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VARIABILITY OF THE PHENOTYPIC COMPOSITION OF *CEPAEA HORTENSIS* (GASTROPODA, HELICIDAE) IN WESTERN UKRAINE: IN SPACE AND TIME

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Variability of the Phenotypic Composition of *Cepaea hortensis* (Gastropoda, Helicidae) in Western Ukraine: in Space and Time. Gural-Sverlova, N. V., Gural, R. I. — The variability of the phenotypic composition of the introduced land snail *Cepaea hortensis* was analyzed in 6 groups of samples (in total, more than 31 thousand specimens), differing in spatial location or collection time. The results obtained confirmed the considerable uniformity of this composition, which has not yet been significantly influenced by the relatively recent repeated introductions of *C. hortensis* associated with the activity of garden centers and contributing to an increase in phenotypic richness at separate, still very spatially limited sites. The Western Ukrainian populations of *C. hortensis* are characterized by low values of the inbreeding coefficient, caused by a common origin and quite possibly by climatic selection favoring unbanded shells.
Key words: land mollusks, white-lipped snail, polymorphism, introduced species, Ukraine.

Introduction

In recent years, a number of publications have appeared trying to relate the level of phenotypic variability in introduced and/or urban populations of land mollusks of the genus *Cepaea* Held, 1838 with the time of their colonization of the corresponding areas (Cameron et al., 2009, 2014; Cameron, von Proschwitz, 2020; Gheoca et al., 2019). It has been suggested that a high level of variability, assessed by the inbreeding coefficient Fst, is characteristic of recently populated areas, regardless of whether they are within the natural ranges of species or outside them (Cameron et al., 2009). However, in addition to the time of colonization of certain areas, Fst values can be influenced by other factors (Cameron, von Proschwitz, 2020; Gural-Sverlova, Egorov, 2021), in particular, whether these areas were colonized by individuals of one or different origins.

In this regard, Western Ukrainian populations of *Cepaea hortensis* (O. F. Müller, 1774) are of particular interest, the common origin of most of which is confirmed not only by the great similarity of their phenotypic composition, but also by such a peculiar phenotypic marker as completely linked inheritance of the presence of bands and the white ground color of the shell (Gural-Sverlova, Gural, 2021 a). Only in recent years, other variants of shell coloration in *C. hortensis* have begun to be recorded in Western Ukraine (Gural-Sverlova, Gural, 2021 a; Gural-Sverlova et al., 2020), clearly associated with relatively recent introductions of this species, independent of its initial introduction, which occurred no later than 1970s (Gural-Sverlova, Gural, 2021 a).

The study of the shell color and banding polymorphism of *C. hortensis* in the largest settlement of Western Ukraine, Lviv city, was begun by us in the late 1990s (Sverlova, 2001 a, b), when this species had already become a common representative of the urban malacofauna (Sverlova, 2002), which made it possible to collect large samples at relatively small sites. During this time, significant statistical material has been accumulated (Gural-Sverlova, Gural, 2021 a), which makes it possible to assess the variability of the phenotypic composition of *C. hortensis* both in different parts of Lviv and beyond, and in different periods of time. So far, only long-term dynamics of the phenotypic composition was analyzed at a number of studied sites in Lviv (Gural-Sverlova, Gural, 2018).

Therefore, the main purpose of this paper was to assess the spatial and temporal variability of the phenotypic composition of *C. hortensis* in Western Ukraine and, first of all, in Lviv, which is especially interesting now, when mixing of individuals, that are descendants of the primary and later introductions, is already recorded at several sites.

Material and methods

The phenotypic composition of *C. hortensis* in Lviv was studied from 1999 to 2021, with a ten-year break (2005-2014). In 1999–2004, quantitative data were obtained for 18 sites located in the northeastern part of Lviv (mainly along Hetman Mazepa and Vyacheslav Lypynsky Streets) as well as for 18 sites south of the historic city center. In the latter case, 12 sites were selected in Stryisky Park and 4 more sites in the Park of Culture and Rest named after Bohdan Khmelnytsky located across the street. Two groups of sites were designated as “Ia” and “IIa” (fig. 1, A).

In 2015–2021, 38 sites were quantitatively studied on the territory of Lviv, combined into three groups (fig. 1, B). Group Ib included 9 re-examined sites and 5 new sites, two of which were located south of the main collection area, but north of the historical center of Lviv. Group IIb, consisting of 13 sites, had only two sites similar to group IIa, one for each of the parks mentioned above. The total collection area was greatly expanded, mainly in the northern and eastern directions. Group III included 11 remaining sites located in the south, south-west and west of Lviv. One of the sites was located on the northern outskirts of the Zubra village, directly adjacent to the administrative border of Lviv (Sykhiv district).

With the exception of group IIa, the studied sites were mostly tree and shrub plantations along residential buildings or highways. Most of them were at least partially shaded by trees, tall bushes, and in some cases also by houses or fences. Only a few sites could be classified as “open”: small wastelands, lawns in parks. In parks, snails gravitated towards ornamental shrubs planted in separate groups or in the form of hedges. Less often, they could be collected in sufficient quantity for counting in forest-like areas or on tall grass (nettles, etc.).

