3 ± 1.8
5	29	36.9 ± 0.7	37.3 ± 0.7	25.9 ± 0.5	23.8 ± 0.5	11.2 ± 0.4	27.6 ± 0.7	0.405 ± 0.014	0.303 ± 0.008	0.99 ± 0.01	1.09 ± 0.01	25805.6 ± 1439.0	485.0 ± 19.4	53.0 ± 1.3
6	656	38.3 ± 0.3	36.3 ± 0.3	26.9 ± 0.2	22.9 ± 0.2	11.4 ± 0.2	26.9 ± 0.3	0.421 ± 0.004	0.294 ± 0.003	1.05 ± 0.01	1.19 ± 0.01	26092.2 ± 615.0	489.4 ± 8.5	52.5 ± 0.6
7	167	35.0 ± 0.6	33.4 ± 0.5	23.7 ± 0.4	20.3 ± 0.4	11.3 ± 0.4	25.5 ± 0.5	0.442 ± 0.012	0.320 ± 0.008	1.05 ± 0.01	1.18 ± 0.02	20043.9 ± 813.3	381.5 ± 13.1	52.4 ± 1.4
8	61	35.3 ± 0.9	35.5 ± 0.7	25.1 ± 0.4	19.1 ± 0.7	10.6 ± 0.9	25.0 ± 0.8	0.422 ± 0.036	0.284 ± 0.013	0.99 ± 0.01	1.32 ± 0.08	22662.7 ± 1387.2	379.1 ± 18.6	61.0 ± 5.0
9	38	40.2 ± 0.9	38.8 ± 1.0	27.6 ± 0.8	25.1 ± 0.6	12.5 ± 0.5	29.4 ± 1.0	0.428 ± 0.017	0.312 ± 0.011	1.03 ± 0.02	1.10 ± 0.02	34040.2 ± 1926.1	581.7 ± 26.0	58.5 ± 1.5
10	31	36.1 ± 1.2	34.5 ± 1.2	25.1 ± 0.7	21.9 ± 0.8	10.9 ± 0.7	25.3 ± 0.9	0.430 ± 0.021	0.301 ± 0.014	1.04 ± 0.01	1.15 ± 0.03	22017.6 ± 2030.4	434.5 ± 26.0	50.0 ± 2.4
11	26	38.3 ± 1.2	40.0 ± 1.1	28.3 ± 0.7	24.0 ± 0.7	10.4 ± 0.6	26.5 ± 0.7	0.390 ± 0.019	0.272 ± 0.016	0.96 ± 0.02	1.18 ± 0.02	30957.4 ± 2345.2	534.7 ± 26.4	57.6 ± 2.7
12	15	36.9 ± 1.5	37.0 ± 1.2	26.6 ± 0.6	23.6 ± 1.3	11.9 ± 0.4	26.5 ± 0.9	0.450 ± 0.003	0.324 ± 0.002	0.995 ± 0.010	1.14 ± 0.04	25944.2 ± 2652.9	498.4 ± 38.7	51.3 ± 1.3

(no. 12) group fell into the same cluster with the adjacent Zhitomir (no. 9), Kiev (no. 10), Kharkiv (no. 8), and Yablonovo (no. 11) populations. But populations in the vicinity of Belgorod (nos. 2, 4, 6, 7) fell into different clusters.

There is an idea that the metric characteristics of the shell are indicators of the climatic zones inhabited by snails. At the same time, it should be noted that this situation is true in those cases, when we consider the natural habitat of aboriginal species, whose gene pools have been developed for a long time. All grape snail populations that we analyzed are adventive and relatively young (although some of them have existed at least 100 years (points 8 and 11)). In addition, these groups are confined to urbanized territories, where the urban environment creates a certain microclimate, which can differ greatly from the zonal climate.

