Ruthenica, 2021, vol. 31, No. 1: 27-38.
Published online January 2, 2021.

Shell colour and banding polymorphism in *Cepaea nemoralis* (Gastropoda, Pulmonata, Helicidae) from the Moscow region

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ABSTRACT. The shell colour and banding were analyzed in more than 2000 specimens of *Cepaea nemoralis* collected in 11 localities in Moscow and the Moscow region. In the colonies studied, snails with pink shells usually predominate, most often represented by the phenotype 00000 (complete absence of the spiral bands). In most cases, yellow unbanded shells were absent or represented by single specimens. Brown shells, almost exclusively unbanded, were found only in 5 sites. On the example of colonies from Zagoryanski and Buch (Berlin), as well as Sheremetievski and Dolgoprudnyi, the phenotypic composition of newly formed colonies and colonies that could have been their origins was compared. A decrease in phenotypic diversity of newly formed colonies was noted.

Полиморфизм окраски раковины у *Cepaea nemoralis* (Gastropoda, Pulmonata, Helicidae) в Московской области

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РЕЗЮМЕ. Проанализирована окраска раковин у более чем 2 тысяч экземпляров *Cepaea nemoralis*, собранных в 11 локалитетах на территории Москвы и Московской области. Показано, что в исследованных колониях обычно преобладают улитки с розовыми раковинами, чаще всего представленными фенотипом 00000 (полное отсутствие спиральных полос). В большинстве случаев желтые бесполосые раковины отсутствовали или были представлены единичными экземплярами. Коричневые раковины, почти исключительно бесполосые, обнаружены только в 5 населенных пунктах. На примере колоний из Загорянского и Буха (Берлин), а также Шереметьевского и Долгопрудного проведено сравнение фенотипического состава новообразованных колоний и колоний, которые могли стать источником интродукции. Отмечено снижение фенетического разнообразия в новообразованных колониях.

Introduction

Cepaea nemoralis (Linnaeus, 1758) is a species of Western European origin [Boettger, 1926; Taylor, 1914]. In addition to southwestern Europe (France, northern Spain), the natural range of *C. nemoralis* also includes a large part of northern and central Europe. In Germany its range extends east of the Elbe River, in some places reaching the Oder River [Boettger, 1926]. Further east, the natural range of *C. nemoralis* extends only along the coast of the Baltic Sea, where it reaches the eastern Baltic states, and in the north it includes Denmark and southern Sweden. These coastal areas have been considered to be the most recently colonized parts of the natural range of *C. nemoralis* [Boettger, 1926]. According to other authors [Cameron *et al*., 2011], the populations living there are introduced.

Today's range of *C. nemoralis* is significantly expanded because of the human activities. Already in the second half of the 19th century, this species was introduced to North America [Alexander, 1952; Howe, 1898], southeastern Poland [Bąkowski, 1880], the Czech Republic [Peltanová *et al*., 2012], the first attempt was made to introduce it into western Ukraine [Łomnicki, 1899]. In the environs of Moscow *C. nemoralis* was first mentioned even earlier [Dwigubsky, 1802]. However, later it was not found in the Moscow region until the early 1980s, when *C. nemoralis* was introduced here from East Berlin, most likely from the Buch district in the northeastern outskirt of the city [Egorov, 2018: 84]. The snails were brought by children on vacations at a summer camp in Buch and released at a private summer cottage between the railway stations of Valentinovka and Zagoryanskaya in the Schelkovsky district in 1980 or 1981. The first mass occurrence of *C. nemoralis* was observed in Zagoryanski settlement in 1984 [Egorov, 2018].

Today *C. nemoralis* is present in various localities in Moscow and the Moscow region [Egorov, 2018]. It is highly likely that not all recorded and observed colonies of this species were formed by descendants of molluscs released in the 1980s near Zagoryanski. The probability of repeated accidental and/or deliberate introductions of *C. nemoralis* to the Moscow region is quite high. It is typical for most known colonies to be confined to either garden nurseries (for example, in Dolgoprudnyi) or places where products grown in nurseries are used, such as parks, squares, summer cottage gardens, and so on.

Until now, the phenotypic composition of only one sample of *C. nemoralis*, collected in June 2006 in the the urban-type settlement of Nakhabino in the Krasnogorsk district of the Moscow region, has been described and analyzed [Sverlova, 2007]. For the rest of the known colonies of *C. nemoralis* in Moscow and the Moscow region, only the predominant phenotypes or phenotype groups have been noted [Egorov, 2018]. Also, no attempt has yet been made to compare the phenotypic composition of *C. nemoralis* in Zagoryanski and other localities in the Moscow region with that in Buch. This study is intended to fill this gap.

Material and methods

In total, 2111 shells of *C. nemoralis* were used in this work, 1620 of adult and 491 of young individuals mostly found alive as well as empty shells. Most samples were collected by R. Egorov, with the exception of 3 shells from Zagoryanski (collected by A. Zubarev in 1985) and one previously described sample from Nakhabino [Sverlova, 2007]. The material was collected from the following 11 localities in Moscow and the Moscow region (Fig. 1), which were described in detail by Egorov [2018].

1) Moscow city, Severnyi Administrative District, Lianozovski forest-park, 55°54.10'N, 37°34.37'E, 2015-2018;

2) Moscow city, Yugo-Zapadnyi Administrative District, Kotlovka Municipal District, ravine of the Kotlovka river (55°39.96'N, 37°35.81'E) and fence of Korobkovski garden (Korobkovki fruit tree nursery, 55°40.05'N, 37°35.97'E – 55°40.09'N, 37°36.05'E), 2016;

3) Moscow region, town of Dolgoprudnyi, east of the railway station Dolgoprudnaya, Dolgoprudnaya Alley, opposite the entrance to the DAOS agro-nursery (now the "Medra Garden Centre") and the park of the former Vinogradovo estate, 55°56.51'N, 37°32.11'E, 2017;

4) Moscow region, town of Dolgoprudnyi, Sheremetievski microdistrict, corner of Pervomaiskaya and Komarova streets, 55°59.52'N, 37°29.60'E, 2017, 2019;

