1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 3, pp. 60–77
https://doi.org/10.21072/mbj.2021.06.3.07

UDC 594.3:591.157

SPECIFICATION OF POLYMORPHISM AND CLASSIFICATION OF SHELL COLORATION IN GASTROPODS BY THE EXAMPLE OF *LITTORINA OBTUSATA* (GASTROPODA: LITTORINIDAE)

© 2021 **E. V. Kozminsky**

Zoological Institute RAS, Saint Petersburg, Russian Federation
E-mail: *ekozminsky@gmail.com*

Received by the Editor 06.04.2021; after reviewing 20.06.2021;
accepted for publication 01.09.2021; published online 20.09.2021.

Understanding the patterns of microevolutionary processes involves a wide range of population genetic studies on different species. However, the number of genetically studied species is limited due to significant methodological difficulties in testing the genetic conditionality of various traits. Developing population phenetics may become an alternative, which allows considering a large number of new species subject to development of morphologically and genetically-based system to describe the variability and classification of phenotypic traits. Gastropods are a classic object for carrying out population genetic studies based on the analysis of polymorphism of shell coloration. The parametric classification system proposed by S. Sergievsky *et al.* (1995) for periwinkles of the genus *Littorina* may serve as a basis for the developing of a universal system for classifying color traits for that taxonomic group. Since a large amount of new data has been published in recent years, this system requires correction and revision. The study aims to revise the system, taking into account new material on the pigment composition, as well as on the peculiarities of the formation and inheritance of color traits, their joint occurrence, *etc.* A revised and modified classification system for shell coloration traits is presented by the example of the White Sea gastropods *L. obtusata*; this system considers the idea of the formation of a phenotype as a combination of several elementary traits. These are traits associated with the formation of: 1) a shell background color (the ability to include different pigments in the ostracum and the hypostracum color); 2) a pattern of spots (the presence of inclusions of white and/or brown pigment); and 3) wide longitudinal bands (brown, white, and orange). Elementary traits are highlighted taking into account the pigments involved, as well as the mechanisms of their formation and inheritance. When describing the shell coloration, elementary traits are first used to describe relatively simple traits (groups of phenes “Shell background color”, “Hypostracum color”, “Pattern of spots”, and “Wide longitudinal bands”), which are subsequently combined to describe the phenotype as a whole. Our study provides an overview of the available data on the qualitative composition of shell pigments in periwinkles and patterns of formation and inheritance of color traits; their possible combinations are described. The phenes isolated by us are described together with their occurrence, color specification in the RGB system, and the peculiarities of the formation, taking into account the distribution of pigments in the shell. Visible traits, that may be used to assess the distribution of pigments in the shell, are indicated. The ontogenetic changes in traits are described. Despite the fact that the proposed classification system is developed by the example of *L. obtusata*, it can be used for same purposes for other periwinkle species and, with some modifications, for a wide range of gastropod species.

Keywords: polymorphism, shell coloration, classification system, gastropods, *Littorina*

Understanding the patterns of microevolutionary processes involves carrying out a wide range of population genetic studies on different species. At the same time, the number of genetically studied species is limited due to significant difficulties in checking out the genetic conditionality of various traits: problems in keeping and breeding animals, long generational change, *etc.* Therefore, the patterns revealed may be insufficiently characteristic of all living nature. The way out lies in the development of population phenetics, which allows extending population genetic methods to species, whose direct genetic study is difficult or impossible (Yablokov, 1987 ; Yablokov & Larina, 1985). This approach should be based on the formation of reasonable systems for describing variability and classifying phenotypic traits.

Gastropods serve as a classical object for carrying out population genetic studies associated with shell coloration polymorphism (Ito & Konuma, 2020 ; Miura et al., 2007 ; Scheil et al., 2014). The emergence of a sufficiently flexible and well-grounded system for classifying formation and inheritance of shell coloration traits will make it possible to include in consideration a large number of new species. Despite the best knowledge of the genetics of pulmonates (Kozminsky, 2014 ; Backeljau et al., 2001), model objects for the development of such a classification system should be sought among the prosobranchias: more numerous and significantly more varied in color. Molluscs of the genus *Littorina*, which are characterized by high polymorphism (Reid, 1996) in shell coloration, are a promising model object; on its basis, an attempt can be made to create a classification system universal for gastropods.

Molluscs of the genus *Littorina* are a popular subject for numerous studies related to the variability of shell coloration (Estévez et al., 2020 ; Rolán-Alvarez et al., 2015 ; Sokolova & Berger, 2000). So far, several systems have been proposed to describe it. The first one – formulated in the early XX century – was a system for describing variability in *Littorina obtusata*, in which each stable combination of coloration traits (morph) had its own name (Dautzenberg & Fischer, 1915, cited from: Reid, 1996). With some modifications, this approach was used for a long time in population genetic studies of periwinkles (Reimchen, 1979 ; Sacchi, 1974). Its key disadvantage is the problems of studying the variability of elementary traits, that are part of a morph. The attempt to solve those problems by isolating additional morphs leads to the unnecessary bulkiness of the system of variability description (Reid, 1996). For this reason, Pettitt (1973) proposed the system of coloration trait classification in periwinkles, based on the imaginary system of genetic control, with six “loci” for description of elementary coloration traits: background, number and coloration of bands, pattern of spots, *etc.* A similar approach was used to describe variability in *L. obtusata* and formed the basis of the parametric system of coloration trait classification in *L. saxatilis* (Sergievsky et al., 1995). Since there were no data on the mechanisms of inheritance of the shell coloration traits in prosobranchias, Sergievsky *et al.* (1995) used the monogenic polyallelic inheritance pattern characteristic of pulmonates. Unfortunately, later studies (Kozminsky, 2014) showed that this assumption is not true. Among the advantages of this approach, due to which it can serve as a basis for the development of a universal classification system for gastropods, are the description of coloration variants as a combination of independent elementary traits and an attempt to consider the mechanisms of their inheritance. Moreover, the peculiarities of coloration traits formation were partly taken into account (Sergievsky et al., 1995).

Considerable time has passed since the emergence of the parametric system for describing variability in periwinkles, and a lot of new data has appeared. By the example of *L. obtusata*, it was shown that the background coloration in periwinkles is a result of the joint activity of several genetic systems, each of which is responsible for the incorporation of a certain pigment into the shell (Kozminsky, 2014).

The inheritance of bands and a pattern of spots on the periwinkle shell was studied (Kozminsky, 2011, 2016 ; Kozminskii et al., 2010). The concepts of the distribution of pigments in the shell and the formation of elementary coloration traits in periwinkles were substantially supplemented, and the composition of the pigments involved was clarified (Kozminskii & Lezin, 2007). Finally, the efficiency of using shell coloration traits when identifying periwinkle phenotypes was assessed; it was shown that for accurate identification, the peculiarities of the distribution of pigments in the shell thickness have to be considered (Lezin & Kozminskii, 2008).

The aim of this work is to revise the previously proposed classification system for shell coloration traits (Sergievsky et al., 1995) for molluscs of the genus *Littorina*, taking into account new data on the composition of pigments, peculiarities of their distribution in the shell, and inheritance of coloration traits.

RESULTS AND DISCUSSION

The work is based on the data obtained in the study of the White Sea molluscs *Littorina obtusata* (Linnaeus, 1758) and, in some cases, *Littorina saxatilis* (Olivi, 1792). The methods used and results obtained are described in detail in the publications of E. Kozminsky *et al.* (Kozminsky, 2011, 2014, 2016 ; Kozminskii & Lezin, 2006, 2007 ; Kozminsky et al., 2008 ; Kozminskii et al., 2010 ; Lezin & Kozminskii, 2008).

