



Variation in the colour pattern of the narrow-headed ant *Formica exsecta* (Hymenoptera: Formicidae) in European Russia

TATYANA S. PUTYATINA¹ , ALEXEY V. GILEV² , VLADIMIR G. GRINKOV¹  and ALEXANDER V. MARKOV¹ 

¹ Department of Biological Evolution, Faculty of Biology, Lomonosov Moscow State University, 119234 Moscow, Russia; e-mails: tsergput@gmail.com, v.grinkov@gmail.com, markov_a@inbox.ru

² Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia; e-mail: gilev@ipae.uran.ru

Key words. Polymorphism, polyphenism, colour variants, adaptation, Ryazan Region, White Sea Coast

Abstract. We determined whether body colour patterns of the narrow-headed *Formica exsecta* ant can serve as a marker of adaptive strategies in heterogeneous environments. The variations in colour were studied in two populations of *F. exsecta* in central and north western areas in European Russia. A generalised colour scheme of the head and three thorax segments is developed based on a comparative analysis of the colouration of *F. exsecta* and red wood ants (*Formica* s. str.). The scheme consists of 23 variants. We found that the colouration of different parts of the body varies independently to a large extent, although a pale (less melanized) head and three thoracic segments often co-occur with paler other parts of the body. We also show that the relationship between colour pattern and body size vary significantly in different complexes. We suggest that these colour variants can be used to study the spatial-temporal phenotypic differentiation of populations and adaptation of ants to microhabitats. The proposed scheme can be used as a convenient tool in future studies to quantify the variation in colour patterns. This scheme should be further tested for its applicability for other species of the subgenus *Coptoformica*.

INTRODUCTION

Insect colouration can be adaptive in terms of protection, signalling and physiology (thermoregulation, UV- and drought-resistance) (Cuthill et al., 2017; Badejo et al., 2020). Both genetically induced variation (Chetvericov, 1926; Dobzhansky, 1951; Zhan, 2014) and phenotypic plasticity (West-Eberhard, 2003; Moczek, 2008, 2012) of each individual trait can be both continuous and discrete. The term ‘polyphenism’ is often used to describe environmentally induced discrete variations (when the reaction norm consists of discrete phenes), while ‘polymorphism’ is a term applicable to mostly genetically (in the classical sense, Ford, 1965) controlled variations. Polyphenic and polymorphic species supposedly can adapt to abrupt changes in environmental conditions more easily and quickly due to a wider general phenotypic variation compared to monophenic and monomorphic species (Dobzhansky, 1951; Cain & Sheppard, 1954; Simpson et al., 2011). For example, among the Old-World flycatchers, the pied flycatcher (*Ficedula hypoleuca*) is a polymorphic and eurytopic species whose territory is several times larger than that of the collared flycatcher (*F. albicollis*), which is a monomorphic and stenotopic species (Qvarnstrom et al., 2010). The fission-dispersing ant *Aphaenogaster senilis* can quickly adjust its body temperature in accordance with

local conditions due to phenotypic plasticity (Oms et al., 2017). Species with discrete phenotypic variation (polymorphic and polyphenic) additionally have another way of responding to environmental changes compared to monomorphic and monophenic species, because each discrete phenotypic state of a trait often reflects a separate adaptive strategy. Populations of polymorphic and polyphenic species can respond to environmental changes through a shift in the frequency of phenotypes (Saccheri et al., 2008; Whitman & Agrawal, 2009; Kerimov et al., 2014; Roulin, 2014).

In natural populations, different adaptive strategies are often linked to melanin-based colouration. For example, the black and brown colouration in most arthropods is melanin based (Fuzeau-Braesch, 1972), including ants (Miyazaki et al., 2014). The variability in melanin colouration is not an exception to the general rule as it is also determined by both genetic variation and phenotypic plasticity.