For comparison, 13 sites examened in 2016–2021 in Western Ukraine outside Lviv were combined into a separate group: Ivano-Frankivsk region, Ivano-Frankivsk (2 sites, 48°55'30.7" N 24°43'39.6" E and 48°56'51.3" N 24°41'47.8" E); Lviv Region, Briukhovychi settlement (49°54'03.0" N 23°57'36.6" E), Dubliany town (49°53'59.0" N 24°05'22.4" E), Horodok town (2 sites, 49°47'12.5" N 23°38'31.1" E and 49°46'40.2" N 23°38'19.2" E), Obroshyne settlement (49°47'13.8" N 23°52'24.6" E), Pidbirtsi village (49°50'30.5" N 24°09'04.8" E), Pustomyty town (49°43'00.3" N 23°54'04.7" E), Solonka village (49°44'56.8" N 24°00'24.2" E), Velyky Liubin settlement (49°43'24.6" N 23°42'49.8" E), Zhovkva town (2 sites, 50°03'13.4" N 23°59'11.4" E and 50°03'12.5" N 23°58'48.3" E). More detailed descriptions of most of the listed sites are given in a previous publication (Gural-Sverlova, Gural, 2021 a).

Table 1. Frequencies of common variants of shell coloration in *C. hortensis* at the studied sites, in percent

Coloration variants		Lviv-Ia	Lviv-Ib	Lviv-IIa	Lviv-IIb	Lviv-III	Outside Lviv
A-0	M ± m	4.9 ±1.50	4.0 ±0.86	7.8 ±1.72	11.2 ±2.87	5.5±2.53	4.6±1.79
	Min-Max	0–27.0	0.5–12.2	1.0–26.4	0–36.9	0–26.9	0–18.6
A-b	M ± m	19.7±4.30	15.8±2.31	15.2±2.66	13.8±2.41	18.2±4.96	21.5±5.69
	Min-Max	0–81.1	3.4–37.8	1.3–39.5	1.9–29.4	3.9–59.3	1.9–68.9
G-0	M ± m	75.4±4.19	80.2±2.41	77.0±2.90	70.1±3.65	76.3±5.19	72.1±5.24
	Min-Max	18.9–94.5	59.5–94.9	54.0–95.9	41.5–98.1	39.8–96.1	31.1–95.3
Nt		8230	6852	10098	2314	1291	2978
Ns		457.2	489.4	561.0	178.0	117.4	229.1

Note. M — arithmetic mean; m — error of the arithmetic mean; Min — the minimum value; Max — the maximum value; Nt — total number of specimens from all sites of one group; Ns — mean number of specimens collected at one site. For other symbols see Material and methods.

At almost all sites, at least 100 adult live snails were collected. Some samples collected outside Lviv also included empty shells of adults with well-preserved coloration, which made it possible to reliably determine the phenotypes (Gural-Sverlova, Gural, 2021 a, table 1). Less than 100 specimens of *C. hortensis* were collected only at two sites in Ivano-Frankivsk and at one site in Briukhovychi. These samples contained 86, 92, and 97 live snails, respectively. In total, more than 31 thousand specimens of *C. hortensis* were included in the analysis.

The length of the collection sites usually ranged from 20–30 to 50–60 m. With a low number of snails, the absence of efficient anthropogenic barriers preventing their free movement (Sverlova, 20002), and the absence of noticeable spatial changes in the frequencies of the main variants of the shell coloration, the length of the collection site in some cases could be increased to 100 m. For comparison: the diameter of the panmictic unit in *Cepaea* is, according to one data, about 50–60 m (Jones et al., 1977), according to other data — up to 100 m (Schnetter, 1950). If several samples were collected at the same site, the data were summarized (Gural-Sverlova, Gural, 2021 a).