Pigments and coloration of shell areas. The coloration of the gastropod shell consists of a background coloration and a pattern of bands or spots (Sergievsky et al., 1995).

The shell areas of *L. obtusata* can be purple[1], orange, yellow, white, and depigmented. Various pigments are responsible for purple, orange, and yellow coloration. Purple pigment is insoluble in water and organic solvents; it remains in the sediment in the form of brownish flakes when the shell is dissolved by weak acids. Chemical stability and color allow suggesting that purple pigment is melanin. This pigment is widespread in animals (Britton, 1986) and is found in mollusc shells (Lucas, 1974 ; Williams, 2017 ; Williams et al., 2016). Yellow and orange colors are caused by pigments insoluble in water, but extractable with organic solvents: ethanol, chloroform, hexane, and toluene, as well as diethyl and petroleum ethers (Kozminskii & Lezin, 2007 ; Sergievsky et al., 1995). When carrying out paper chromatography, these pigments behave similarly to carotenoids (Zabelensky, Kozminsky, unpublished data). By their visible color, they can belong to both carotenoids and polyenes also found in mollusc shells (Délé-Dubois & Merlin, 1981 ; Hedegaard et al., 2006 ; Williams, 2017). Apparently, guanine is responsible for white coloration, which, strictly speaking, is not a pigment. White color caused by its presence is structural and associated with the reflection of light by ordered guanine microcrystals (Britton, 1986). As known, guanine is responsible for the formation of white coloration in many animals, *inter alia* molluscs (Britton, 1986 ; Lucas, 1974). The incorporation of guanine into the periwinkle shell can explain the formation of a denser than usual microstructure of white areas as well (Kozminskii & Lezin, 2007 ; Sergievsky et al., 1995). Discolored areas of the shell with normal microstructure result from true depigmentation.

Formation of the shell coloration. The major contributor to the formation of the periwinkle shell coloration is the irregular-prismatic layer (ostracum) (Kozminskii & Lezin, 2007). The background shell color can be formed due to the incorporation of one, two, and three pigments into the shell.

[1]It would be more correct to speak of brown. The latter, with a high pigmentation intensity, looks red-brown and even almost black and was designated by Sergievsky *et al.* (1995) as purple. In this work, the original designation is used.

Elementary traits associated with the formation of a background coloration are the ability to incorporate various pigments into the shell (melanin, two carotenoids) and the ability to incorporate guanine into the shell. Depending on which pigments are incorporated into the shell, different variants of the background color are formed (Fig. 1A–M): monochromatic (pure yellow, orange, and purple), dichromatic (two-layer yellow-purple, yellow-orange, orange-purple, and white-purple), and trichromatic ones (three-layer yellow-orange-purple, yellow-white-purple, and orange-white-purple). In yellow-purple molluscs, the coloration of the shell background depends on the ratio of yellow and purple pigments in the outer ostracum area (Fig. 1H–J). With a higher concentration of yellow pigment, the visible shell color is almost yellow; with its lower concentration, the shell looks brown. With an intermediate concentration of pigment, the shell is olive (greenish). It should be noted that the proper (layer-by-layer) distribution of pigments, which is characteristic of *L. obtusata*, is not necessary. In *L. saxatilis*, in the presence of the same elementary traits, the distribution of pigments in the shell is often chaotic. The pattern of spots is formed on the basis of white or purple pigments (Fig. 1O–R). Separate pattern elements are lenticular incorporations of pigment located in the upper ostracum area. The relative location of the pattern elements varies, as well as the degree of their fusion. The elementary trait highlighted at this stage is the presence of a pattern of white or purple spots or their absence. Considering high variability of the pattern of spots, further studies are required of the formation of this color trait and a highlight of additional elementary traits, *e. g.* the ability to form a zigzag pattern. Bands form as interlayers of brown, white, and orange pigments (Fig. 1S–U). As in the previous case, the elementary trait is the presence of bands of different colors or their absence. The hypostracum of the periwinkle shell can be purple or depigmented (Fig. 1N); elementary trait is colored or depigmented hypostracum.

No one has studied the formation of mollusc shell coloration at histological and biochemical levels. As known, dorsal and ventral glands, as well as the cells of the dorsal epithelium of the mantle margin, are involved in the formation of the periwinkle shell (Bevelander & Nakahara, 1970). The ventral and dorsal glands are responsible for the periostracum formation. The ostracum and hypostracum formation involves the cells of the dorsal epithelium, which are formed in the proliferation zone located at the mantle margin and are gradually displaced in the dorsal direction. It stands to reason that during this displacement, the cells of the dorsal epithelium at certain points in time ensure the synthesis of different pigments and their incorporation into the shell. Moments of switching modes of functional activity of pigment-forming cells are obviously genetically determined. As a result, there are an ordered incorporation of pigments into the shell and a formation of various coloration elements.

When a monochromatic background color is formed, the cells of the dorsal epithelium of the mantle margin synthesize the same pigment. As these cells are displaced from the proliferation zone, their functional activity may decrease. In this case, a pigmentation gradient is formed, with a decrease in intensity from the outer to the inner ostracum area. If this does not occur, the prismatic layer is colored evenly. With the formation of di- and trichromatic variants of the background color, the cells of the dorsal epithelium synthesize one kind of pigment. Then, a synchronous switching of their functional activity occurs from the formation of one pigment to the formation of another. Since the boundaries between areas of different pigmentation are blurred, it can be concluded that functional activity of pigment-forming cells changes smoothly. Apparently, we can talk about a certain pattern in the change in the modes of functional activity of pigment-forming cells: first, yellow and orange pigments and guanine are synthesized; only then, purple pigment is synthesized.