It is known that an increase in the body (and, accordingly, shell) size in mesophilic mollusk species (which include the *H. pomatia*) indicates more favorable values of the type of humidification, temperature, and food supply (Likharev and Rammel'meier, 1952; Goodfriend, 1986). In our case, by following this rule, it is possible to expect an increase in the grape snails' body sizes as they move from the northeast to the southwest. However, we observed no such clinal variability. The largest *H. pomatia* individuals were registered in the eastern population of Kursk (point 3) and in the most western of the studied Zhitomir populations (point 9). In other large cities (Kiev, Kharkiv, Belgorod), the snails have significantly smaller morphometric indices similar to northern groups from Tver (point 1) and Moscow region (point 12). This partly confirms the assumption, according to which the shell size decreases in the *H. pomatia* in urbanized landscapes (Khlus, L.N. and Khlus, K.M., 2001). At the same time, the data exist that refuse this assumption (Sverlova, 2005). The reason of such inconsistency lies in the fact that variations in the shell size are determined by the peculiarities of the population's gene pools.[2] The origin of adventive colonies can be different, and their allelic composition (and, accordingly, the size characteristics of individuals) can be largely determined by the gene pools of parental populations developed in other climatic conditions.

The soil's friability can have some influence on the shell shape. The snails are buried deep in the soil in the

[2] It is known that the heritability level for the interpopulation variability of the shell sizes in ground mollusks can be 50–70%.

Table 3. Results of the single-factor analysis of the variance of morphometric traits

Trait	Variability source	*SS*	*df*	*MS*	*F*	*P*
SH	Between groups	2536.5	11	230.6	13.69	7.4×10^{-25}
	Within groups	18281.1	1086	16.8		
LSD	Between groups	2874.3	11	261.3	19.11	2.1×10^{-35}
	Within groups	14849.7	1086	13.7		
AH	Between groups	2079.5	11	189.0	26.94	5.0×10^{-50}
	Within groups	7620.8	1086	7.0		
AW	Between groups	2395.2	11	217.7	26.48	1.66×10^{-45}
	Within groups	9660.1	1086	8.9		
SpH	Between groups	335.7	11	30.5	4.79	3.1×10^{-7}
	Within groups	6912.2	1086	6.4		
CW	Between groups	1201.2	11	109.2	11.03	1.5×10^{-19}
	Within groups	10751.3	1086	9.9		
SpH/SW	Between groups	0.238	11	0.022	4.15	5.06×10^{-6}
	Within groups	5.664	1086	0.005		
SpH/SH	Between groups	0.222	11	0.020	9.79	4.4×10^{-17}
	Within groups	2.235	1086	0.002		
SH/LSD	Between groups	6.037	11	0.548	6.84	3.2×10^{-11}
	Within groups	87.160	1086	0.080		
AH/AW	Between groups	2.015	11	0.183	13.13	9.9×10^{-24}
	Within groups	15.143	1086	0.014		
V	Between groups	1.04×10^{10}	11	9.42×10^{8}	18.58	2.2×10^{-34}
	Within groups	5.51×10^{10}	1086	50716948		
S	Between groups	3025812	11	275073	27.9	8.4×10^{-52}
	Within groups	10699578	1086	9852.3		
V/S	Between groups	19819.1	11	1801.7	5.09	8.21×10^{-8}
	Within groups	384112.4	1086	353.7		

period of summer drought and in the winter. Therefore, the selection for more elongated shell occurs in the solid ground. In addition, this favors an increase in the main locomotor organ (leg), with which the snails are buried, and the increase in the leg is accompanied by an increase in the relative aperture size. Individuals with the most elongated shells with the largest values of the *SH/LSD* index were registered at the Maiskii and Belgorod points. Individuals with a relatively larger aperture were registered at the Tver and Kiev points. The snails with the most crowded curl and flattened shell are typical for Yablonovo populations. This group living in the Valuiskii region of Belgorod oblast was registered at the beginning of the 20th century (Velichkovskii, 1910) and is the only one of the studied populations naturalized in a natural environment. At present, this population is probably depressed. We found here only three living specimens; therefore, we were able to conduct the comparative analysis using only the shells of dead mollusks.