5) Moscow region, near the town of Lobnya, a sawmill in the forest opposite the village of Nosovo, on abandoned railway tracks and undergrowth around them, 55°59.63'N, 37°25.84'E, 2018-2019;

6) Moscow region, town of Pushchino, Park Pobedy (Victory Park), 54°50.27'N, 37°36.83'E, 2016;

7) Moscow region, Dmitrov district, town of Dmitrov, along Pochtovaya street (56°20.44'N, 37°30.97'E) and at the beginning of Professional'naya street (56°20.83'N, 37°31.09'E), 2014-2017;

8) Moscow region, Krasnogorsk district, Nakhabino settlement, 7 sites along Panfilova street (Fig. 2), described in more detail below, 2006, 2017-2018 (the greatest numbers of snails were collected in 2017);

9) Moscow region, Lyubertsy district, Malakhovka settlement, along Kirov street (55°38.56'N, 37°59.88'E) and Fevral'skaya street (55°38.57'N, 37°59.66'E), 2015-2017;

10) Moscow region, Mytishchi district, town of Mytishchi, wasteland between the Perlovskaya bus stop (MKAD) and Zapadnaya street (55°53.62'N, 37°42.21'E – 55°53.61'N, 37°42.27'E) and north of Zapadnaya street (55°53.64'N, 37°42.30'E), 2017-2018;

11) Moscow region, Schelkovo district, Zagoryanski village, along the Zelenaya street (55°55.47'N, 37°55.24'E), Kooperativnaya street (55°55.54'N, 37°55.00'E), Lenin street (55°55.48'N, 37°54.94'E), 2016, 2018, and Yuzhnaya street, 1985.

At the beginning of September 2017, six samples were collected in Nakhabino. The collection sites are shown in Fig. 2.

Site 1 – the southern wall of building 19 (about 97 m long), lawn and shrubs along it as well as concrete fence around a closed military area (total length of fencing is about 320 m), 55°50.17'N, 37°10.89'E – 55°50.12'N, 37°10.82'E. Limited additional collecting took place in May 2018.

Site 2 – the southern wall of building 12a (length about 74 m) and trees along it, concrete fence around a closed military area (total length of the fence about 450 m), 55°50.16'N, 37°10.72'E –55°50.09 'N, 37°10.68'E.

Site 3 – concrete fence of the city ambulance substation on Panfilova street, length about 35 m, 55°50.22'N, 37°10.57'E.

Site 4 –concrete fence of the Nakhabinsk Engineering Networks LLC in the yard of building 13a and opposite building 9a (total length about 200 m) as well as trees and shrubs near the fence, 55°50.23'N, 37°10.62 ' E – 55°50.20'N, 37°10.72'E.

Site 5 – the north-eastern wall of building 24 (about 70 m long), lawn and garden with trees alongside it, 55°50.24'N, 37°10.53'E.

Site 6 – the eastern wall of building 22 (length about 70 m), lawn with trees and bushes alongside it and on the southern corner of the building, 55°50.20'N, 37°10.56'E.

The site where molluscs were collected in June 2006 [Sverlova, 2007] is located near site 4 and designated as site 7. This site is a planted with trees and shrubs and located along the eastern wall of the building No.13 (55°50.27'N, 37°10.77'E).

In many cases, the dimensions of the surveyed sites exceeded the diameter of the panmictic unit in *Cepaea* [Lamotte, 1951; Schnetter, 1950].

Phenotypes were scored based on the colour and banding pattern of the body 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 bands 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

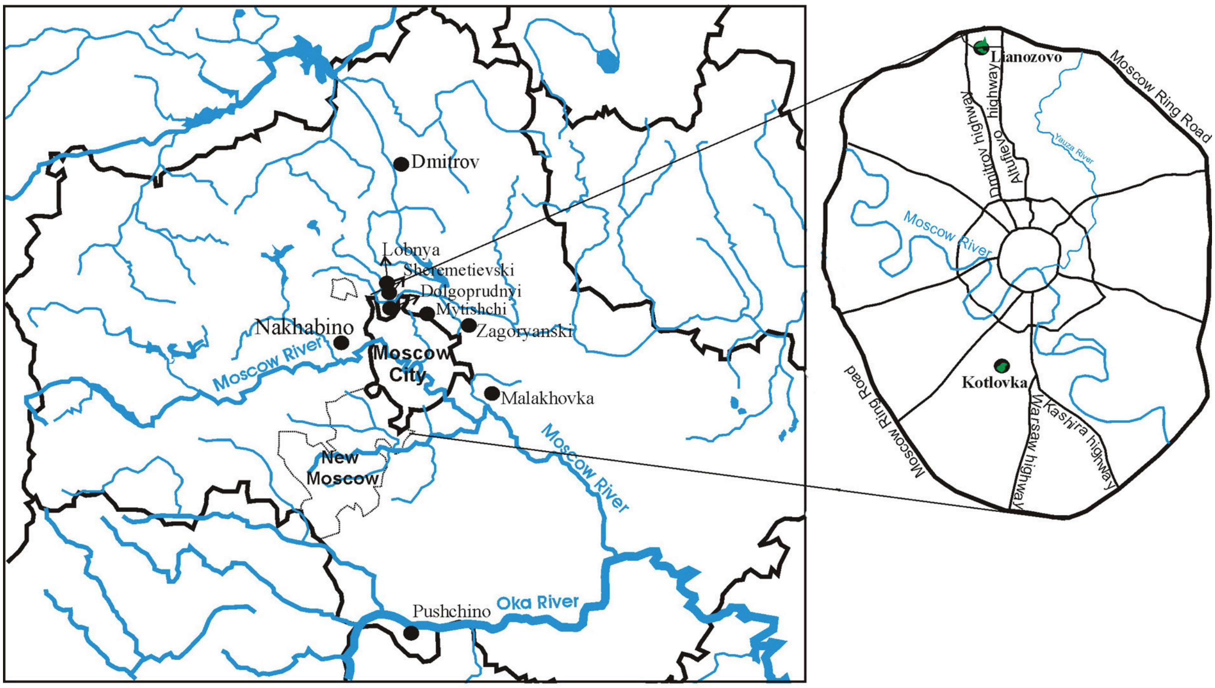

FIG. 1. The locations of the collecting sites in the Moscow region.