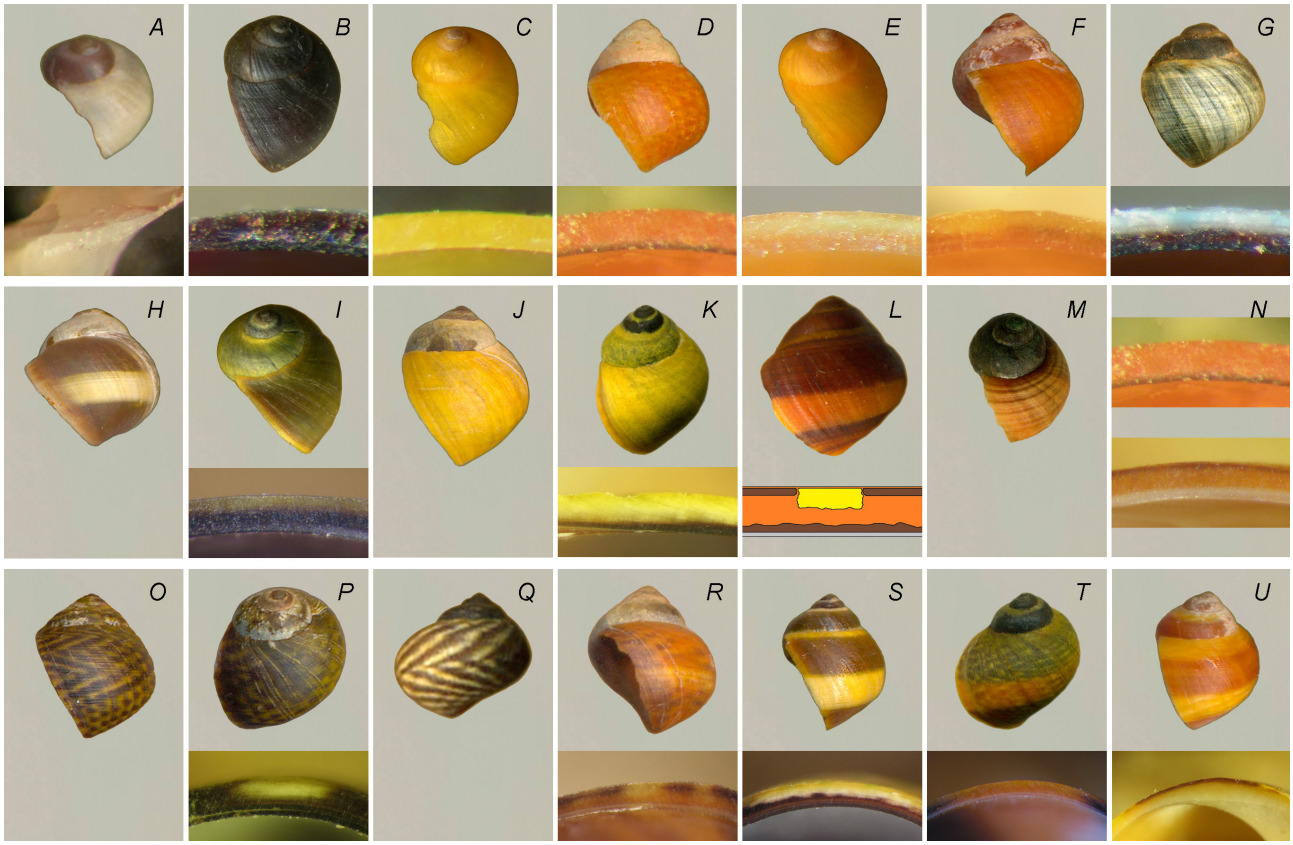

Fig. 1. Variants of the shell background color and the pattern of spots and bands in *Littorina obtusata*. Shell background color: A – depigmented; B – purple; C – yellow; D – orange; E – yellow-orange; F – orange-purple; G – white-purple; H–J – variants of yellow-purple shell background color; K – yellow-white-purple; L – yellow-orange-purple; M – orange-white-purple (by the example of *L. saxatilis*). Hypostracum: N – purple and depigmented. Pattern: of white (O–Q) and purple (R) spots. Wide longitudinal bands: S – white; T – orange; U – brown. The general view of the shells (top panel) and the distribution of pigments in the shell (bottom panel) are presented. This illustrative material is freely available on the website of the Zoological Institute RAS, Saint Petersburg: http://www.zin.ru/projects/litphen/index.html

With the formation of brown (= purple), white, and orange bands, the cells of the dorsal epithelium, located in certain areas of the mantle margin, switch at the appropriate time to the formation of the pigment of the bands. Interestingly, the system sometimes fails: the formation of the background coloration stops, but the synthesis of the pigment of the bands does not occur. In this case, depigmented bands are formed, which we noted in yellow *L. obtusata*; these are homologous to the *hyalozonata* phenotype of other molluscs (Cook, 1967 ; Cook & King, 1966). When a pattern of spots is formed, a change in functional activity of pigment-forming cells in certain areas of the mantle margin occurs periodically. The linear dimensions of such areas are much smaller, and their number is much larger than in the case of bands. As a result, a pattern of pigment spots is formed, the shape and relative position of which vary (Fig. 1O–R).

Switching of the mantle margin cells from the formation of an irregular-prismatic layer to the formation of a lamellar layer is associated, apparently, with a decrease in the number of possible variants of functional activity of pigment-forming cells. In the ostracum formation zone, cells can synthesize purple, orange, and yellow pigments and guanine; in the hypostracum formation zone, purple pigment alone (Fig. 1N).

Possible variants and combinations of color traits. In the series of our research, most of the theoretically possible variants of the background coloration were revealed, which can be formed on the basis of three pigments and guanine. Individuals with depigmented shell were obtained under experimental conditions. *In vivo*, three out of four possible monochromatic color variants (purple, yellow, and orange) were found; all the possible dichromatic color variants involving purple, yellow, and orange pigments and guanine; and three out of four possible trichromatic color variants. At the same time, no molluscs were revealed with monochromatic white, dichromatic yellow- or orange-white, and trichromatic yellow-white-orange background coloration; obviously, white pigment is found only in conjunction with purple one.

White bands were registered in periwinkles with the most varied background colors. Brown bands are characteristic of molluscs, which have a background coloration with orange pigment. Less commonly, they can be found in periwinkles with a background color formed with yellow pigment (yellow and yellow-purple molluscs) or – only under experimental conditions – in depigmented individuals. In *L. saxatilis*, brown bands are found in white-purple individuals alone. Orange bands are recorded mainly in molluscs with yellow-purple background color; occasionally, with pure purple one. Bands of different types can occur simultaneously. Individuals with brown and white bands on the shell are quite common. Periwinkles bearing both white and orange bands were found as well; however, this color variant is very rare. This is probably due to the fact that white and orange bands are formed on the same shell area and "interfere" with each other.

A pattern of white spots blends freely with all the background colors and bands. At the same time, a pattern of purple spots is associated with the presence of orange pigment in the background coloration and often with the presence of brown bands. Both pattern variants can occur at the same time.

Inheritance of shell coloration traits. The information on the inheritance of shell coloration traits in periwinkles is limited. The data of Reimchen (Reimchen, 1974, cited from: Reid, 1996) testify to the hereditary nature of some color variants. In the work (Kozminsky et al., 1995), an attempt was made to explain the inheritance of the background shell coloration in periwinkles based on a single-locus polyallelic inheritance pattern, which is characteristic of pulmonates (Murray, 1975). Unfortunately, in the publication mentioned, little attention was paid to the fact that several different pigments are involved in the formation of the periwinkle shell coloration, and the possibility of multiple paternity was not considered, which doubts the conclusions drawn. In later works (Kozminsky, 2011, 2014, 2016 ; Kozminskii et al., 2010), those shortcomings were eliminated; the results obtained form the basis of the following scheme of inheritance of shell coloration traits in periwinkles (Table 1).

With the formation of the background color in *L. obtusata*, three different groups of genes are responsible for the incorporation of purple, yellow, and orange pigments into the shell (Kozminsky, 2014). The incorporation of pigments into the shell is dominant. In the case of purple and yellow pigments, each group incorporates at least two complementary interacting genes. We have not studied the inheritance of white coloration in *L. obtusata* due to the rarity of the corresponding phenotypes in the White Sea populations[2]. By analogy with pigments, it stands to reason that a separate genetic system is responsible for the incorporation of guanine into the shell as well (it is shown in the table as a separate biallelic gene). In general, the background color of the periwinkle shell is formed as a result of the interaction of several groups of genes, with each being responsible for a certain pigment (synthesis, transport, and incorporation into the shell).

[2]The corresponding study was carried out by the example of *L. saxatilis*.