The allele frequencies and heterozygosity levels for the used loci are presented in Table 4. A significant ($P \geq 0.05$) deficiency of the heterozygous alleles[3] was registered in 27.2% cases; and homozygosity for one of the alleles, in 29.5% cases. A significant excess of heterozygotes was registered only in one case (point 5, the *EST4* locus). No significant differences between the actual and theoretical heterozygosity were registered in other cases.

[3] The significance of the heterozygote deficiency was estimated according to the formula $\chi^2 = F^2N(k-1)$, $df = k-1$, where F is the inbreeding coefficient; N, sample size; and k, number of alleles for this locus (Li, 1978).

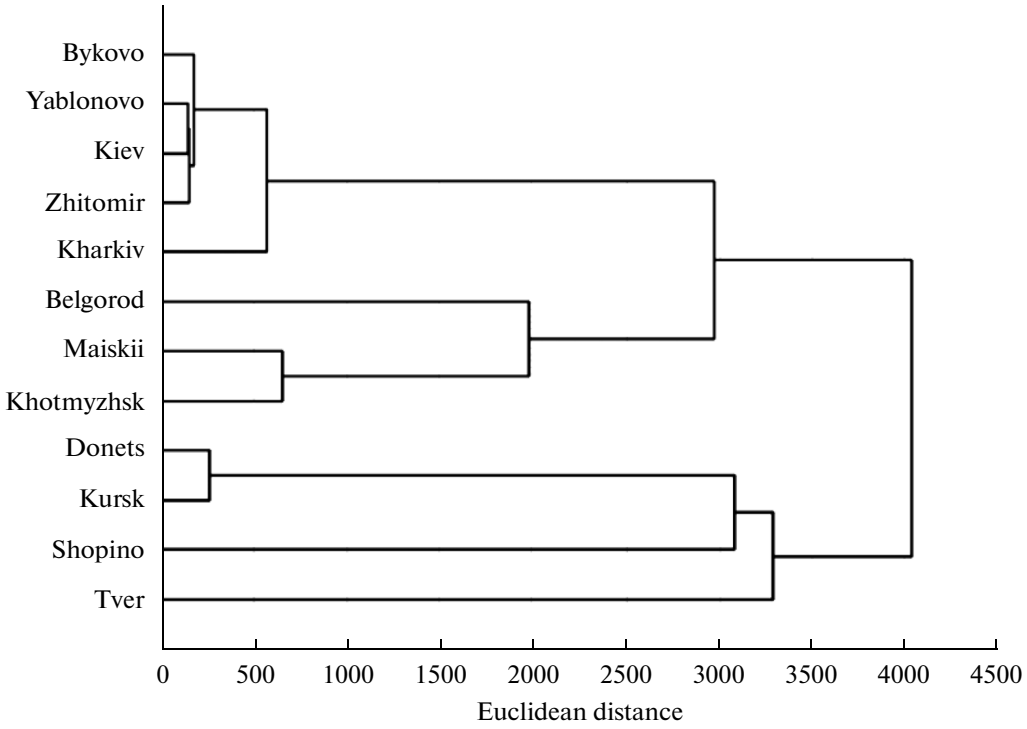

Fig. 3. Dendrogram of the morphometric traits of the *H. pomatia* populations.

The most western of the studied groups living in Zhitomir was the most monomorphic (Table 5). The reduced values of the genetic variability indices were also typical for populations from points 2 and 8, while the largest values of the inbreeding coefficient were registered in populations from points 3, 9, and 10. The heterozygosity level was not the same in different populations for different loci. Heterozygous variants are most frequently found in the *EST4* ($H_0 = 0.398$) and *MDH1* ($H_0 = 0.271$) loci. These loci contribute most to the interpopulation diversity estimated according to the F_{st} inbreeding coefficient (Table 6). This is probably associated with the different selective significance of the studied loci and with the different selection directions in the studied populations. In addition, it is possible that this phenomenon is the result of a genetic drift due to the "founder effect" (Mair, 1968).