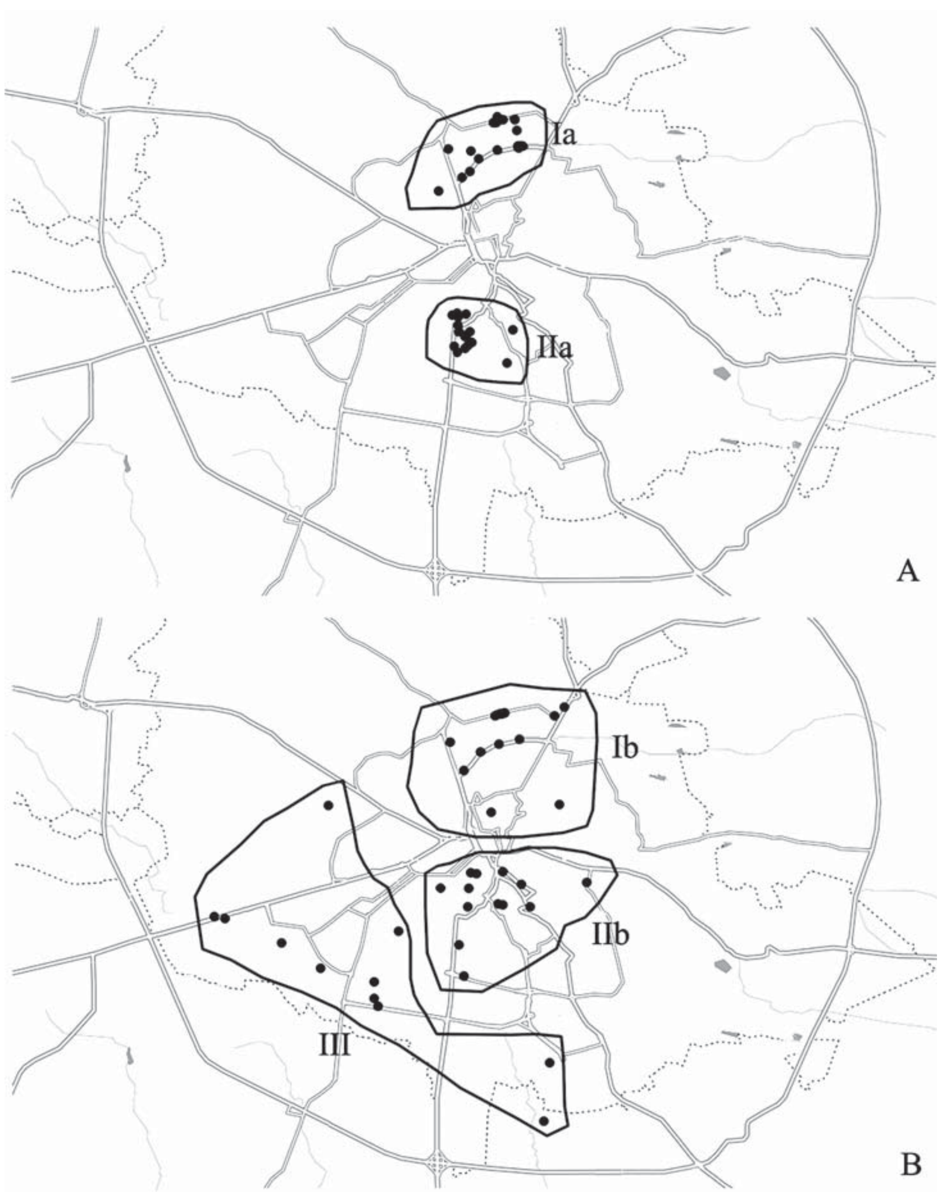

Fig. 1. Location of the sites in Lviv used for the quantitative study of the shell coloration variability of *C. hortensis* in 1999–2004 (A) and in 2015–2021 (B).

Phenotypes were scored based on the ground color of the shells and the banding pattern of their ultimate whorl according to the standard method (Clarke, 1960). Spiral dark bands were designated by Arabic numerals from 1 to 5, counting them from the apex to the base of the shell. The absence of band(s) was indicated as "0" in place of the corresponding numeral(s). The fusion of adjacent bands was indicated with parentheses. The bands were considered to be fused if they were fully or partially merged for no less than a quarter of a whorl before the aperture. The shell ground color was designated as "A" (white, no traces of yellow or other pigment even at the apex), "Y" (yellow), "P" (pink, including orange shells) or "B" (brown).

In addition to the main sites in Lviv, which were used for quantitative registration of phenotypes (fig. 1), 146 additional sites were examined in the city in 2020–2021, where large or smaller concentracion of *C. hortensis* was found, but insufficient for quantitative collection. At these sites, only the presence of the main variants of the shell coloration in adults and enough large juveniles was scored, designated as "A-0" — white unbanded, "A-b" — white banded, "Y-0" — yellow unbanded, "Y-b" — yellow banded, "P-0" — pink unbanded, "P-b" — pink banded, "B-0" — brown unbanded. No brown banded shells have yet been found in *C. hortensis* from Western Ukraine.

When finding rare for Western Ukraine variants of shell coloration in *C. hortensis*, first of all, shells with a dark lip, reminiscent of *C. nemoralis*, the species identification of such individuals was confirmed anatomically. The size of the stylophore, which is much shorter in *C. hortensis* (Schileyko, 1978), was considered to be a main distinguishing feature. In adults of *C. nemoralis*, often found in Lviv together with atypically colored *C. hortensis* (Gural-Sverlova, Gural, 2021 a, table 5; Gural-Sverlova et al., 2020, 2021), the length of the stylophore was usually approximately equal to the length of the penis, as shown in the monograph by Schileyko (1978, fig. 433), while in *C. hortensis* it was half the length (Schileyko, 1978, fig. 434).

To assess the variability of the phenotypic composition of *C. hortensis* between Lviv the inbreeding coefficient Fst was used, calculated based on the frequencies of phenotypic manifestation of some inherited traits (Cameron et al., 2009), in this case, banded/unbanded, yellow and white shells, or the frequencies of the corresponding alleles (Gural-Sverlova et al., 2021). Allele frequencies were calculated conditionally, using the Hardy-Weinberg formula for an ideal panmictic population.

Unfortunately, we are not aware of experimental data on the inheritance of the white shell ground color in *Cepaea*. However, a yellow ground color is known to be recessive to darker coloration variants (pink or brown), and a light yellow color is recessive to dark yellow (Murray, 1975). Therefore, it can be assumed that the white color of the shell in *C. hortensis* is recessive with respect to the yellow one (Gural-Sverlova, Gural, 2021 a).

Due to the low frequencies of banded shells at most of the studied sites, their number in samples was often too small to reliably estimate the occurrence of phenotypes with fused bands among them. Therefore, the proportion of shells with fused bands as well as the frequencies of individual phenotypes were calculated from the total number of banded shells collected at all sites of one group.

For two sites on the edge of the Old Park in Horodok and near the city park in Pustomyty, the frequencies of banded shells among collected live snails and empty shells were compared. Both sites are completely or almost completely shaded by trees. In both cases, the frequencies of banded shells, which are unusually high for Western Ukraine (Gural-Sverlova, Gural, 2021 a), were noted. The length of the collection site in Horodok was about 30 m, in Pustomyty about 60 m.

Part of the collected material is stored in the malacological collection of the State Museum of Natural History of the National Academy of Sciences of Ukraine in Lviv (Gural-Sverlova, Gural, 2020). Different variants of the shell coloration in *C. hortensis*, recorded in Western Ukraine, were partially illustrated in a previous paper (Gural-Sverlova, Gural, 2021 a). They are shown to a greater extent in the illustrated database "Land mollusks of Ukraine" (Gural-Sverlova, Gural, 2012–2022).