Table 1. Inheritance of the shell coloration traits in *Littorina obtusata*

Trait	Gene	Alleles	Genotype / phenotype accordance
Background coloration (see Kozminsky, 2014)			
Purple pigment participates / does not participate in the coloration formation	P_1	P_1 p_1	P_1–P_2– – the pigment is incorporated into the shell (C^P, C^{YP}, C^{WP}, C^{OP}, C^{YOP}, C^{YWP}, C^{OWP})
	P_2	P_2 p_2	$p_1p_1P_2$–; P_1–p_2p_2; $p_1p_1p_2p_2$ – no pigment incorporation into the shell
White pigment participates / does not participate in the coloration formation	W	W w	W– – the pigment is incorporated into the shell (C^{WP}, C^{YWP}, C^{OWP}) ww – no pigment incorporation into the shell
Yellow pigment participates / does not participate in the coloration formation	Y_1	Y_1 y_1	Y_1–Y_2– – the pigment is incorporated into the shell (C^Y, C^{YP}, C^{YO}, C^{YOP}, C^{YWP})
	Y_2	Y_2 y_2	$y_1y_1Y_2$–; Y_1–y_2y_2; $y_1y_1y_2y_2$ – no pigment incorporation into the shell
Orange pigment participates / does not participate in the coloration formation	O	O o	O– – the pigment is incorporated into the shell (C^O, C^{YO}, C^{OP}, C^{YOP}, C^{OWP}) oo – no pigment incorporation into the shell
Bands (see Kozminsky, 2011, 2016 ; Kozminsky, unpublished data)			
Presence/absence of brown bands	B^p	B^p b^p	B^p– – brown bands are formed (B^P) b^pb^p – no brown bands
Presence/absence of white bands	B^w	B^w b^w	B^w– – white bands are formed (B^W) b^wb^w– – no white bands
Presence/absence of orange band	B^o	B^o b^o	B^o – no orange band (B^O) b^ob^o – orange band is formed
Pattern of white spots (see Kozminskii et al., 2010)			
Presence/absence of the pattern	S_1^w	S_1^w s_1^w	S_1^w– S_2^w– – the pattern of spots is formed (S^W)
	S_2^w	S_2^w s_2^w	s_1^w s_1^w S_2^w–; S_1^w– s_2^w s_2^w; s_1^w s_1^w s_2^w s_2^w – no pattern

Note: designations of phenotypes and further explanations are given in the text; pairs of complementary genes are shown in blue color, and pairs of linked genes, in red color.

One gene with two alleles is responsible for the formation of brown bands in periwinkles (Kozminsky, 2011). The inheritance of white (Kozminsky, 2016) and orange (Kozminsky, unpublished data) bands is also consistent with the hypothesis of a monogenic inheritance pattern. While the presence of brown and white bands is dominant, the presence of orange bands is most likely a recessive trait.

At least two complementary genes are responsible for the presence of a pattern of white pigment spots on the shell (Kozminskii et al., 2010). The high variability of the shape, relative position, and degree of fusion of the pattern elements (Fig. 1O–Q) suggests that the number of genes associated with its formation can be much larger. It is also possible that the manifestation of this trait may depend on environmental factors.

Analysis of the available data indicates a linkage of the loci responsible for the incorporation of orange pigment (*O*) into the shell and the formation of brown bands (B^{p}). Within the framework of the corresponding hypothesis, the formation of four types of gametes (OB^{p+}, OB^{p-}, oB^{p+}, oB^{p-}) is possible, on the basis of which 10 unique genotypes can be formed. The corresponding phenotypes of individuals can be combined into four groups ("orange banded", "non-orange banded", "orange bandless", and "non-orange bandless"), and each of them should be characterized by its own peculiarities of joint inheritance of background color and bands. In the experiment, in the offspring of orange banded females, banded individuals with orange background color alone split off. In the offspring of yellow banded females, banded individuals had yellow background coloration as well. In the offspring of orange bandless females, only orange bandless individuals were recorded. In the offspring of females of the fourth group, banded individuals (if present) always had orange background color; the corresponding alleles were obtained, apparently, from fathers.

It should be noted as follows: in the cases studied by us, in the offspring of yellow banded females, individuals with bands had yellow background color alone. At first glance, this indicates the emergence of a new linkage pair resulting from a crossing over and the fact that alleles of one gene are responsible for the formation of yellow and orange coloration, which contradicts the data on the inheritance of the background color. However, this is not the case. Let the genes, responsible for the inheritance of orange pigment and bands, and the genes, responsible for the formation of yellow and purple background colors, be in different chromosomes. Then, the crossover gametes oB^{p+} can freely combine with all kinds of gametes, that give different variants of the background color with the participation of yellow and purple pigments (yellow, purple, yellow-purple, and depigmented; we can neglect gametes giving white-purple background color, since this variant is rare in *L. obtusata*). In purple molluscs, the bands will be invisible. We recorded depigmented individuals with brown bands in the experiment (under natural conditions, depigmented individuals most likely do not survive). In reality, only combinations of oB^{p+} gametes with variants of yellow background color (yellow-purple and yellow) will be observed, since they are the most abundant in the population, giving rise to the sensation of apparent linkage of yellow color with brown bands.

There are no data on the inheritance of the pattern of purple pigment spots and hypostracum coloration. A pattern of purple pigment spots is found only in individuals with a background color with the participation of orange pigment, both banded and bandless. It can be both a result of the activity of a separate gene, closely linked to the gene for brown bands, and a by-product of the activity of a "banded" gene. The formation of hypostracum is associated with a change in the mode of functional activity of the mantle margin cells; at the same time, a change in the programs of synthesis

and incorporation of pigments into the shell can occur (Fig. 1N). Therefore, it stands to reason that a separate gene (or a group of genes) is responsible for the hypostracum color. It has to be noted that a separate genetic system is probably also responsible for the embryonic coloration of the shell, since the embryonic shell in periwinkles is always purple, even in depigmented individuals.

System for describing variability of shell coloration traits in *Littorina obtusata*

Group of phenes "Shell background coloration" – C.

C^D – "Depigmented" (Fig. 1A). A rare coloration variant: two individuals studied were obtained under laboratory conditions when analyzing the inheritance of shell coloration traits. The shell is depigmented, partially translucent, dull white, and horny in color (RGB[3]: $197\pm_{13}$: $180\pm_{12}$: $146\pm_{14}$). Border and underside are not expressed; shell microstructure is common. The hypostracum is depigmented. The embryonic shell contains a small amount of purple pigment. This phenotype can be confused with very old yellow periwinkles, in which the incorporation of pigment into the shell may have stopped.

C^P – "Purple" (Fig. 1B). One of the most common phenotypes in the White Sea populations (about 40 %). The visible shell color is dark brown, almost black ($77\pm_{19}$: $62\pm_{15}$: $56\pm_{18}$); border and underside are not expressed. The coloration intensity varies slightly. The prismatic layer is colored with purple pigment. In one part of individuals, the ostracum color is even; in other part, a gradient is observed, with a decrease in the pigmentation intensity from the outer to inner shell area (in some cases, the inner ostracum area is practically depigmented). Shell microstructure is common. The hypostracum may be purple or depigmented.

C^Y – "Yellow" (Fig. 1C). Quite a rare phenotype (0.3 %). The visible color is yellow ($195\pm_{32}$: $155\pm_{31}$: $22\pm_{23}$); the intensity varies relatively little. Border and underside are not expressed. The ostracum is colored with yellow pigment; microstructure is common. The coloration of the prismatic layer can be even, or there can be a gradient, with a decrease in the pigmentation intensity from the outer to inner shell area. The hypostracum is usually depigmented, occasionally purple.

C^O – "Orange" (Fig. 1D). Common phenotype (2 %). Visible color is orange, with varying intensity ($179\pm_{27}$: $96\pm_{12}$: $21\pm_{13}$); border and underside are not expressed. The irregular-prismatic layer is colored with orange pigment; microstructure is common. A gradient is possible, with a decrease in the pigmentation intensity from the outer to inner shell area. The hypostracum can be depigmented or colored with purple pigment (the latter variant is common in *L. saxatilis*). The shell may have a pattern of purple spots, which makes the visible coloration darker. On the contrary, a pattern of white spots makes the coloration lighter.

C^{YO} – "Yellow-orange" (Fig. 1E). Quite a rare phenotype (0.3 %). The visible shell color is yellow-orange ($176\pm_{31}$: $119\pm_{28}$: $9\pm_{7}$). There is no expressed border; underside has the same color as other shell areas. Shell microstructure is common. In some individuals, the outer area of the prismatic layer is colored predominantly with yellow pigment, and the inner area is predominantly orange; in most individuals, the pigments are blended. The color of yellow-orange molluscs varies from almost orange to almost yellow, and this can cause problems in identifying the phenotype (Lezin & Kozminskii, 2008). The presence of a white checker lightens the coloration and can also cause identification problems. The hypostracum is usually colorless.