It should be noted that we registered a significant correlation between the shell volume and the inbreeding coefficient in the studied populations ($R_{F/V} = 0.598 \pm 0.214$, $t = 2.79$, $P < 0.05$). It is rather difficult to explain this phenomenon. It is possible to suggest the following as a working hypothesis.

Contrary to the common opinion, according to which an increase in inbreeding leads to a decrease in the life potential of populations (for example, Willis, 2009), many organisms (especially slow-moving species and species capable of self-fertilization (that include ground mollusks)) have mechanisms preventing the inbreeding depression. For example, it was registered during the artificial breeding of the giant oyster (*Crassostrea gigas*) that the inhibitory effect of the inbreeding was usually manifested in a decrease in the survival rate at the larval stages, and poorly adapted individuals were not involved in subsequent crossings (and thus harmful recessive alleles were deleted from the population). The individuals of inbred lines that survived and reached sexual maturity did not differ in the growth survival rate from oysters from natural habitats (Kholodov et al., 2010). Similar data were obtained in the experiments with the *Stator limbatus* beetles (Fox et al., 2008). In addition, it was registered in the parasitic trematode (*Coitocaecum parvum*) that individuals obtained as a result of self-fertilization did not differ in viability from individuals born during outcrossing (Lagrue and Poulin, 2009). In the case of the grape snail, it is possible that some adventive groups could be founded by single individuals. During the inbreeding (and possibly automixis[4] after reaching the inbred minimum, selection could favor the survival of snails with the most successful genetic combinations (the side effect of which was an increase in the animal's size). The competitive vacuum, in which the adventive groups[5] were found, could favor the preservation of inbred lines. Further, it is quite possible that

[4] There is evidence that hermaphrodite mollusks are capable of self-fertilization (Baur and Klemm, 1989).

[5] The inbred lines within the natural area would be gradually replaced by outbred groups due to the large embryonic mortality.

Table 4. Allele frequencies and heterozygosity levels of loci in *H. pomatia* populations

Locus–allele	Points										
	Tver	*Shopino*	*Kursk*	*Donets*	*Khotmyzhsk*	*Maiskii*	*Belgorod*	*Kharkiv*	*Zhitomir*	*Kiev*	*Bykovo*
SOD2-1	0.571	1.000	0.839	1.000	0.845	0.749	1.000	1.000	1.000	0.500	0.717
SOD2-2	0.429	0.000	0.161	0.000	0.155	0.251	0.000	0.000	0.000	0.500	0.283
H_0	0.571	0.000	0.258	0.000	0.103	0.305	0.000	0.000	0.000	0.429	0.478
H_e	0.490	0.000	0.271	0.000	0.262	0.376	0.000	0.000	0.000	0.500	0.405
F	–0.167	–	0.046	–	0.605*	0.189*	–	–	–	0.143	–0.179
MDH1-1	1.000	0.021	0.645	0.150	0.569	0.430	0.089	0.629	0.071	0.625	0.761
MDH1-2	0.000	0.979	0.355	0.850	0.431	0.570	0.911	0.371	0.929	0.375	0.239
H_0	0.000	0.042	0.258	0.250	0.517	0.517	0.107	0.484	0.143	0.321	0.391
H_e	0.000	0.041	0.458	0.255	0.490	0.490	0.163	0.467	0.133	0.469	0.364
F	–	–0.021	0.436*	0.020	–0.055	0.049	0.341	–0.037	–0.077	0.314	–0.075
EST 3-1	0.000	0.000	0.161	0.000	0.000	0.016	0.000	0.000	0.086	0.143	0.000
EST 3-2	1.000	0.958	0.839	1.000	1.000	0.955	1.000	1.000	0.886	0.821	1.000
EST 3-3	0.000	0.042	0.000	0.000	0.000	0.029	0.000	0.000	0.029	0.036	0.000
H_0	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000
H_e	0.000	0.080	0.271	0.000	0.000	0.087	0.000	0.000	0.207	0.304	0.000
F	–	1.000*	1.000*	–	–	0.741*	–	–	1.000*	1.000*	–
EST 4-1	0.143	0.521	0.065	0.175	0.379	0.300	0.500	0.000	0.086	0.107	0.370
EST 4-2	0.857	0.438	0.210	0.450	0.621	0.628	0.393	1.000	0.914	0.286	0.217
EST 4-3	0.000	0.042	0.726	0.375	0.000	0.072	0.107	0.000	0.000	0.607	0.413
H_0	0.286	0.625	0.226	0.475	0.690	0.350	0.679	0.000	0.057	0.429	0.565
H_e	0.245	0.536	0.425	0.626	0.471	0.510	0.584	0.000	0.157	0.538	0.646
F	–0.167	–0.167	0.469*	0.242*	–0.465*	0.315*	–0.162	–	0.635*	0.204	0.124
N	7	24	31	40	29	223	28	31	35	28	23