РИС. 1. Расположение мест сбора в Московской области.

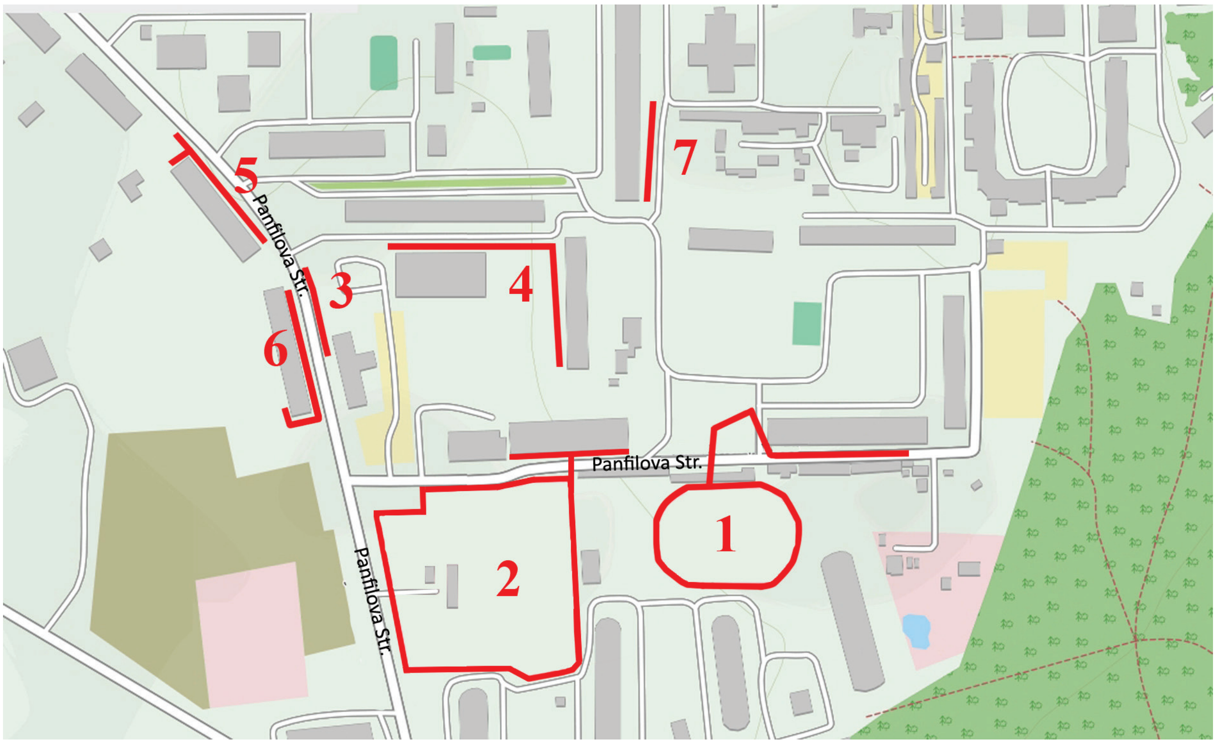

FIG. 2. The locations of the collecting sites in Nakhabino.

РИС. 2. Расположение мест сбора в Нахабино.

before the aperture. The presence of additional weak, blurry bands (modification) on some shells that have a genetically determined phenotype 00000 or 00300, was indicated by placing the numeral representing the band(s) in square brackets. The splitting of one of the bands was denoted by repeating its number in the formula. The ground colour of the shell was scored as Y – yellow, P – pink, B – brown.

In the subsequent analysis of the phenotypic composition of the studied colonies of *C. nemoralis*, individual phenotypes were combined into 4 groups. The formulas of the most common morph and the "+" sign were used for each group, as follows.

1) 00000+ – almost exclusively the phenotype 00000, but also genetically unbanded shells with 1-2 unclear bands (modifications).

2) 00300+ – the same as for the phenotype 00000.

3) 00345+ – the phenotype 00345 and its variations with the absence or fusion of some bands.

4) 12345+ – the same as for the phenotype 00345.

The character of the shell banding in *Cepaea* may change during ontogenesis. This is associated with the sequential appearance of dark spiral bands in young shells, and their subsequent expansion and possible fusion [Sverlova, 2004a]. Therefore, the use of juvenile shells in the study of the phenotypic composition of populations can lead to incorrect results, for example, underestimation of the proportion of individuals with fused bands and even a decrease in the observed level of phenotypic diversity [Sverlova, 2004b]. To avoid potential problems, juvenile shells (i.e. without a reflected apertural margin) but with a shell diameter of more than 10 mm, were scored only to the level of phenotypic groups, while the adult shells were scored to the level of specific phenotypes. Juvenile shells smaller than 10 mm, were not scored, as their eventual adult banding pattern may not have had time to appear.

For comparison, we used data on two large samples of *C. nemoralis* from the Buch district in Berlin collected around the summer camp in 1952 and along the highway near the hospital in 1951 [Schilder, Schilder, 1957, tab. 10], a total of 1200 specimens. The exact composition of banding phenotypes and the percentage of yellow shells was taken from the file cabinets of these researchers stored in the Museum of Natural History in Berlin.

To assess the variability of the phenotypic composition between the studied colonies the inbreeding coefficient Fst was used, calculated based on the frequencies of some phenotype groups [Cameron *et al.*, 2009]. The frequencies of yellow and unbanded shells were calculated from the total sample size, and the frequencies of shells with a central band from the number of banded shells. The calculations did not take into account a small sample from Pushchino, which included only 7 shells of adults and 3 shells of juvenile snails. For comparison, we used published data on urban populations of *C. nemoralis* from different parts of the present species range [Cameron *et al.*, 2009, 2014; Ożgo, 2005].

The shells from Moscow and the Moscow region used in this study were deposited in the malacological collection of the State Museum of Natural History of the National Academy of Sciences of Ukraine in Lviv (inventory Nos. 2660, 4376-4384, 4386-4399, 4401-4412, 4416, 4424, 4427, 4428).