Results

Although the frequencies of three common for Western Ukraine variants of the shell coloration in *C. hortensis* (according to the frequency of occurrence — yellow unbanded, white banded, white unbanded) varied noticeable in each of the compared groups of samples, their ratio, on average, was relatively stable regardless of the location and collection time (table 1). The average frequency of the predominant phenotype (yellow unbanded) did not fall below 70 % in any of the groups.

The ratio of sites with different frequency intervals of these three coloration variants remained relatively stable in Lviv at different periods of time (fig. 2). However, in later collections, there were fewer samples in which the frequency of yellow shells (always unbanded in older samples and almost always unbanded in later ones) exceeded 80 %, and more sites in which the frequency of this trait ranged from 71 to 80 %.

All three coloration variants listed above were found at almost all sites in Lviv used for the quantitative study of shell color and banding polymorphism in *C. hortensis* (table 2).

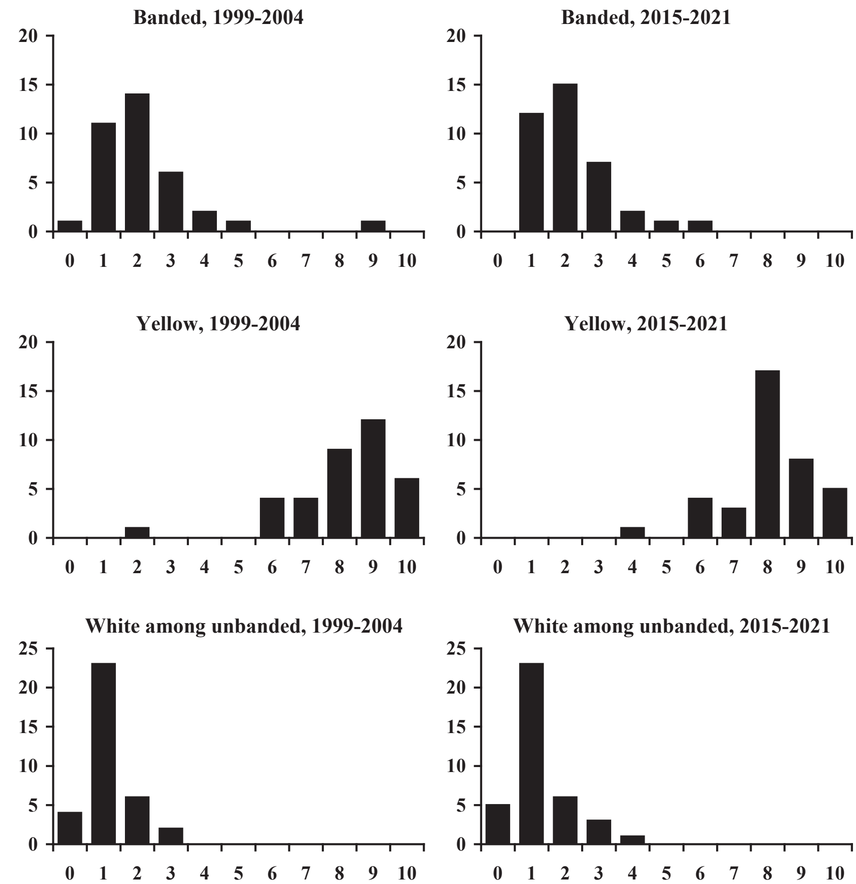

Fig. 2. The number of studied sites in Lviv with different frequencies of banded, yellow and white unbanded shells. The numerals on the abscissa indicate the frequency intervals: 0 — absent, 1 — up to 10 %, 2 — from 11 to 20 %, etc.

White unbanded shells were absent at few sites, and banded shells were not found only in one case. Shells with a different coloration (yellow banded, pink) were completely absent in older samples from Lviv (1999–2004), and in later samples they were found only at three quantitatively studied sites from group IIb. When examining additional sites with a smaller number of snails, carried out in 2020–2021, the absence of one of the three common col-

Table 2. The number of studied sites in Lviv with a different combination of shell coloration in *C. hortensis*

Shell coloration	Older samples (1999–2004)	Newer samples or observations		
		main sites (2015–2021)	additional sites (2020–2021)	all sites (2015–2021)
Only common variants of shell coloration				
A-0, A-b, Y-0	31	31	66	97
A-b, Y-0	4	4	56	60
A-0, Y-0	1	–	14	14
Only Y-0	–	–	6	6
With yellow banded and/or pink shells				
All combinations	–	3	4	7
Number of sites	36	38	146	184

oration variants was recorded in 52 % of cases, and the presence of shells with a different coloration only in 4 cases.

Despite our long-term studies of the shell color and banding polymorphism in *C. hortensis*, so far only 13 sites with yellow banded and/or pink shells have been found in Western Ukraine (table 3), of which 7 are located in Lviv (fig. 3, B). In all cases, snails with shell coloration atypical for Western Ukraine were found at limited sites, the size of which did not exceed the size of the panmictic unit in *Cepaea* (see Material and methods). In many cases, it was only a few tens of meters, or even single individuals.

At two sites in Lviv and at one site in Pidbirtsi, all found pink shells (both unbanded and banded) had a dark lip (table 3). At another site in Lviv, only a single snail with a pink unbanded shell and a dark lip has been found. A darkly colored lip in yellow unbanded shells has so far been recorded in only two individuals from Lviv and Pidbirtsi. Pink shells

Fig. 3. Location of all (main and additional) sites in Lviv, where only the variants of shell coloration common for Western Ukraine (A) or any other coloration variants (B) were registered in 2015–2021.