[3]Color coordinates characterizing the shell coloration elements according to the RGB color model (Kozminskii & Lezin, 2006).

C^{OP} – "Orange-purple" (Fig. 1F). Quite a rare phenotype (0.5 %). The visible shell color is orange ($176\pm_{16}$: $93\pm_{16}$: $38\pm_{18}$); it seems darker due to purple coloration of the inner ostracum area and often incorporation of purple pigment in the form of spots. There is an orange border along the mouth edge. Shell underside is darker and may have a distinct purple tint. The outer area of the prismatic layer is colored with orange pigment, and the inner one is purple. Typically, purple sublayer is very thin. Phenotype identification is difficult, since the incorporation of orange pigment into the shell seems to inhibit the incorporation of purple one, and only a thin, inconspicuous purple sublayer retains at the border with the hypostracum. The latter is colorless or colored with purple pigment.

C^{WP} – "White-purple" (Fig. 1G). A relatively rare coloration variant in the White Sea *L. obtusata* (0.5 %). Unlike the color of depigmented individuals, white color is saturated, shiny ($206\pm_{17}$: $181\pm_{16}$: $111\pm_{24}$). The shell is dense, opaque. Border along the mouth edge is white; shell underside is purple. The outer area of the prismatic layer is colored white, and the inner area is purple. The thickness of a white sublayer varies; accordingly, several variants of white-purple background color, with different intensity, can be distinguished. On thin sections and chips, the shell in the white sublayer area has a denser than usual microstructure with a bluish tint. The hypostracum is always colored with purple pigment.

C^{YP} – "Yellow-purple" (Fig. 1H–J). The most common variant of the background coloration in the White Sea populations (55 %). The visible coloration varies from brown ($116\pm_{8}$: $88\pm_{9}$: $60\pm_{8}$) to deep yellow ($219\pm_{28}$: $181\pm_{17}$: $61\pm_{12}$), but most individuals have olive shell coloration ($123\pm_{25}$: $101\pm_{23}$: $51\pm_{23}$), with yellow border along the mouth edge and purple underside. The outer ostracum area is colored with yellow pigment, the inner one is purple. Shell microstructure is common. Coloration depends on the thickness of yellow pigment sublayer and its pigmentation intensity. In brown individuals, trace amounts of yellow pigment are incorporated into the shell; the outer ostracum area contains traces of purple pigment and appears to be discolored[4]. Olive individuals have a relatively high amount of yellow pigment; when overlaid with traces of purple pigment, a visible greenish coloration is formed in the outer ostracum area. In individuals with deep yellow color, the yellow sublayer is very thick, and the pigmentation intensity is very high; purple pigment is noticeable only in the lower area of the prismatic layer and in the hypostracum. Brown molluscs have a colorless border along the mouth edge; olive and yellow molluscs have a yellow one. The hypostracum is usually purple.

C^{YWP} – "Yellow-white-purple" (Fig. 1K). A very rare variant of the background coloration (0.01 %). The visible color varies from white-purple with a slight admixture of yellow pigment to lemon one ($209\pm_{34}$: $179\pm_{28}$: $33\pm_{14}$). Border along the mouth edge is white or yellowish; underside is purple. The distribution of white and purple pigments corresponds to that of white-purple individuals. White sublayer has a denser microstructure. In the outer ostracum area, a distinct admixture of yellow pigment is noticeable. The hypostracum is purple.

C^{YOP} – "Yellow-orange-purple" (Fig. 1L). Apparently, a rather rare phenotype (0.5 %), but its identification is difficult, since purple sublayer is usually very poorly expressed. The visible color of the shell ($173\pm_{30}$: $116\pm_{29}$: $16\pm_{10}$) corresponds to that of yellow-orange molluscs (if the purple sublayer is weakly expressed) or is slightly darker (if the purple sublayer is expressed enough). In the latter case, a yellow-orange border along the mouth edge and dark shell underside become noticeable. Shell microstructure is common. This variant of the background coloration is characteristic of periwinkles with brown bands.

[4]Apparently, this is due to the fact that the activity of the gene (or genes) providing the incorporation of yellow pigment into the shell simultaneously inhibits the incorporation of purple pigment into the shell.

Interestingly, in this case, a fairly clear separation of yellow and orange pigments is observed as well: the outer area of the prismatic layer is distinctly yellow outside the brown bands, and the middle area of the prismatic layer is orange; at the border with the hypostracum, the area is purple. The hypostracum can be colorless or colored with purple pigment.

C^{OWP} – "Orange-white-purple" (Fig. 1M). This variant of the background color was found in *L. saxatilis* alone (0.01 %). The detection of similar *L. obtusata* is possible as well, but their occurrence should be very low, since white-purple *L. obtusata* are extremely rare. The visible coloration ($220\pm_{21}$: $148\pm_{25}$: $40\pm_{36}$) varies from white-purple with streaks of orange pigment to reddish-cream. Border is white; shell underside is purple. The distribution of guanine and purple pigment corresponds to that of white-purple individuals; in the outer ostracum area, there is an admixture of orange pigment. The prismatic layer at the location of a white sublayer has a denser than usual microstructure with a bluish tint. The hypostracum is purple.

Group of phenes "Hypostracum coloration" – H.

The hypostracum of the periwinkle shell (Fig. 1N) can be purple (H^P; 66 %) or colorless (H^D; 34 %). The hypostracum discoloration is not associated with the formation of a denser microstructure, which is characteristic of white-colored shell areas. The visible coloration of the hypostracum is affected by the color of the irregular-prismatic layer: for example, in molluscs with yellow and orange ostracum coloration, the colorless hypostracum seems yellowish and orangey. The effect of the hypostracum color on the shell background color is small: in yellow and orange periwinkles, it makes the visible shell coloration darker. No unambiguous relationship was found between the colors of the hypostracum and ostracum. If the color of the prismatic layer is yellow or orange, the hypostracum is usually colorless; if it is purple, the hypostracum is colored purple.

Group of phenes "Pattern of spots" – S.

S^W – "Pattern of white spots" (Fig. 1O–Q). Most periwinkles (55 %) are characterized by the presence of a pattern of white spots on the shell ($198\pm_{31}$: $158\pm_{32}$: $90\pm_{31}$). Separate elements of the spotted pattern are lenticular incorporations of white pigment interconnected by thin layers-anastomoses. The pattern elements are located in the upper area of the prismatic layer. At the location of the pattern elements, the shell has a denser microstructure than in other areas. The shape, relative location, and degree of fusion of the spotted pattern elements are very variable. The traits are found in molluscs with a wide variety of phenotypes. No relationship was found between the presence of a pattern of white spots, bands, and shell background coloration. In periwinkles with yellow and orange shell background color, spots are yellowish. A pattern of white spots is not formed at the location of brown bands, but may be formed at the location of an orange band.

S^P – "Pattern of purple spots" (Fig. 1R). As in the case of a pattern of white spots, separate elements are lenticular incorporations of purple pigment interconnected by thin layers-anastomoses ($125\pm_{43}$: $66\pm_{27}$: $22\pm_{29}$). The pattern elements are located at the top of the ostracum. Microstructure of the irregular-prismatic layer at the location of the pattern elements is common. The trait is typical for periwinkles, which have a background color with the participation of orange pigment and brown bands on the shell. It is found in orange and orange-purple bandless molluscs as well (0.4 %). It appears much later than brown bands and is noticeable only on the last whorls of the shell.

S_o – "No pattern of spots".