Results

In total, 40 phenotypes differing in shell colour and banding were recorded in adult *C. nemoralis* from the Moscow region (Table 1). Some of them were represented by such modifications as the presence of weak additional bands on the shells of phenotype 00300, or the absence of a central band in five-banded shells. P00000 (27.4% of the total number of shells), P00300 (16.2%), B00000 (8.4%), Y00300 (8.1%), Y12345 (7.2%) were the predominant phenotypes. All other phenotypes combined represented 32.7% of the total.

Among the group of five-banded shells (12345+), specimens with 5 discrete bands constituted 36.7%, with one band missing 1.1%, with fusion of 2 to 5 bands 62.2%. Among the shells with fused bands, phenotypes with one fusion (38.9%) or two fusions (35.3%) of two adjacent bands prevailed. In each of these groups, only one phenotype was predominant (more than 80%): 123(45) or (12)3(45). Among the variations with a larger number of fusions, phenotypes (123)(45) and (12345) prevailed, being, respectively, 67.0% and 28.2%. More than 90% of shells with fused bands had bands 4 and 5 fused, and about 60% had bands 1 and 2 fused.

Almost everywhere within the study area, the shell ground colour varied. The only exception was a relatively small (44 specimens) sample from the Sheremetievski microdistrict, in which only pink shells were present (Table 2). Individuals with yellow and pink shells were found in five localities, another five localities contained shells of all three colours. At almost all sites, pink shell colour prevailed, brown colour was dominant in only one sample (from Zagoryanski).

After additional research, shells from Lianozovo, designated as "pinkish brown" by Egorov [2018, fig. 6Q], were scored as pink. Their ground colour was determined to be "dirty pink" rather than "light brown". Among unbanded and mid-banded specimens from this area, continuous variation in ground colour from intense to dirty pink was observed. In the latter case, the colour of empty shells may even look grayish or light beige. At the same time, in the samples from Kotlovka, Malakhovka, Mytishchi, and the above-mentioned Zagoryanski, definitely brown unbanded shells were present [Egorov, 2018, fig. 6R], from 0.4% in Mytishchi to 56.8% in Zagoryanski (Table 2).

Polymorphism in colour of the apertural margin in adult *C. nemoralis* was not found in the Moscow region. Nor did we find shells with depigmented (hyalozonate) bands.

Five-banded phenotypes were predominant in yellow shells, and unbaded phenotypes in pink shells (Table 2). Brown specimens were represented almost exclusively by the B00000 phenotype. Only in the

Table 1. The phenotypic composition of the samples of *C. nemoralis* (adults only) from Moscow and the Moscow region.

Табл. 1. Фенетическая структура выборок *C. nemoralis* (только половозрелые особи) из Москвы и Московской области.

Phenotypes	Moscow		Moscow region									Total
	Lianosovo	Kotlovka	Dmitrov	Dolgoprudnyi	Lobnya	Malakhovka	Mytishchi	Nakhabino	Pushchino	Sheremetievski	Zagoryanski	
Yellow unbanded												
Y00000	1	9	–	1	–	–	–	–	–	–	1	12
Yellow with one central band and their modifications												
Y00300	77	1	2	6	–	2	33	7	3	–	1	132
Y0030[5]	–	–	–	–	–	–	1	1	–	–	–	2
Y003[4]0	1	–	–	–	–	–	–	–	–	–	–	1
Yellow with 3 lower bands												
Y00345	–	–	–	2	5	–	4	42	–	–	–	53
Y003(45)	–	–	–	1	–	–	2	14	–	–	–	17
Yellow with 5 unfused bands and variants with the absence or splitting of one band												
Y12345	29	–	–	14	–	3	14	57	–	–	–	117
Y123445	1	–	–	–	–	–	–	–	–	–	–	1
Y02345	1	–	–	1	–	–	–	–	–	–	–	2
Y10345	–	–	–	–	–	–	–	1	–	–	–	1
Y12045	–	–	–	1	–	–	–	–	–	–	–	1
Yellow with 5 bands and bands fusion												
Y(12)345	–	–	–	1	1	–	1	1	–	–	–	4
Y1(23)45	–	–	–	1	–	–	–	3	–	–	–	4
Y123(45)	28	–	–	15	–	–	6	14	1	–	–	64
Y(12)3(45)	7	1	–	10	–	–	6	8	1	–	–	33
Y(123)(45)	9	–	–	10	–	–	3	5	–	–	1	28
Y1(23)(45)	3	–	–	2	–	–	1	2	–	–	–	8
Y1(2345)	1	–	–	–	–	–	–	–	–	–	–	1
Y(12)(345)	–	–	–	3	–	–	–	–	–	–	–	3
Y(12345)	4	–	–	3	–	–	1	–	–	–	–	8
Pink unbanded and modification												
P00000	61	13	20	18	8	–	89	232	–	–	3	444
P00[3]00	1	–	–	–	–	–	–	–	–	–	–	1
Pink with one central band and their modifications												
P00300	79	–	87	11	1	13	20	1	1	28	21	262
P0[2]3[4]0	–	–	–	–	–	–	–	–	–	–	1	1
P10300	–	–	–	–	–	–	–	–	–	–	1	1
Pink with 3 lower bands												
P00345	–	–	–	6	–	–	2	4	–	9	–	21
P003(45)	1	–	–	6	–	–	–	–	–	–	–	7
Pink with 5 unfused bands and variants with the absence of one band												
P12345	16	1	2	14	–	16	13	3	–	3	8	76
P10345	–	–	–	–	–	–	1	–	–	–	–	1
P12045	–	–	–	1	–	–	–	–	–	–	–	1
Pink with 5 bands and band fusion												
P(12)345	–	–	–	3	–	2	1	–	–	–	5	11
P1(23)45	1	–	1	4	–	–	–	–	–	–	–	6
P123(45)	13	2	3	9	–	3	4	1	1	–	3	39
P(123)45	2	–	–	–	–	–	–	–	–	–	–	2
P(12)3(45)	25	6	4	14	–	3	3	1	–	–	6	62
P(123)(45)	14	–	1	4	–	–	2	–	–	–	8	29
P1(23)(45)	6	–	1	4	–	–	–	–	–	–	–	11
P(12345)	7	–	–	2	–	–	1	–	–	–	6	16
Brown unbanded												
B00000	–	13	–	–	–	21	–	14	–	–	88	136
Brown with one central band												
B00300	–	–	–	–	–	–	–	–	–	–	1	1
Total	388	46	121	167	15	63	208	411	7	40	154	1620

Table 2. Percentages of main phenotypes and groups of phenotypes in the samples of *C. nemoralis* (adults and juvenile individuals) from Moscow and the Moscow region.