H_0, average observed heterozygosity; H_e, average expected heterozygosity; *F*, fixation index (inbreeding coefficient); *N*, number of individuals; *, cases of significant difference between H_0 and H_e are designated.

Table 5. Genetic variability indices and efficient size values in the studied *H. pomatia* populations

Points	N	P %	A	A_e	I	H_o	H_e	F	N_e	N_e/N
1. *Tver*	7	50.0	1.50	1.32	0.273 ± 0.167	0.214 ± 0.137	0.184 ± 0.117	–0.167	7	1.000
2. *Shopino*	24	75.0	2.00	1.32	0.277 ± 0.189	0.167 ± 0.153	0.164 ± 0.125	–0.018	24	1.000
3. *Kursk*	31	100.0	2.25	1.58	0.568 ± 0.075	0.185 ± 0.062	0.356 ± 0.050	0.488	21	0.672
4. *Donets*	40	50.0	1.75	1.50	0.364 ± 0.244	0.181 ± 0.114	0.220 ± 0.148	0.131	35	0.884
5. *Khotmyzhsk*	29	75.0	1.75	1.55	0.445 ± 0.159	0.328 ± 0.164	0.306 ± 0.114	0.029	28	0.972
6. *Maiskii*	223	100.0	2.50	1.68	0.575 ± 0.134	0.286 ± 0.094	0.366 ± 0.098	0.323	169	0.756
7. *Belgorod*	28	50.0	1.75	1.40	0.313 ± 0.225	0.196 ± 0.163	0.187 ± 0.138	0.090	26	0.917
8. *Kharkiv*	31	25.0	1.25	1.22	0.165 ± 0.165	0.121 ± 0.121	0.117 ± 0.117	–0.037	31	1.000
9. *Zhitomir*	35	75.0	2.00	1.15	0.242 ± 0.088	0.050 ± 0.034	0.124 ± 0.044	0.519	23	0.658
10. *Kiev*	28	100.0	2.50	1.87	0.703 ± 0.072	0.295 ± 0.101	0.453 ± 0.052	0.415	20	0.707
12. *Bykovo*	23	75.0	2.00	1.77	0.553 ± 0.218	0.359 ± 0.124	0.354 ± 0.133	–0.043	23	1.000

N, number of individuals in the sample; A, average number of alleles; A_e, efficient number of alleles; I, Shannon index; H_o, observed heterozygosity; H_e, expected heterozygosity; F, fixation index (inbreeding coefficient).

Table 6. Values of locus heterozygosity values, inbreeding coefficients, and gene flow levels in the studied *H. pomatia* populations

Locus	H_e	H_o	F_{is}	F_{it}	F_{st}	N_m
SOD 2	0.209	0.195	0.069	0.281	0.227	0.849
MDH1	0.303	0.271	0.105	0.454	0.389	0.392
EST3	0.086	0.002	0.976	0.978	0.087	2.627
EST4	0.431	0.398	0.075	0.334	0.280	0.644
Average	0.257	0.217	0.306	0.512	0.246	1.128

F_{it}, inbreeding coefficient of an individual relative to a large population; F_{is}, inbreeding coefficient of an individual relative to a subpopulation; F_{st}, inbreeding coefficient of a subpopulation relative to a large population; N_m, the level of gene flow between populations expressed by the number of migrating individuals per generation.

the assortative crossing (during which the largest individuals preferred to pair with similar individuals) occurred.