Group of phenes "Bands" – B.

B^W – "White bands" (Fig. 1S). A common color variant (2 %). The shell has one or two wide white bands ($232\pm_{20}$: $203\pm_{21}$: $110\pm_{42}$). The main band is located along the whorl periphery; the narrower one, in the suture area of the shell. The bands are layers of additional pigment located in the outer area of the irregular-prismatic layer. At the location of the bands, the shell has a denser microstructure than in other areas. The trait is found in periwinkles with yellow, orange, purple, and yellow-purple background coloration. Since the bands are immersed in the ostracum, their visible color may differ from pure white: in periwinkles with yellow-purple and yellow ostracum, it is yellowish; in orange and orange-purple molluscs, it is orangish. The variability of the trait manifests itself in a change in the pigmentation intensity of the bands (down to complete depigmentation) and in narrowing or complete disappearance of the additional band. In the case of complete depigmentation, in molluscs with a yellow background coloration of the shell, band silhouettes are visible in the lumen in the spots of their usual location.

B^O – "Orange bands" (Fig. 1T). A relatively rare color variant (0.8 %). The shell has one wide orange band ($155\pm_{44}$: $95\pm_{30}$: $29\pm_{24}$) at the whorl periphery. The bands are interlayers of orange pigment with well-defined edges. The location of the bands in the shell thickness is characterized by expressed ontogenetic variability. At the time of their appearance (in the first year of life of a mollusc), those are located in the inner ostracum area; subsequently, they spread to almost the entire ostracum and disappear in the fourth-fifth year shifting to the hypostracum. At the location of the bands, shell microstructure is common. Orange bands are characteristic of molluscs with a yellow-purple shell; sometimes, the trait was observed in individuals with a purple background coloration.

B^P – "Brown (purple) bands" (Fig. 1U). A rare color variant (0.2 %). The shell has two wide bands of different shades of brown, sometimes almost black ($81\pm_{35}$: $50\pm_{23}$: $23\pm_{20}$), located in the upper and lower whorl areas. The bands can either be organized as interlayers of purple pigment in the upper ostracum area, below which a more or less expressed pigment "trail" extends, or occupy the entire shell thickness. At the location of the bands, shell microstructure is common. Brown bands are usually found in *L. obtusata* with a background coloration with orange pigment. Less commonly, this trait is observed in yellow and yellow-purple molluscs; in rare cases, in periwinkles with a depigmented shell[5]. The variability of the trait manifests itself in a change in the coloration intensity of the bands (down to complete depigmentation) and their width, in reduction of separate bands, and in fusion and distribution of bands over the entire shell surface or its part. In yellow-orange periwinkles, depigmentation of the bands results in a color variant when the individuals look like yellow molluscs with two bands. A pattern of spots of white or purple pigment, if present, is always formed outside the area of the bands.

B_o – "No bands".

Differences between two classification systems. The main difference from the previous version of the classification system for coloration traits (Sergievsky et al., 1995) is the correspondence between selected phenotypes and actually existing groups of genes responsible for the formation of various elementary traits. The pre-existing groups of phenes "white color" and "pigment coloration of ostracum" are combined into one group "background shell coloration", since the background color arises as a result of the interaction of a number of substances, some of which (melanin and carotenoids) are pigments, and some (guanine) serve as the basis for the formation of structural color. Quantitative gradations of traits – similar to the previously identified variants W_1–W_3 – are not specially highlighted in the classification system proposed by us. However, they can be useful; if necessary, the corresponding numeric indices can be easily entered into the model.

[5]In *L. saxatilis*, the trait is always associated with white-purple background coloration.

In the group of phenes “bands”, several new phenes were identified, since the results of morphological and genetic studies showed that brown, white, and orange bands are independent structures, which differ in terms of organization, ontogenesis, and inheritance. The “bandless” phenotype is now common to all banded variants, meaning that the genetic systems responsible for the formation of three band types are not active.

The group of phenes associated with the formation of a spotted pattern underwent relatively small changes. However, the data on genetics (Kozminskii et al., 2010) show that the mechanism of inheritance of the trait is more complicated than previously assumed: at least two genes are responsible for the presence/absence of a pattern of white spots. The extremely high variability of the trait allows suggesting that its formation is affected by a much larger number of genes. The emergence of new data on genetics will require clarification or even a significant revision of this group of phenes, with the involvement of new elementary traits. Moreover, this group includes now the previously undescribed “purple-spotted” phenotype. It should be emphasized as follows: although the mechanisms of formation of two variants of the pattern are similar, different genes are most likely responsible for their inheritance.

Conclusion. Despite the widespread use of molecular biological methods, the studies on population and phenetics are still relevant: in particular, when it comes to the investigation of microevolutionary processes directly related to coloration, *e. g.* when the latter performs a protective or thermoregulatory function. A prerequisite for such studies is knowledge of the mechanisms of formation and inheritance of coloration traits. The involvement into the analysis of new species, the genetic study of which is difficult for some reasons, is possible only on the basis of generalization of the available information on the species investigated morphologically and genetically, *i. e.* by methods of population phenetics. In carrying out that kind of research, molecular biological markers rather perform an auxiliary function, since our ideas about the molecular genetic mechanisms underlying the formation and inheritance of complex traits (such as color) are still insufficient to fully replace population-genetic and phenetic methods with molecular biological ones. Moreover, given the high diversity of pigments involved in the formation of shell coloration (Comfort, 1950, 1951 ; Hedegaard et al., 2006 ; Lucas, 1974 ; Williams, 2017), it is hardly possible to speak of the existence of a single “model” of its formation and inheritance. To identify common patterns, it is necessary to use methods of population phenetics and to carry out their joint analysis with molecular biological data as well.

A key prerequisite for the development of the population-phenetic direction of studies is the formation of reasonable systems for description and classification of coloration traits. The system of classification of coloration traits in molluscs of the genus *Littorina* proposed by us can serve as a good basis for the development of such systems.

As noted above, the variety of pigments involved in the formation of shell color in molluscs is great (Comfort, 1950, 1951 ; Hedegaard et al., 2006 ; Lucas, 1974 ; Williams, 2017). Like in periwinkles, in many prosobranchias, the background shell color is formed with the participation of several pigments. It is doubtful whether the incorporation of different pigments into the shell was controlled by a single gene. The implementation of a polygenic inheritance scheme seems to be much more probable (Kozminsky, 2014), with a separate (and, possibly, polyallelic) gene responsible for the incorporation of each pigment into the shell. The monogenic polyallelic inheritance pattern, which is characteristic of pulmonates (Backeljau et al., 2001), is apparently a special case of this more general and universal inheritance pattern. Therefore, any system of classification of coloration traits that claims to be universal should be based on a polygenic scheme for the formation and inheritance of background coloration.

The coloration of the shell of gastropods can be represented as a combination of a number of elementary traits. The elementary traits identified in periwinkles are common in molluscs. Each color trait corresponds to a certain distribution of pigments in the shell, which is a consequence of a regular change in functional activity of pigment-forming cells of the mantle margin (Sergievsky et al., 1995). The peculiarities of the distribution of pigments found in periwinkles with the formation of the background color, bands, and pattern of spots are quite common for molluscs.

Taking into account the variety of shell coloration of gastropods, it is obvious that the elementary traits of coloration in periwinkles and corresponding variants of functional activity of chromatophores do not exhaust all the possible diversity. Specifically, in order to describe a high variety of modes of the pattern of spots on the shell, it is necessary to highlight additional elementary traits and corresponding modes of functional activity of pigment-forming cells, as well as to study the mechanisms of their inheritance. Other possible areas of research are the study of ontogenetic changes in coloration traits and the analysis of the relationship between possible modes of functional activity of pigment-forming cells and formation of different calcium layers of the shell. The introduction of new elementary color traits into consideration will significantly expand the capabilities of the classification system proposed by us.