Табл. 2 Частоты основных фенотипов и групп фенотипов в выборках *C. nemoralis* (половозрелые и молодые особи) из Москвы и Московской области, %.

Phenotypes and their groups	Moscow		Moscow region									In total
	Lianosovo	Kotlovka	Dmitrov	Dolgoprudnyi	Lobnya	Malakhovka	Mytishchi	Nakhabino	Pushchino	Sheremetievski	Zagoryanski	
Ground colour and banding												
Y00000	0.2	21.6	–	1.2	–	–	–	–	–	–	0.6	0.8
Y00300+	21.0	2.0	1.9	4.1	4.2	3.0	16.2	2.2	**30.0**	–	1.3	8.0
Y00345+	–	–	–	2.4	33.3	–	3.5	14.9	–	–	–	5.8
Y12345+	21.5	2.0	–	**40.7**	4.2	4.5	17.1	23.8	20.0	–	0.6	19.2
P00000+	14.8	**27.5**	16.3	8.5	**54.2**	–	**41.2**	**50.9**	10.0	–	1.9	**27.7**
P00300+	19.5	2.0	**71.3**	6.5	4.2	21.2	9.2	0.6	20.0	**70.**4	14.8	14.9
P00345+	0.2	–	–	7.3	–	–	0.9	1.3	–	20.4	–	1.8
P12345+	**22.8**	19.6	10.6	29.3	–	**37.9**	11.4	1.3	20.0	9.1	23.2	14.3
B00000	–	25.5	–	–	–	33.3	0.4	5.0	–	–	**56.8**	7.5
B00300	–	–	–	–	–	–	–	–	–	–	0.6	0.05
Ground colour of the shell												
Yellow	42.7	25.5	1.9	48.4	41.7	7.6	36.8	40.9	**50.0**	–	2.6	33.7
Pink	**57.3**	**49.0**	**98.1**	**51.6**	**58.3**	**59.1**	**62.7**	**51.4**	**50.0**	**100.0**	40.0	**58.7**
Brown	–	25.5	–	–	–	33.3	0.4	5.0	–	–	**57.4**	7.5
Bands number												
00000+	15.0	**74.5**	16.3	9.8	**54.2**	33.3	**41.7**	**55.9**	10.0	–	**59.4**	**35.9**
00300+	40.5	3.9	**73.1**	10.6	8.3	24.2	25.4	2.8	**50.0**	**70.4**	16.8	22.9
00345+	0.2	–	–	9.8	33.3	–	4.4	16.2	–	20.4	–	7.7
12345+	**44.3**	21.6	10.6	**69,9**	4.2	**42.4**	28.5	25.1	40.0	9.1	23.9	33.5
With 0–1 or with 3–5 bands												
00000+ and 00300+	**55.5**	**78.4**	**89.4**	20.3	**62.5**	**57.6**	**67.1**	**58.7**	**60.0**	**70.4**	**76.1**	**58.8**
00345+ and 12345+	44.5	21.6	10.6	**79.7**	37.5	42.4	32.9	41.3	40.0	29,5	23.9	41.2
N	447	51	160	246	24	66	228	680	10	44	155	2111

Notes: + includes other phenotypes that can be considered as variations of this (see methods), N – sample size. The maximum values are bold and underlined.

sample from Zagoryanski did one specimen have a mid band (Table 1).

If we exclude small (up to 25 specimens) samples from Lobnya and Pushchino, because the phenotypic composition of these samples could have been significantly affected by random collection factors, the colonies of *C. nemoralis* from Moscow and the Moscow region could be divided into 3 groups according to their banding (Table 2), as follows.

1) With a distinct predominance (from 42 to 74%) of unbanded shells, due to the high proportion of the phenotype P00000 in Mytishchi and Nakhabino, B00000 in Zagoryanski, and both morphs in Kotlovka.

2) The same distinct predominance (from 70 to 73%) of mid-banded shells of the phenotype P00300 in Dmitrov and Sheremetievski.

3) With a predominance (42 to 70%) of a group of five-banded shells in Dolgoprudnyi, Lianozovo and Malakhovka. In Dolgoprudnyi the frequencies of all other phenotype groups was correspondingly reduced. In Lianozovo mid-banded yellow and pink shells, and in Malakhovka brown unbanded shells were found almost as often as five-banded shells.

The total proportion of shells with 3 to 5 bands exceeded 50% only in Dolgoprudnyi. In all other localities, shells with a more homogeneous colouring prevailed, i.e. without contrasting dark bands or with one central band. The predominance of these phenotypes was most noticeable in Dmitrov, where

Table 3. The phenotypic composition of the samples of *C. nemoralis* (adults and juvenile individuals) from Nakhabino, Moscow region.

Табл. 3. Фенетическая структура выборок *C. nemoralis* (половозрелые и молодые особи) из Нахабино, Московская область.