Table 3. Known records of *C. hortensis* with regionally rare variants of shell coloration made in Western Ukraine in recent years

Localities and coordinates	N	Phenotypes or phenotype groups						
		common			rare			
		A-0	A-b	Y-0	Y-b	P-0	P-b	B-0
Lviv Region, 2019-2021								
Lviv, 49°50'06.2" N 24°01'34.6" E	159	14	17	66	30	9*	23*	–
Lviv, 49°49'38.7" N 24°02'48.8" E	107	–	10	70	7	16	4	–
Lviv, 49°48'42.3" N 24°01'24.6" E	375	76	15	282	2	–	–	–
Ibidem, additional sample of banded shells	11	–	11	–	–	–	–	–
Lviv, 49°50'09.6" N 24°02'29.8" E	20	–	–	6**	10	2*	2*	–
Lviv, 49°49'47.8" N 24°01'32.2" E	SO	–	+	+	+	–	–	–
Lviv, 49°49'19.9" N 23°59'59.0" E	SO	–	–	+	–	+*	–	–
Lviv, 49°49'47.2" N 23°57'50.5" E	SO	–	–	+	+	–	–	–
Solonka, 49°44'57.5" N 23°59'40.5" E	SO	–	–	+	+	–	+	–
between Lviv and Davydiv, 49°45'57.3" N 24°06'29.2" E	14	–	2	5	6	1	–	–
Pidbirtsi, 49°50'30.5" N 24°09'04.8" E	110	4	3	77**	18	1*	4*	3
Zhovkva, 50°03'20.8" N 23°58'36.3" E	21	–	1	11	9	–	–	–
near Zhovkva, 50°04'30.4" N 23°59'28.6" E	SO	–	–	+	+	–	–	–
Transcarpathian Region, 2015–2018								
Uzhgorod, 48°37'28.3"N 22°17'48.1" E	24	–	–	11	–	13	–	–

* All with a dark lip; ** among them one shell with a dark lip; SO — single observations. For other symbols see Material and methods.

with a light colored lip were scored at 4 sites of Western Ukraine (table 3): in Lviv, Lviv Region (Solonka, between Lviv and Davydiv) and Transcarpathian Region (Uzhgorod).

In three cases, snails with regionally rare variants of shell coloration were found near garden centers: two operating in Pidbirtsi and between Lviv and Davydiv, one recently closed in Lviv. However, in the latter case, we were able to find only two individuals with yellow banded shells. Another introduced species of the same genus, *C. nemoralis*, was found near the same garden centers. In total, in 8 cases out of 13, atypically colored individuals of *C. hortensis* were found together with *C. nemoralis*.

The greatest diversity of shell coloration in *C. hortensis* was recorded near the garden center in Pidbirtsi, where, in addition to variability in lip coloration and the presence of yellow banded, pink unbanded and banded shells, three unbanded shells with a brown ground color and a light lip were also found (table 3). The color of these shells varied from light brown to yellowish brown (Gural-Sverlova, Gural, 2021–2022) and differed well from pink shells from the same or other sites. This is the first finding of brown shells of *C. hortensis* in Western Ukraine.

The phenotype 12345 clearly prevailed among banded shells in all groups of samples; its share was below 70 % only in group IIb (table 4). The phenotype (12)345 was usually no less distinctly predominant among five-banded shells with fused bands. Its share remained very stable in groups of samples Ia, Ib and III, varying from 64.2 to 64.8 %, and increased to 74.6 % outside Lviv. Only in the area II adjacent to the historical center of Lviv from the south (fig. 1), the share of this phenotype in the total number of shells with fused bands decreased to 45.2 % in group IIb and 28.8 % in group IIa. In both cases, this was associated with a increase in the frequency of phenotype 1(23)45 among banded shells, which in group IIb was accompanied by an increase in the frequencies of a number of other phenotypes with fused bands, and in group IIa by a more rare occurrence of phenotype (12)345 (table 4).

The total proportion of phenotypes with fused bands ranged from 11.9 % outside Lviv to 49.7 % in group IIb. In the northeast of Lviv, the ratio of phenotypes among the collected

Table 4. Percentages of the phenotypes among banded shells, regardless of their ground color

Phenotypes	Lviv-Ia	Lviv-Ib	Lviv-IIa	Lviv-IIb	Lviv-III	Outside Lviv
12345	72.6	72.4	75.9	50.3	78.5	86.2
(12)345	17.4	17.6	6.8	22.5	13.3	8.8
1(23)45	2.0	2.4	6.9	7.3	3.0	0.3
(123)45	3.2	3.0	2.0	5.9	1.7	0.2
(12)3(45)	2.3	2.3	1.9	5.9	0.9	1.2
(123)(45)	1.3	0.5	1.2	2.5	0.4	0.3
123(45)	0.6	1.0	1.8	2.5	0.9	0.7
(12345)	–	0.3	1.1	1.4	0.4	0.1
1(234)5	< 0.1	0.1	0.6	0.3	–	0.1
1(23)(45)	–	0.1	0.7	1.1	–	–
(1234)5	–	0.1	0.1	–	–	–
12(34)5	0.1	–	–	0.3	–	–
1(2345)	–	–	0.6	–	–	–
12(345)	–	–	–	–	–	0.1
12045	0.4	0.1	0.3	–	0.4	1.5
(12)0(45)	< 0.1	0.1	–	–	–	–
(12)045	–	0.1	–	–	–	–
10345	< 0.1	–	–	–	0.4	0.4
103(45)	–	–	–	–	–	0.1
All fused	27.0	27.5	23.8	49.7	20.6	11.9
Nb	2047	1054	2089	356	233	1001
Frequencies of fusion of band pairs, calculated from the number of five-banded shells with fused bands						
Bands 1 and 2	90.0	86.8	54.9	76.8	81.3	89.8
Bands 2 and 3	24.1	23.6	55.7	37.3	27.1	8.5
Bands 3 and 4	0.5	1.7	10.3	4.0	2.1	2.5
Bands 4 and 5	15.6	15.3	30.6	27.1	12.5	20.3
Nfb	551	288	497	177	48	118