There are a lot of examples of polymorphism in insects. The well-known examples of colour polymorphism in insects are the peppered moth (Van't Hof et al., 2016), the swallowtail butterfly (Nishikawa et al., 2015), the Colorado potato beetle (Boiteau, 1994; Grizenko et al., 1998; Benkovskaya et al., 2008; Benkovskaya & Udalov, 2011) and ladybirds (Yassin et al., 2016; Holloway et al., 1995).

The melanisation of cuticle can be modified by environmental factors, demonstrating the high phenotypic plasticity of this trait in some insects (Simpson et al., 2011). One of the first studies of this kind was the work of Timofeev-Resovskiy et al. (1965). They modified the colour of the ladybug *Epilachna chrysomelina* by varying the temperature, which affected the degree of pigmentation. The order Lepidoptera is an abundant source of examples of polyphenism (Brakefield & Frankino, 2009). Other classic examples of adaptive phenotypic plasticity include the butterflies *Manduca sexta* (Kingsolver et al., 2012) and *Araschnia levana* (Vilcinskas, 2016). Many insects have diet-induced morphs (Simpson et al., 2011). For example, quantitative and qualitative features of larval nutrition of the vespine *Polistes dominula* influence their size and percentage melanisation of the adult (Rusina et al., 2011).

In general, melanin colouration in insects is highly variable in the extent to which it is controlled by genes, environment and genotype-environment interactions (Stoehr 2006; Roulin, 2016).

The heritability of colour variation in mound-building *Formica* ants (and many other ant species) is unknown. The well-studied differences in colouration of different families of red wood ants (Alpatov, 1924) do not unequivocally indicate the dominance of hereditary or environmental components in the variation. A recent investigation (Skaldina & Sorvari, 2020) found no associations between the diversity of phenotypic traits and worker kinship (assessed by the average relatedness (r) between the nest mates) in *Formica rufa*. In Finland, however, the wood ant *Formica rufa* is mainly monogynous, and thus the variation in a colony is mostly due to multiple paternity (Skaldina & Sorvari, 2020). Most red forest ants (*Formica polyctena*, *F. aquilonia*, *F. lugubris*) are mostly polygynous (there are several females in a colony) and each female can store the sperm of many males (Seifert, 2007; Zakharov, 2015). This is not the case in *Diacamma* ants (Hymenoptera: Ponerinae), in which there is a distinct genetically based colour dimorphism in both males and females (Miyazaki et al., 2014). Thus, the determinants of variation in colouration in *Formica* ants remain to be resolved.

The most common feature that indicates a trait is adaptive, may be regular geographical and/or spatial change in its phenotypic state or frequency in populations (for example, Ilik et al., 2008). If there is a clear geographic trend in the variation of a trait (for example, darkening in northern populations), this indicates a trait-fitness covariation (connection of a trait to one of the two main components of fitness, for example, fertility and/or vitality), since the trait changes are associated with changes in the most common climatic characteristics of the territories. For example, the geographical variation in morph frequency in populations of pied flycatchers is associated with the temperature when males are involved in territorial behaviour and is reflected in the extent of brown or black colour in their breeding plumage (Ilyina & Ivankina, 2001; Kerimov et al., 2014; Grinkov et al., 2019).

There are ecological races of red wood ants associated with different types of forests (Zakharov, 2015), which may vary in colour. Phenotypically homogeneous groups in closely located areas that differ sharply from neighbouring groups were identified based on differences in the frequencies of the colour types of pro- and mesonotum in populations of the northern wood ant *F. aquilonia* (Gilev, 2003; Korochkina et al., 2014). A clear phenotypic differentiation among populations occurs in the northwest of European Russia and Fennoscandia, which probably indicates the path of the postglacial spread of this species (Gilev et al., 2015). Significant differences in the frequencies of colour variants depending on the degree of industrial pollution (Belsky & Gilev, 2007; Skaldina et al., 2018) are thought to indicate that colour variability is adaptive. It is also known that in the North American *Formica neorufibarbis* living in the mountainous tundra, small dark workers forage in the morning, when there is little insolation, while the larger red specimens forage in the afternoon, when insolation is at higher levels (Bernstein, 1976).