Phenotypes and their groups	Sites							Total
	1	2	3	4	5	6	7	
Ground colour and banding								
Y00300+	2	–	1	2	–	8	2	15
Y00345+	15	4	16	41	–	2	23	101
Y12345+	26	6	**32**	52	11	7	28	162
P00000	**63**	**63**	23	**93**	**17**	11	**76**	**346**
P00300	–	–	–	–	–	4	–	4
P00345+	–	–	–	–	–	9	–	9
P12345+	–	–	–	–	–	9	–	9
B00000	–	–	–	–	2	**32**	–	34
Ground colour of the shell								
Yellow	43	10	**49**	**95**	11	17	53	278
Pink	**63**	**63**	23	93	**19**	**33**	**76**	**370**
Brown	–	–	–	–	–	32	–	32
Bands number								
00000+	**63**	**63**	23	**93**	**19**	**43**	**76**	**380**
00300+	2	–	1	2	–	12	2	19
00345+	15	4	16	41	–	11	23	110
12345+	26	6	**32**	52	11	16	28	171
N	106	73	72	188	30	82	129	680

Notes: as in table 2.

their frequency was 8.4 times higher than that of the combined three- and five-banded shells, in Kotlovka (3.6 times) and Zagoryanski (3.2 times).

There was a spatial differentiation in phenotypic composition among the *C. nemoralis* colonies in Nakhabino (Fig. 3). Brown shells, all B00000, were observed only on one side of the street, at sites 5 and 6 (Table 3), where they constituted 6.7% and 39.0% of the samples, respectively. Snails with pink banded shells, represented by phenotypes P00300, P00345, P12345, as well as some five-banded shells with band fusion were found only at site 6, where they constituted 26.8% of the sample. Much less phenotypic diversity than at site 6, was observed at sites 1, 2, 3, 4 and 7, caused by the absence of snails with brown unbanded and pink banded shells, and at site 5, caused by the absence of pink banded shells and yellow shells with one or three bands (Fig. 3, Table 3). Site 3 differed from all other sites by the predominance of not unbanded but five-banded shells.

The data from the Schilder's file cabinet regarding the phenotypic composition of two samples from the Buch district in Berlin (see Material and methods), used for comparison, are completely given in Table 4. As in Zagoryanski, more than half of the snails from Buch had unbanded shells (Table 5). Three-banded shells, rarely found in the Moscow region and possibly completely absent in Zagoryanski, were also rare in Buch. At the same time, the frequency of yellow shells in Zagoryanski is significantly lower than in Buch.

Unfortunately, the shells with both pink and brown ground colour were indicated in the Schilder's card file and monograph [Schilder, Schilder, 1957] as "rot" (red). In addition, in the file cabinet you can find out only the total percentage of yellow and non-yellow shells in the samples, regardless of their banding. Considering the data given in the monograph [Schilder, Schilder, 1957, tab. 10], it was possible to calculate approximate frequencies of the phenotype Y00000 (about 12-13%) and the phenotypes P00000 and B00000 combined (about 46%).

Similarly to colonies of *C. nemoralis* from the Moscow region (see above), phenotypes with band fusion prevailed among five-banded shells in Buch (74.1% of the total number of such shells). The frequence of shells with 5 discrete bands was 24.1%, and of shells without one (mostly 10345) or two (10305) bands was 2.4%. Phenotypes with fusion of two adjacent bands predominated (40.6%) among the shells with fused bands. The most frequently encountered fusion variants were (12)3(45), (123)(45), 123(45) and (12345), listed in decreasing order of occurrence frequencies; 91.1% of the shells with fused bands had bands 4 and 5 fused, and 76.4% had bands 1 and 2 fused.

In the Moscow region, the Fst values calculated for different settlements were higher than the analo-

Table 4. The phenotypic composition of two samples of *C. nemoralis* in Buch district of Berlin, Germany (data from the Schilder's file cabinet).

Табл. 4. Фенетическая структура двух выборок *C. nemoralis* из района Бух в Берлине, Германия (данные из картотеки Шильдеров).

Phenotypes	Summer camp, in 1952	Near the hospital in 1951	Total
00000	505	198	703
00300	166	118	284
10300	1	–	1
02300	2	–	2
00340	5	2	7
00305	9	3	12
00345	6	4	10
003(45)	7	5	12
00(345)	1	2	3
12345	21	19	40
10345	–	2	2
10305	1	–	1
(12)0(45)	1	–	1
(12)345	4	2	6
1(23)45	2	1	3
(123)45	2	–	2
123(45)	17	7	24
(12)3(45)	36	9	45
(123)(45)	23	4	27
(12345)	10	2	12
12(345)	–	1	1
(12)(345)	1	–	1
1(23)(45)	1	–	1
Total	821	379	1200
Percentage of yellow shells	42.3	33.2	39.4

gous values calculated for the colonies in Nakhabino. A similar pattern is observed in the southeast of Poland, the Subcarpathian Voivodeship (Table 6).

Discussion

C. nemoralis is one of the best-known species of European land molluscs; many publications are devoted to the colour and banding polymorphism of its shells. Long-term studies in England, France, Germany and other European countries already in the second half of the 20th century demonstrated that the phenotypic composition of populations of this species is the result of a complex interaction of selective and non-selective (stochastic) factors [Jones *et al.*, 1977]. At the initial stages of introduction, the latter may be of particular importance, first of all, the founder effect.

The initial restriction of genetic and phenotypic diversity in introduced colonies, caused by a limited number of founding individuals and the possibility of accidental disappearance of some traits in the initial stages of establishment, cannot be compensated for by immigration, but only by repeated introduction of the snails by people from the main part of the species range or from other introduced colonies. Such periodic unintentional introduction can theoretically occur via nurseries that regularly receive plant material from other countries. In other types of habitats inhabited by snails, the probability of repeated introduction may be much lower.

Therefore, with the greatest probability in introduced colonies there will be no inherited traits that are locally and/or relatively rare in the natural range of the species. These traits include, for example, a white lip and hyalozonate bands which were not found in the Moscow region and in other introduced colonies of *C. nemoralis* in Eastern Europe [Gural-Sverlova *et al.*, 2020]. Such shells are also absent in most natural populations of this species, although locally they can reach relatively high frequencies [Schilder, Schilder, 1957]. The colonies studied by us also did not have shells with punctate bands.