Note. Nb — total number of banded shells for a group of sites; Nfb — the same for five-banded shells with fused bands.

banded snails was very stable regardless of the collection time (compare groups of samples Ia and Ib in table 4). For groups of samples IIa and IIb, this was not observed, which could be due to the different location of the studied sites (fig. 1), which in 1999–2004 were concentrated

Table 5. The variability of the phenotypic composition of *C. hortensis*

Site groups (number of sites)	Time period	Inbreeding coefficient Fst calculated from the frequencies of					
		phenotypes			alleles		
		banded/ unbanded	white	yellow	banded/ unbanded	white	yellow
Lviv-Ia (18)	1999–2004	0.198	0.161	0.161	0.137	0.098	0.098
Lviv-Ib (14)	2015–2021	0.052	0.047	0.047	0.045	0.041	0.041
Lviv-IIa (18)	1999–2004	0.094	0.081	0.081	0.090	0.071	0.071
Lviv-IIb (13)	2017–2021	0.090	0.060	0.049	0.085	0.067	0.059
Lviv-III (11)	2019–2021	0.165	0.149	0.149	0.121	0.112	0.112
Lviv-all (36)	1999–2004	0.154	0.122	0.122	0.116	0.085	0.085
Lviv-all (38)	2015–2021	0.101	0.085	0.084	0.081	0.072	0.071
Lviv-part* (11)	1999–2004	0.035	0.033	0.033	0.036	0.024	0.024
Lviv-part* (11)	2015–2018	0.025	0.041	0.041	0.029	0.039	0.039
Outside Lviv (13)	2016–2021	0.202	0.187	0.175	0.146	0.141	0.136

* Only for sites studied in both time periods.

Table 6. Differences in the frequencies of banded among empty shells and living snails at two sites with an unusually high proportion of banded shells

Sample type	Years	N	Percentage of banded	Difference
		Horodok, Lviv Region		
Empty shells	2018–2019	484	72.3	
Live snails	2021	112	53.6	$t = 3.86$, $p < 0.001$, significant
		Pustomyty, Lviv Region		
Empty shells	2021	156	40.4	
Live snails	2021	61	29.5	$t = 1.49$, $p > 0.1$, insignificant

mainly in two parks, and in 2017–2021 along the streets (see Material and methods).

The calculated values of the inbreeding coefficient Fst are given in table 5. As in *C. nemoralis* in Lviv (Gural-Sverlova et al., 2021), they were higher when used in calculations the frequencies of phenotypic manifestation of some inherited traits (ground color of the shell, the presence/absence of dark spiral bands), rather than the alleles of the corresponding genes. The greatest contribution to the phenotypic and genetic variability of *C. hortensis* in Lviv and in general in Western Ukraine is made by such a trait as the presence/absence of bands.

In 1999–2004, the genetic subdivision in *C. hortensis*, assessed on the basis of phenotype frequencies, was approximately twice as high in the first group of sites (Ia), which was represented mainly by street tree and shrub plantations (table 5). This could be due to the smaller number of anthropogenic barriers limiting the free movement of snails in the parks (the second group of sites – IIa). The Fst values calculated from the frequencies of the corresponding alleles differed less, by 1.4–1.5 times.

During repeated studies in the northeast of Lviv (area I), the Fst values decreased by 3.4–3.8 times (calculated based on phenotype frequencies) or 2.4–3.0 times (the same for alleles). In area II, the Fst values also decreased over the same period of time, but only slightly (table 5). This could be caused by a significant increase in the area on which the studied sites were located (fig. 1) as well as by their complete isolation from each other, in contrast to earlier samples from park biotopes.

When comparing all samples from Lviv, made in 1999–2004 and in 2015–2021, the Fst values decreased 1.4–1.5 times (for phenotypes) or 1.2–1.4 times (for alleles). However, if we take into account only those sites where we managed to collect samples in both time periods, the Fst values, firstly, were much lower (table 5), and secondly, they decreased only for banded shells (respectively, in 1.4 and 1.2 times), and even slightly increased for the ground color (in 1.2 and 1.6 times).

If we compare the samples of recent years, the greatest genetic subdivision in Lviv was noted for group III (table 5), which unites the most spatially distant sites (fig. 1). However, when comparing this group with samples from other settlements in Lviv and Ivano-Frankivsk Regions, it can be seen that a significant increase in the collection area did not affect the Fst values so much.

At both analyzed sites in Lviv Region with the proportion of banded shells atypically high for Western Ukraine, the frequencies of this trait were lower among living snails and higher among collected empty shells (table 6). However, this difference reached a statistically significant level only in Horodok.