The analysis of molecular variance (AMOVA) demonstrates a rather strong genetic differentiation of the studied populations (Excoffier et al., 1992). The differentiation index $\Phi_{st} = 0.295$ insignificantly exceeds the similar index obtained by means of the Wright F-statistics ($F_{st} = 0.254$) (Table 7). Moreover, the portion of interpopulation diversity (V_{bp}) accounts for 30%, while the portion of intrapopulation (V_{wp}) accounts for 70%; this corresponds to the differentiation level in other aboriginal background species of ground mollusks (that we previously studied) living in natural biotopes of the studied region (*Bradybaena fruticum*: $\Phi_{st} = 0.300$, $F_{st} = 0.228$, $V_{bp}/V_{wp} = 30/70\%$; *Chondrula tridens*: $\Phi_{st} = 0.295$, $F_{st} = 0.198$, $V_{bp}/V_{wp} = 30/70\%$) (Snegin, 2012).

The results of the cluster analysis based on genetic distances (Nei, 1978) by the unweighted pair group method (UPGMA) (Fig. 4) demonstrate rather an ambiguous picture of the population distribution by the groups. The degree of population similarity by isoenzyme allele frequencies (as opposed to the shell phenotypes) particularly depends on their geographical localization. This fact is reflected in the regression analysis between the pairwise values of the logarithms of the gene flow level between populations ($\log Nm$) and the logarithms of the geographical distances between them ($\log D$) (Fig. 5). The correlation coefficient $R = -0.525 \pm 0.117$ ($t = 4.5$ $P < 0.001$) indicates the existing inverse dependence between these indices (that corresponds to the effect of isolation by distance, which is typical for selectively neutral genes). According to this model, the degree of genetic differences between populations increases with an increase in the geographical distances between them. In the case that

Table 7. Values of molecular variance (AMOVA) according to the allozymes in the *H. pomatia* populations

Variability source	Number of degrees of freedom (*df*)	Sum of squares (*SS*)	Mean square (*MS*)	Dispersion (*V*)	%	Φ_{st}	*P*	*Nm*
Between populations	10	251.076	25.108	0.618	30	0.295	0.010	0.597
Within populations	488	719.263	1.474	1.474	70			
In total	498	970.339	26.581	2.091				

the loci are exposed to the effect of a stabilizing selection, the effect of isolation by distance should be absent (Slatkin, 1993). Thus, according to these ideas, it can appear that the genetic markers that we are using are selectively neutral, and the observed differences between the populations are a consequence of genetic drift. However, this model is typical for the description of geographically and historically related populations and is hardly applicable for randomly arising adventive *H. pomatia* colonies.

For example, populations living in Belgorod and its vicinities were divided into two clusters distant from each other; one of them included groups from points 2, 4, and 7; the other, from points 5 and 6. The population from Zhitomir (point 9) also fell into one of these clusters, while populations from Kharkiv (point 8) and Tver (point 1) fell into another cluster. Another cluster was generated by populations from Kursk (point 3), Kiev (point 10), and Moscow region (point 12) that were considerably distant from each other. We assume that such an atypical population distribution by different clusters can be explained by gene drift and different directions of natural selection under the influence of the microclimatic and biotopic conditions of the urban environment (which, as we already noted can differ significantly from zonal characteristics.[6]

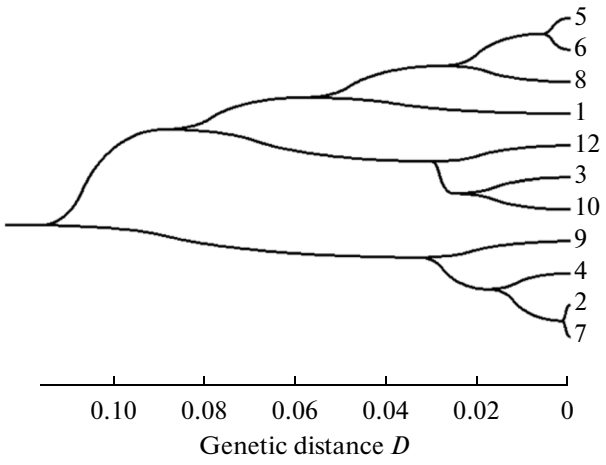

Fig. 4. Dendrogram of genetic distances according to Nei (1972) (UPGMA).