The inherited traits that are more common for *C. nemoralis* (varied ground colour, presence and number of bands, their fusion) are quite well represented in the Moscow region (Tables 1, 2) as well as in introduced colonies of this species in Belarus [Kolesnik, Kruglova, 2016; Ostrovsky, Prokofieva, 2017] and in western Ukraine [Gural-Sverlova *et al.*, 2020]. Brown shells were not found in many eastern European colonies. However, in different parts of

Table 5. Percentages of groups of phenotypes in Buch (Berlin, Germany) and in Moscow region.

Табл. 5. Частоты групп фенотипов в Бухе (Берлин, Германия) и в Московской области, %.

Groups of phenotypes	Germany	Moscow region	
	Buch	Zagoryanski	Other localities
Yellow shells	39.4	1.9	34.0
00000+	58.6	59.7	34.2
00300+	24.5	16.2	24.6
00345+	3.1	–	6.2
12345+ total	13.8	24.0	35.0
12345+ no fusion	3.6	5.2	13.5
12345+ fused	10.3	18.8	21.5
N	1200	154	1336

Table 6. The variability of the phenotypic composition of *C. nemoralis* under urban conditions.

Табл. 6. Изменчивость фенетической структуры *C. nemoralis* в урбанизированной среде.

City (region), country, literature source	Comments	Inbreeding coefficient Fst		
		Yellow	00000+	00300+
Sheffield, England [Cameron *et a*l., 2009]	Within the natural range, but actively colonizing the city only in the last decades	0.207	0.350	0.284
Göteborg, Sweden [Cameron *et al*., 2014]	Outside the natural range, introduced no later than the middle of the 19th century, increased spread in the last decades	0.212	0.302	0.277
Wrocław, Poland [Cameron *et a*l., 2009]	Outside the natural range, introduced over a century ago	0.089	0.092	0.123
Rzeszów, Poland, calculated based on data in Ożgo [2005]	Similar to Wrocław	0.163	0.193	0.153
Subcarpathian Voivodeship, Poland, calculated based on data in Ożgo [2005]	5 settlements, including Rzeszów (see above)	0.194	0.218	0.186
Nakhabino, Russia, own data	Introduced, first recorded in 2006	0.118	0.094	0.178
Moscow region, Russia, own data (includes Nakhabino as one sample)	Introduced in the early 1980s 8 settlements, excluding Pushchino	0.187	0.243	0.265

the natural range of *C. nemoralis*, the average frequencies of brown shells are also usually lower than yellow and pink [Arnold, 1970, 1971; Cain, Sheppard, 1954: Cameron, 2001; Cameron, Dillon, 1984; Cameron, Pannet, 1985; Ramos, 1984, 1985; Wolda, 1969a, 1969b and others]. Brown banded shells are found in the natural range much less frequently than brown unbanded ones. Therefore, they are most often completely absent in introduced colonies.

Among the variants of shell colouration, more common for *C. nemoralis* than brown banded shells, the phenotype Y00000 is often absent or very rare in introduced colonies from the Moscow region (Table 2), Belarus [Ostrovsky, Prokofieva, 2017] and western Ukraine [Gural-Sverlova *et al*., 2020]. Among all the eastern European colonies studied so far, only one small colony with a predominance of yellow unbanded shells was found in a wasteland near Lviv, western Ukraine [Gural-Sverlova *et al*., 2020]. At the same time, the phenotype Y00000 can reach high frequencies and even prevail in some parts of the natural range of *C. nemoralis* [Ramos, 1984] and especially in certain types of biotopes (open, with high insolation) [Wolda, 1969b]. It is also often found in some introduced colonies in North America [Brussard, 1975; Clench, 1930; Johnson, 1927; Landman, 1956; McConnell, 1936].

The phenotypic composition of the colonies of *C. nemoralis* in Nakhabino (Fig. 3, Table 3) can be considered as an example of the random disappearance of some phenotypes and phenotype groups during the formation of new colonies. If the snail colonies living on different sides of Panfilov Street were not formed more or less simultaneously by the founding individuals, brought in by people from some other territory, located in or outside the city, then a colony should first have been formed at site 6 because it possesses the greatest number of phenotypes and phenotype groups, including all phenotype groups present at the other sites. The accidental transfer of a small number of snails across the road (for example, by children [Sverlova, 2002]) or genetic drift that followed in the newly formed colony could have led to the disappearance of individuals with brown unbanded and pink banded shells that make up more than half the colony at site 6. In the related species *C. hortensis*, roads 5 to 10 m wide can be effective barriers to the dispersal of snails and may lead to phenotypic differentiation of the colonies separated by them [Sverlova, 2001, 2002]. The width of the roadway on Panfilov Street is about 6 m. A similar disappearance of pink banded shells on one side of the street was observed also in Bogorodochany, Ivano-Frankivsk region, Ukraine [Gural-Sverlova *et al*., 2020]. As in Nakhabino, it led to a significant reduction of the phenotypic diversity (only yellow banded and pink unbanded).

Given the known origin of one of the colonies of *C. nemoralis* in the Moscow region [Egorov, 2018], it is of particular interest to compare the phenotypic