Discussion

Despite the fact that the primary introduction of *C. hortensis* into Western Ukraine took place, most likely, not earlier than the middle of the 20th century (Gural-Sverlova, Gural, 2021 a), its descendants managed to settle quite widely in the settlements of the Lviv region, as evidenced by our observations as well as some information from databases (iNaturalist, 2022; UkrBIN, 2022), confirmed by high-quality photographs. Known, although not so numerous, findings of this species in other administrative regions of Western Ukraine: in Ivano-Frankivsk, Khmelnytsky, Transcarpathian, and Volyn Regions (Gural-Sverlova, Gural, 2021 a) as well as in Rivne and Chernivtsi Regions (iNaturalist, 2022), most of which apparently also have the same origin.

The populations formed by the descendants of the above-mentioned primary introduction are distinguished by a restricted phenotypic composition, in the most complete version represented by only three variants of shell coloration: yellow unbanded, white banded, and white unbanded (Gural-Sverlova, Gural, 2021 a, fig. 3, A). The latter variant is less common (table 1) and is most often absent in certain samples (table 2). However, the most characteristic feature of such populations is the presence of banded shells with only a white ground color (Gural-Sverlova, Gural, 2021 a). Therefore, the finding at some sites of at least single snails with yellow banded shells, which are quite common in other parts of the present range of *C. hortensis*, can be considered as reliable evidence of repeated introduction, independent of the primary one.

While the primary dispersal of *C. hortensis* in Lviv (Sverlova, 2002) and other urbanized areas in Western Ukraine (Gural-Sverlova, Gural, 2021 a) was facilitated by their planned landscaping with ornamental shrubs, the repeated introductions of this species observed now are mainly associated with the activities of garden centers, purchasing part of the sold products abroad. A similar process has now been noted also for the related species *C. nemoralis* (Gural-Sverlova et al., 2021).

The different origins of *C. hortensis*, both now living near the garden centers, and brought from them to some urbanized habitats in Lviv and its immediate environs (Solonka), is clearly demonstrated by the different coloration of the lip in pink shells: only dark at some sites, only light at others (table 3). The dark lip in *C. hortensis* is a quite rare hereditary trait, found only locally even in the natural range of this species (Ożgo, 2010; Schilder, Schilder, 1957). We managed to find out that the spreading of such snails comes from the garden center "Club of Plants", which opened near Lviv (Pidbirtsi) in 2008 and is now one of the largest garden centers in the Lviv Region. The official website of this garden center indicates that imported plant varieties are brought from Germany, Holland, Italy and Poland.

Thus, in Western Ukraine there are already two phenotypic markers corresponding to two different introductions of *C. hortensis*: completely linked inheritance of the presence of bands and white shell color in the descendants of the primary introduction and the presence of a dark lip in all pink specimens (Gural-Sverlova, Gural, 2021 a, fig. 5, C, D), the distribution of which originates from the garden center "Club of Plants".

So far, repeated introductions of *C. hortensis* into Western Ukraine, which occurred through the above-mentioned "Club of Plants" and other garden centers, provided only small "infusions" of other coloration traits into populations, formed by the descendants of the primary introduction and often very abundant. Even in Lviv, where the largest number of such cases was recorded (table 3), they remain "a drop in the ocean" (fig. 3) and do not affect the general patterns of phenotypic variability of *C. hortensis* (table 1, fig. 2).

The great uniformity of the phenotypic structure of *C. hortensis* in Lviv and beyond, noted by us earlier (Gural-Sverlova, Gural, 2021 a) and caused, at least partially, by a common origin, affects the low values of the inbreeding coefficient, which are considered not typical for relatively recently colonized areas (Cameron et al., 2009; Gheoca et al., 2019). In particular, the Fst value calculated for the frequencies of unbanded shells in the Romanian city of Sibiu, where *C. hortensis* was introduced more than a century ago, was 0.445 (Gheoca et al., 2019, table 5), which is 2.9–4.4 times higher than similar values calculated for all sites from Lviv in different periods of time (table 5).

When analyzing the Fst values obtained by us for different groups of sites (table 5), there was also a certain dependence of this indicator on the total size of the compared areas and the degree of isolation of studied sites located on them (see Results). The calculation results were also greatly influenced by the presence in some groups of single sites with frequencies of banded shell atypically high for Western Ukraine. For example, in the northeast of Lviv (area I) in the late 1990s–early 2000s, two sites were studied, the frequency of banded shells at which exceeded 80 % and 40 %. By the beginning of repeated studies in 2015, mollusks of this species became completely extinct at the first site and were present in a small number not sufficient for quantitative collection at the second site. In both cases, this was not caused by the destruction of urban habitats inhabited by snails.

Recently, it has been suggested that low Fst values even in areas outside the natural range recently colonized by *Cepaea* may be related either to a common origin or to "a relatively uniform and rigorous selection regime" (Cameron, von Proschwitz, 2020). The first can already be considered a proven fact for the Western Ukrainian populations of *C. hortensis* (Gural-Sverlova, Gural, 2021 a), the second is also very likely and may be the result of climatic selection in a more continental climate compared to the natural range of *C. hortensis* (Gural-Sverlova, Gural, 2018).

We have already noted (Gural-Sverlova, Gural, 2021 a) that the average frequency of unbanded shells of *C. hortensis* in Western Ukraine, usually more than 80 % (table 1), exceeds that in any part of the natural range of this species (Cameron, 2013, table 6). This is even more significant because in Lviv, for example, snails are most often found at sites at least partially shaded by trees, tall bushes, houses, etc., while higher frequencies of unbanded, and therefore lighter shells should be more typical for open biotopes. The advantage that individuals with lighter shells can receive in a more continental climate has already been discussed by us earlier (Gural-Sverlova, Gural, 2021 b).