The above-described classification system for coloration traits has been successfully tested in the study of the inheritance of shell coloration traits in periwinkles (Kozminsky, 2011, 2014, 2016 ; Kozminskii et al., 2010) and the distribution of pigments in the mollusc shell (Kozminskii & Lezin, 2007 ; Lezin & Kozminskii, 2008), as well as in carrying out population biological monitoring in the Kandalaksha State Nature Reserve in 2005–2019 (Kozminsky, 2006, 2020). Due to the use of universal principles of classification of coloration elements (analysis of the diversity of pigments, isolation of elementary traits, and study of the mechanisms of their formation and inheritance), the system developed can be used to describe the shell color not only in periwinkles, but also in the widest range of gastropods.

This work was carried out with partial financial support from the Russian Foundation for Basic Research (project No. 05-04-48056), the Basic Research Programs of the Russian Academy of Sciences "Dynamics of the gene pools of plants, animals, and humans" and "Biological diversity: Inventory, functions, and conservation", and state research assignment of the Zoological Institute RAS No. AAAA-A19-119022690122-5.

Acknowledgement. I am deeply grateful to P. Lezin and M. Fokin for their help in studying the inheritance of shell coloration traits and to P. Lezin for help in analyzing the formation of coloration traits and carrying out color measurements. I am sincerely grateful to prof. S. Zabelensky for his help in identifying the pigments in periwinkle shell. I am deeply grateful to the administration of the White Sea Biological Station of the Zoological Institute RAS, in particular to V. Berger and A. Sukhotin, for their support in the research. I am grateful to Yu. Kurzikov for invaluable technical assistance and to other persons who provided their assistance and support in the research.

REFERENCES

1. Britton G. *The Biochemistry of Natural Pigments.* Moscow : Mir, 1986, 422 p. (in Russ.)
2. Kozminskii E. V. Conditions of *Littorina saxatilis* (Olivi, 1792) and *L. obtusata* (Linnaeus, 1758) populations and structure of parasite invasions in these species in Yuznaya inlet of Ryashkov Island in 2006. In: *The Chronicle of Nature by the Kandalaksha Reserve for 2005.* Vol. 2 / A. S. Koryakin (Ed.). Kandalaksha, 2006, pp. 100–114. (in Russ.)

3. Kozminsky E. V. Inheritance of longitudinal shell bands in the snails *Littorina obtusata* and *Littorina saxatilis* (Gastropoda, Prosobranchia). *Russian Journal of Genetics*, 2011, vol. 47, no. 8, pp. 987–993. (in Russ.). https://doi.org/10.1134/S1022795416080068
4. Kozminsky E. V. Inheritance of the background shell color in the snails *Littorina obtusata* (Gastropoda, Littorinidae). *Russian Journal of Genetics*, 2014, vol. 50, no. 10, pp. 1038–1047. (in Russ.). https://doi.org10.7868/S0016675814100051
5. Kozminsky E. V. Inheritance of longitudinal white stripes on the shell of mollusks *Littorina obtusata* (Gastropoda, Prosobranchia). *Russian Journal of Genetics*, 2016, vol. 52, no. 8, pp. 882–886. (in Russ.). https://doi.org/10.7868/S0016675816080063
6. Kozminsky E. V. Conditions of *Littorina saxatilis* (Olivi, 1792) and *L. obtusata* (Linnaeus, 1758) populations and structure of parasite invasions in these species in Yuznaya inlet of Ryashkov Island in 2017 and 2018. In: *The Chronicle of Nature by the Kandalaksha Reserve for 2019*. Vol. 2 / E. L. Tolmacheva (Ed.). Kandalaksha, 2020, pp. 62–79. (in Russ.)
7. Kozminskii E. V., Lezin P. A. Method of color measurements of the elements of color in gastropod shell. *Russian Journal of Marine Biology*, 2006, vol. 32, no. 5, pp. 316–318. (in Russ.). https://doi.org/10.1134/S1063074006050105
8. Kozminsky E. V., Lezin P. A. Distribution of pigments in the shell of the gastropod *Littorina obtusata* (Linnaeus, 1758). *Russian Journal of Marine Biology*, 2007, vol. 33, no. 4, pp. 238–244. (in Russ.). https://doi.org/10.1134/S1063074007040062
9. Kozminsky E. V., Granovitch A. I., Sergievsky S. O. Inheritance of shell colour morphs of *Littorina saxatilis* (Olivi) (Gastropoda: Prosobranchia). *Trudy Zoologicheskogo instituta*, 1995, vol. 264, pp. 19–34. (in Russ.)
10. Kozminsky E. V., Lezin P. A., Fokin M. V. A methodology of studying the inheritance of shell color in mollusks of the genus *Littorina* (Gastropoda, Prosobranchia). *Zoologicheskii zhurnal*, 2008, vol. 87, no. 5, pp. 614–619. (in Russ.)
11. Kozminskii E. V., Lezin P. A., Fokin M. V. A study of inheritance of white spots on the shell of *Littorina obtusata* (Gastropoda, Prosobranchia). *Russian Journal of Genetics*, 2010, vol. 46, no. 12, pp. 1455–1461. (in Russ.). https://doi.org/10.1134/S1022795410120082
12. Lezin P. A., Kozminskii E. V. Color characteristics of *Littorina obtusata* (Gastropoda, Prosobranchia) shells and their use for distinguishing its phenotypic groups. *Zoologicheskii zhurnal*, 2008, vol. 87, no. 1, pp. 29–35. (in Russ.)
13. Sergievsky S. O., Granovitch A. I., Kozminsky E. V. Shell colour polymorphism of *Littorina saxatilis* (Olivi): Classification principles. *Trudy Zoologicheskogo instituta*, 1995, vol. 264, pp. 3–18. (in Russ.)
14. Yablokov A. V. *Populyatsionnaya biologiya.* Moscow : Vysshaya shkola, 1987, 303 p. (in Russ.)
15. Yablokov A. V., Larina N. I. *Vvedenie v fenetiku populyatsii.* Moscow : Vysshaya shkola, 1985, 159 p. (in Russ.)
16. Backeljau T., Baur A., Baur B. Population and conservation genetics. In: *The Biology of Terrestrial Molluscs* / G. M. Barker (Ed.). Wallingford, UK : CAB International Publishing, 2001, pp. 383–412.
17. Bevelander G., Nakahara H. An electron microscope study of the formation and structure of the periostracum of a gastropod, *Littorina littorea*. *Calcified Tissue Research*, 1970, vol. 5, no. 1, pp. 1–12. https://doi.org/10.1007/BF02017528