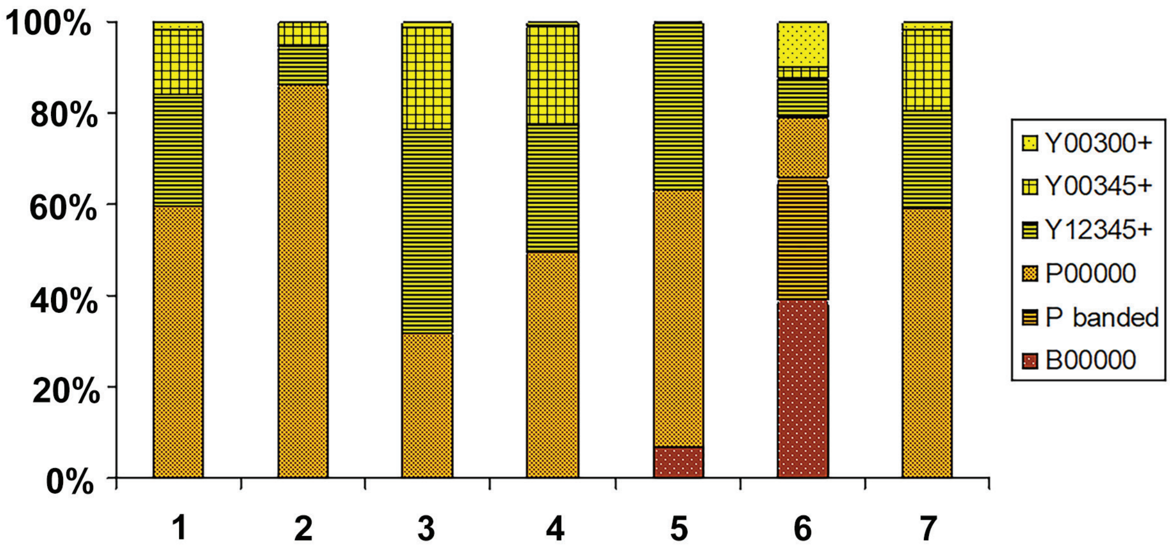

FIG. 3. The ratio of the main phenotypes and groups of phenotypes in the studied colonies of *C. nemoralis* in Nakhabino. Site numbers as in Figure 2.

РИС. 3. Соотношение основных фенотипов и групп фенотипов в исследованных колониях *C. nemoralis* в Нахабино. Номера участков аналогично рисунку 2.

composition of this species in Zagoryanski and in Buch district in Berlin (Table 5). With the exception of more than a 20-fold decrease in the frequency of yellow and yellow unbanded shells, as well as the absence of three-banded shells, samples from Zagoryanski have much in common with those from Buch. As in Buch, not only in Zagoryanski but also in other colonies from the Moscow region, there are often unbanded shells with a darker (non-yellow) ground colour, and among the five-banded shells, those with the band fusion prevail. The quantitative distribution of phenotypes and the relative frequencies of fusions of different pairs of bands among five-banded shells with fused bands are also similar.

Neither in the Zagoryanski nor in other localities of the Moscow region was found the phenotype 00305, which Schilder, Schilder [1957, p. 189, 191, map 75] consider to be derived from 00345 and typical in Germany, with the exception of its northern coastal areas. According to these researchers, the phenotype 00305 is common in diluvium of Thuringia, and currently it is most often found between Bodensee and West Mecklenburg, as well as in places between Savoie in France and Portugal. The only two similar shells found in Nakhabino and Mytishchi (Table 1) were clearly modifications from 00300 with a unclear 5th band. In samples from Buch, the phenotype 00305 was found with a frequency of about 1% (Table 4), but it accounted for about a third of all shells from the 00345+ group.

The absence of three-banded shells in Zagoryanski and their presence in some other colonies from the Moscow region can obviously be considered indirect confirmation that at least part of these colonies were not formed by snails from Zagoryanski. Although it is impossible to completely exclude the possibility that three-banded shells were present in Zagoryanski at the initial stages of introduction. The heterogeneous origin of the known colonies of *C. nemoralis* from the Moscow region is also supported by the present distribution of this species in the region, the connection of most colonies with garden nurseries or places where products grown in nurseries are used, as mentioned above.

In the colony from Sheremetievski founded most likely in 2005 by snails or their eggs brought along with seedlings of apple trees from the plant-nursery in Dolgoprudnyi [Egorov, 2018], the phenotypic composition is very reduced. Apparently, due to the limited number of founding individuals, the newly formed colony became monomorphic in the shell ground colour (pink). Also, the phenotype P00000, which was registered in most of the studied colonies and amounted to 8.5% in Dolgoprudnyi, has not yet been discovered here (Table 2). Instead of five-banded shells, which had more than two-thirds of snails in Dolgoprudnyi, the lightest of the remaining phenotypes, P00300, prevails here, whose share in Dolgoprudnyi was only 6.5% (Table 2).

The origin of most colonies of *C. nemoralis* in the Moscow region (except Zagoryanski and Sheremetievski) is unknown. Therefore, it is impossible to make such a comparison for them. The examples of the colonies from Zagoryanski, Sheremetievski, and Nakhabino, however, clearly suggest that the initiation of new colonies caused by intentional or unintentional transfer of snails by humans is usually associated with a reduction of the initial genetic and phenotypic diversity to various degrees. At most sites in Nakhabino, this leads not only to the absence of brown shells, but also to the linked inheritance the pink colour of the shell and the absence of bands on it, and in Sheremetievski – to the formation of a colony monomorphic in the shell ground colour. The ratio of the remaining groups of phenotypes may remain relatively stable (Buch and Zagoryanski, Table 5), or change significantly (Dolgoprudnyi and Sheremetievski, Table 2).

It is considered that Fst values may depend on the time of colonization of cities by snails [Cameron *et al*., 2009]. However, the data obtained for some not very great cities [Cameron *et al*., 2009, 2014] given in table 6 are difficult to compare with the relatively few samples collected over a much larger area and at larger sites. Even in the Subcarpathian Voivodeship, the colonization of which by *C. nemoralis* began already in the second half of the 19th century [Bąkowski, 1880], when comparing samples from several settlements, the Fst values slightly increase. This increase is even more pronounced in the Moscow region, especially for unbanded shells (Table 6). The relatively high Fst values calculated for Moscow and its environs can be caused not only with a relatively small number of spatially isolated colonies and their relative youth, but also, very likely, with their different origins (see above).

Since the process of anthropohoric spreading of *C. nemoralis* throughout the Moscow Region will continue further, we can expect the discovery of new colonies with a reduced phenotypic composition caused by the unintentional transfer of a small number of individuals from other introduced colonies and primarily from colonies associated with nurseries.

The data on the phenotypic composition of the introduced colonies of *C. nemoralis* in the Moscow region, as well as the previous publication dedicated to distribution of the species in this region [Egorov, 2018], can be used for subsequent monitoring of known colonies. In particular, such observations can answer the question of whether the phenotypic composition of the studied colonies remains relatively stable for a long time, maintaining the founder effect, or whether it will gradually change under the influence of selective and non-selective factors. Nakhabino can become especially indicative in this respect, since colonies with different phenotypic composition live here on a relatively small area and under similar conditions, separated by anthropogenic barriers.

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