According to Kirchhoff's law of thermal radiation, light-colored shells, like any other light-colored surfaces, must not only heat up more slowly in the sun but also cool more slowly as a result of their own thermal radiation (Arnason, Grant, 1976; Sverlova, 2004). Therefore, it is assumed that the dark-colored phenotypes of *Cepaea* should have a selective advantage in cooler, but at the same time relatively stable climatic conditions: in forests, on coasts, etc. (Sverlova, 2004). And a lighter coloration can prevent not only overheating of shells in the sun but also their cooling too quickly with a sharp decrease in ambient temperature (Arnason, Grant, 1976). Therefore, snails with light-colored shells can theoretically receive a selective advantage also with sharper temperature fluctuations characteristic of a more continental climate.

When we recently analyzed the long-term dynamics of the phenotypic composition of *C. hortensis* in Lviv (Gural-Sverlova, Gural, 2018), a statistically significant decrease in the frequency of banded shells was recorded in four out of 10 studied sites after a 10-year break in 2005–2014. Simultaneously, an increase in the average daily temperature, daily temperature fluctuations, and the number of days with a maximum temperature of +30 °C or more in the summer were observed in the city due to global warming (Gural-Sverlova, Gural, 2018, table 3), with the result that the climate has become even more continental.

A gradual decrease in the frequency of banded shells can also occur at the present time at two sites in the Lviv region studied by us, where such individuals are more common (possibly as an accidental consequence of the founder effect). The lower proportion of banded shells among live snails compared to empty shells collected from the same sites (table 6) may probably indicate an increased mortality of snails with such shell coloration and/or that a gradual decrease in the frequency of dark colored shells occurs in successive generations of snails. However, to confirm this assumption, further observations are needed in Horodok and, especially, in Pustomyty, where it has not yet been possible to collect a sufficiently representative sample of live snails (table 6).

Despite climatic selection, which may act against specimens of *C. hortensis* with dark-colored shells in Western Ukraine, a certain number of banded shells persist at

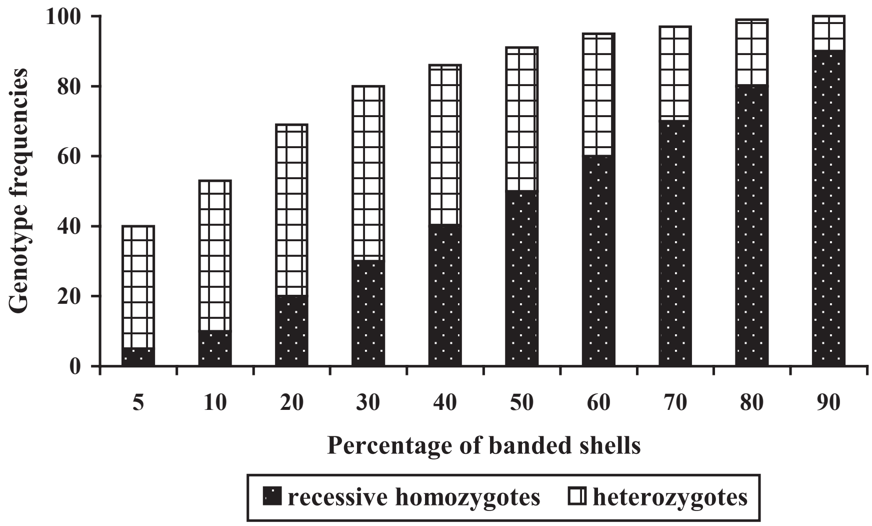

Fig. 4. Theoretical ratio of genotype frequencies among carriers of the recessive allele (presence of bands) depending on the percentage of banded individuals at the site.

most sites (table 2), even at open ones with a high level of insolation. As is known, the presence of dark spiral bands on *Cepaea* shells is a recessive trait inherited monogenously (Murray, 1975), which makes it possible to theoretically calculate the frequencies of homozygous and heterozygous individuals in the absence of genetic subdivision. At areas with a low occurrence of banded snails, there are not only fewer individuals carrying the corresponding allele, but also the quantitative ratio between its homozygous and heterozygous carriers changes significantly (fig. 4). For example, at sites where 5 to 10 % of banded snails are observed, theoretically about 40–50 % of individuals are carriers of the recessive allele, but it is phenotypically manifested in only 10–20 % of them (Gural-Sverlova, Gural, 2018). The remaining 80–90 % of carriers are heterozygotes with a unbanded shell, the thermoregulatory properties of which (Sverlova, 2004) are no different from those of dominant homozygotes.

Conclusions

Currently, in Western Ukraine (both in Lviv, the most studied in this respect, and beyond), there is a significant uniformity of the phenotypic composition in the introduced land snail *C. hortensis*. This results in the relatively low values of the inbreeding coefficient, which are considered uncharacteristic for relatively recently colonized areas. The observed pattern is due to the common origin of most of the Western Ukrainian populations of *C. hortensis*, formed by the descendants of the primary introduction of this species to the west of Ukraine, which most likely occurred in the second half of the 20th century. In recent years, individuals with different shell coloration have also begun to be recorded in Western Ukraine, indicating the presence of later and independent introductions of this species associated with the activities of some garden centers. So far, they do not have a considerable effect on the general pattern of the spatial variability of the phenotypic composition in the studied species, although they can significantly increase the phenotypic richness at separate, still very spatially limited sites.

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