18. Comfort A. Biochemistry of molluscan shell pigments. *Journal of Molluscan Studies*, 1950, vol. 28, iss. 2-3, pp. 79–85. https://doi.org/10.1093/oxfordjournals.mollus.a064570
19. Comfort A. Observations on the shell pigments of land pulmonates. *Journal of Molluscan Studies*, 1951, vol. 29, pp. 35–44. https://doi.org/10.1093/oxfordjournals.mollus.a064598
20. Cook L. M. The genetics of *Cepaea nemoralis*. *Heredity*, 1967, vol. 22, no. 3, pp. 397–410. https://doi.org/10.1038/hdy.1967.49
21. Cook L. M., King J. M. B. Some data on the genetics of shell-character polymorphism in the snail *Arianta arbustorum*. *Genetics*, 1966, vol. 53, iss. 3, pp. 415–425. https://doi.org/10.1093/genetics/53.3.415
22. Délé-Dubois M.-L., Merlin J.-C. Étude par spectroscopie Raman de la pigmentation du squelette calcaire du corail. *Revue de Gemmologie*, 1981, vol. 68, pp. 10–13.
23. Estévez D., Kozminsky E., Carvajal-Rodríguez A., Caballero A., Faria R., Galindo J., Rolán-Alvarez E. Mate choice contributes to the maintenance of shell color polymorphism in a marine snail via frequency-dependent sexual selection. *Frontiers in Marine Science*, 2020, vol. 7, art. no. 614237 (15 p.). https://doi.org/10.3389/fmars.2020.614237
24. Hedegaard G., Bardeau J.-F., Ghateigner D. Molluscan shell pigments: An *in situ* resonance raman study. *Journal of Molluscan Studies*, 2006, vol. 72, no. 2, pp. 157–162. https://doi.org/10.1093/mollus/eyi062
25. Ito S., Konuma J. Disruptive selection of shell colour in land snails: A mark–recapture study of *Euhadra peliomphala simodae*. *Biological Journal of the Linnean Society*, 2020, vol. 129, no. 2, pp. 323–333. https://doi.org/10.1093/biolinnean/blz168
26. Lucas M. Pigments in Mollusca. *Conchiglia*, 1974, vol. 6, pp. 5–10.
27. Miura O., Nishi S., Chiba S. Temperature-related diversity of shell colour in the intertidal gastropod *Batillaria*. *Journal of Molluscan Studies*, 2007, vol. 73, no. 3, pp. 235–240. https://doi.org/10.1093/mollus/eym019
28. Murray J. The genetics of the Mollusca. In: *Handbook of Genetics.* Vol. 3. *Invertebrates of Genetic Interest* / R. C. King (Ed.). New York : Plenum Press, 1975, pp. 3–31.
29. Pettitt C. W. A proposed new method of scoring the colour morphs of *Littorina saxatilis* (Olivi, 1772) (Gastropoda: Prosobranchia). *Journal of Molluscan Studies*, 1973, vol. 40, no. 6, pp. 531–538. https://doi.org/10.1093/oxfordjournals.mollus.a065247
30. Reid D. G. *Systematics and Evolution of Littorina.* Dorchester, England, UK : Dorset Press, 1996, 463 p.
31. Reimchen T. E. Substratum heterogeneity, crypsis, and colour polymorphism in an intertidal snail (*Littorina mariae*). *Canadian Journal of Zoology*, 1979, vol. 57, no. 5, pp. 1070–1085. https://doi.org/10.1139/z79-135
32. Rolán-Alvarez E., Austin C. J., Boulding E. G. The contribution of the genus *Littorina* to the field of evolutionary ecology. In: *Oceanography and Marine Biology: An Annual Review* / D. J. Hughes, A. C. Dale, R. N. Hughes, I. P. Smith (Eds). Boca Raton : CRC Press, 2015, vol. 53, pp. 157–214. https://doi.org/10.1201/b18733
33. Sacchi C. F. Le polychromatisme des littorines (Gasteropodes, Prosobranches) d'Europe: points de vue d'un ecologiste. *Mémoires de la Société zoologique de France*, 1974, vol. 37, pp. 75–84.
34. Scheil A. E., Hilsmann S., Triebskorn R., Köhler H.-R. Shell colouration and parasite tolerance in two helicoid snail species. *Journal of Invertebrate Pathology*, 2014, vol. 117, pp. 1–8. https://doi.org/10.1016/j.jip.2014.01.003

35. Sokolova I. M., Berger V. Ja. Physiological variation related to shell colour polymorphism in White Sea *Littorina saxatilis*. *Journal of Experimental Marine Biology and Ecology*, 2000, vol. 245, iss. 1, pp. 1–23. https://doi.org/10.1016/S0022-0981(99)00132-X
36. Williams S. T. Molluscan shell colour. *Biological Reviews*, 2017, vol. 92, iss. 2, pp. 1039–1058. https://doi.org/10.1111/brv.12268
37. Williams S. T., Ito S., Wakamatsu K., Goral T., Edwards N. P., Wogelius R. A., Henkel T., de Oliveira L. F. C., Maia L. F., Strekopytov S., Jeffries T., Speiser D. I., Marsden J. T. Identification of shell colour pigments in marine snails *Clanculus pharaonius* and *C. margaritarius* (Trochoidea; Gastropoda). *PLoS ONE*, 2016, vol. 11, no. 7, art. no. e0156664 (25 p.). https://doi.org/10.1371/journal.pone.0156664

ОПИСАНИЕ ПОЛИМОРФИЗМА И КЛАССИФИКАЦИЯ ПРИЗНАКОВ ОКРАСКИ РАКОВИНЫ У БРЮХОНОГИХ МОЛЛЮСКОВ НА ПРИМЕРЕ *LITTORINA OBTUSATA* (GASTROPODA: LITTORINIDAE)

Е. В. Козминский

Зоологический институт РАН, Санкт-Петербург, Российская Федерация
E-mail: *ekozminsky@gmail.com*

Понимание закономерностей микроэволюционных процессов предполагает проведение широкого спектра популяционно-генетических исследований на разных видах. Однако количество видов, изученных в генетическом отношении, ограничено вследствие значительных методических трудностей при проверке генетической обусловленности различных признаков. Выход из этого положения заключается в развитии фенетики популяций, которая позволяет включить в рассмотрение большое количество новых видов при условии разработки обоснованных систем описания изменчивости и классификации фенотипических признаков. Классическим объектом для проведения популяционно-генетических исследований, связанных с полиморфизмом по окраске раковины, служат брюхоногие моллюски. В качестве основы для разработки универсальной для этой группы системы описания и классификации признаков окраски может быть использована параметрическая система классификации, предложенная С. О. Сергиевским с соавторами для моллюсков рода *Littorina*. В связи с появлением в последние годы большого количества новых данных, эта система нуждается в коррекции и пересмотре. Целью нашего исследования было провести ревизию этой системы с учётом новых данных о составе пигментов, особенностях формирования и наследования признаков окраски, их совместной встречаемости и т. д. В настоящей работе на примере беломорских моллюсков *L. obtusata* представлена переработанная система классификации признаков окраски раковины, в основу которой положено представление о формировании фенотипа как совокупности ряда элементарных признаков. К их числу отнесены признаки, связанные с формированием: 1) фоновой окраски раковины (способность к включению в остракум разных пигментов и окраска гипостракума); 2) рисунка из пятен (наличие включений белого и/или коричневого пигмента); 3) широких продольных полос (коричневых, белых и оранжевых). Элементарные признаки выделены с учётом задействованных пигментов, механизмов их формирования и наследования. При описании окраски раковины элементарные признаки сначала используются для описания относительно простых признаков (группы фенов «Фоновая окраска раковины», «Окраска гипостракума», «Рисунок из пятен», «Широкие продольные полосы»), которые в последующем комбинируются для описания фенотипа в целом. В работе приведён обзор данных по качественному составу пигментов раковины у литторин и закономерностям формирования и наследования признаков окраски, описаны их возможные варианты и комбинации. Приведены описания выделенных фенов с указанием их встречаемости,

количественной характеристики цвета в системе МКО RGB, особенностей их формирования с учётом распределения пигментов в толще раковины. Указаны видимые признаки, которые могут быть использованы при оценке распределения пигментов в раковине; описаны особенности изменения признаков в онтогенезе. Несмотря на то, что предложенная система классификации описана на примере *L. obtusata*, она может быть также использована при описании изменчивости у других видов литторин, а с некоторыми доработками — у самого широкого спектра брюхоногих моллюсков.

Ключевые слова: полиморфизм, окраска раковины, система классификации, брюхоногие моллюски, *Littorina*