The efficient size of the studied snail populations was calculated according to the formula which takes into account the inbreeding level in the population (Li, 1978):

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

In order to obtain comparable data, we calculated the ratio of the efficient sample size to its total volume (Table 5). According to the obtained values, the *Ne*/*N* ratio on the average is 0.870 ± 0.043, which fits in the total range of the *Ne* portion suggested by Crow, Morton, and Kimura (Crow and Morton, 1955; Crow and Kimura, 1970)[7] In addition, the average value of the *Ne* portion obtained for the *H. pomatia* significantly exceeds the similar values obtained for aboriginal background species of ground mollusks living in the studied region: *Br. fruticum*, 0.800 ± 0.021, *Ch. tridens*, and 0.661 ± 0.013 (Snegin, 2012), which indirectly indicates the high level of viability of the studied grape snail populations.

CONCLUSIONS

The results obtained give an idea about the structure of settlement and the state of the *H. pomatia* population gene pools under the conditions of urbanized landscapes of the south-eastern and eastern parts of the modern area. According to the data obtained, most populations of this mollusk are in satisfactory condition. This is indicated by the large values of the efficient size, the high heterozygosity level, and decreased inbreeding. The increased homozygosity of some populations can be caused by different reasons. Either these are relatively old groups (and, accordingly, have existed for a longer time in an isolated state) or these groups appeared recently but were founded by

[6] The followers of the "balance theory" (Ayala, 1977; Kirpichnikov, 1981) consider that the protein polymorphism is the basis of biochemical plasticity, which ensures a wide adaptation of the species to a certain spectrum of environmental conditions and is supported by different forms of selection.

[7] The authors determined that the *Ne* portion for most organisms is on the average 0.75.

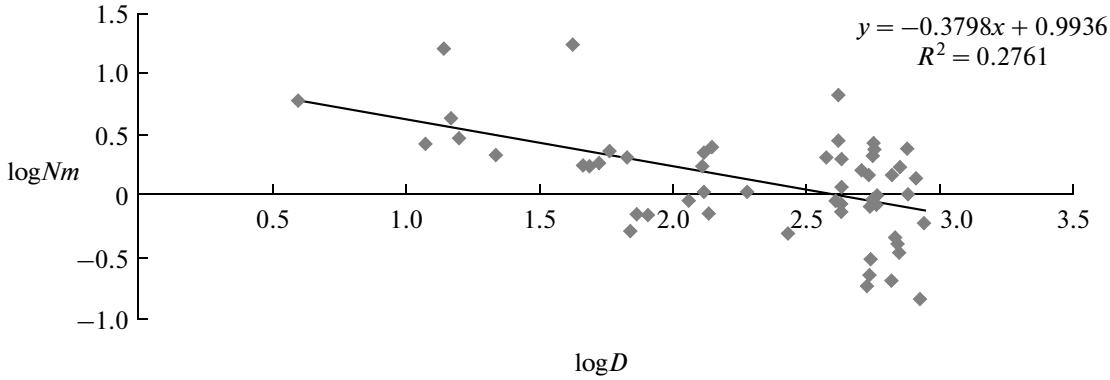

Fig. 5. Dependence of *Nm* gene flow level between pairs of *H. pomatia* populations on the geographical distance between them (*D* (km)).

a small number of individuals. It should be noted that the presented results can be considered as a starting point for the subsequent monitoring of this species in order to clarify the peculiarities of the evolutionary processes occurring in its populations.

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