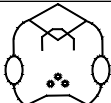



































This study seeks to investigate previously undescribed colour variation in one of the most common mound-building ants, *Formica exsecta* (subgenus *Coptoformica*). There are many studies on this species (Pisarski & Banert, 1982, Berman et al., 2007; Goryunov, 2007, 2010, 2015; Zakharov, 2015). The workers of *F. exsecta* are two-coloured and exhibit a diverse colouration of the head and thorax. This colouration may vary from completely pale (yellow or red) to completely black, with a variety of intermediate states. The pigment on the head and thorax is deposited in spots of varying shape and size. This species inhabits territories with a clear gradient in environmental conditions because it is a widespread trans Palearctic species found in many natural zones, from forest-tundra in the north to steppes in the south. It prefers open biotopes throughout its range. The link between colouration and adaptation to an environmental gradient in *F. exsecta* can be deduced from the results obtained by Dlussky (1967). He reports that the colouration of the workers of *F. exsecta* becomes paler in a north-easterly direction.

This work aims to develop a unified classification system for the description of the variation in colour of an ant's body based on the analysis of colour variants in *F. exsecta* from different geographic populations. We also aimed to analyse combinations of the colour patterns on different parts of the body and assess the association of the pattern in melanin colouration with the size of the individuals. Ant populations from north-western and central European Russia were used as model systems.

MATERIALS AND METHODS

In 2016, we collected and analysed 662 specimens of *F. exsecta* from two populations at three locations. Varvarovka is located in central European Russia (Ryazan Region, Kadomsky District, near the village Varvarovka, 54°54'N, 42°24'E), the other two locations are in the eastern part of the Kuzokotsky Peninsula on the White Sea Coast (66°46'N, 33°56'E). At Varvarovka, 40–50 specimens were collected from each of 9 nests. On the Kuzokot-

Table 1. The proportion of the different colour variants of *F. exsecta* at the different locations. H – head, Pn – pronotum, Mn – mesonotum, Pr – propodeum. The standard error of each sample is in brackets. ^a Combines 6 (from 2a to 2f) variants (only Pr2b is shown).

Variant	Schematic diagram	Photograph	Location (No. of individuals / No. of nests)		
			Pink Rocks (142/4)	Cape (131/3)	Varvarovka (389/9)
H1			0.021 (0.012)	0.038 (0.017)	0
H2			0.070 (0.022)	0.298 (0.040)	0.041 (0.010)
H3			0.909 (0.024)	0.664 (0.041)	0.864 (0.017)
H4			0	0	0.095 (0.015)
Pn1			0.106 (0.026)	0.023 (0.013)	0.080 (0.014)
Pn3			0.754 (0.036)	0.970 (0.015)	0.804 (0.020)
Pn4			0.056 (0.019)	0	0.018 (0.007)
Pn5			0.070 (0.022)	0.008 (0.008)	0.098 (0.015)
Pn6			0.014 (0.010)	0	0
Mn1			0.416 (0.041)	0.473 (0.044)	0.337 (0.024)
Mn2			0.105 (0.025)	0.199 (0.035)	0.190 (0.020)
Mn3			0.430 (0.042)	0.328 (0.041)	0.500 (0.025)
Mn4			0.014 (0.010)	0	0.015 (0.006)
Mn5			0.035 (0.016)	0	0.008 (0.004)
Pr1			0.937 (0.021)	0.954 (0.018)	0.964 (0.009)
Pr2 ^a			0.021 (0.012)	0.023 (0.013)	0.013 (0.006)
Pr3			0.014 (0.010)	0.023 (0.013)	0.023 (0.008)
Pr4			0.028 (0.014)	0	0

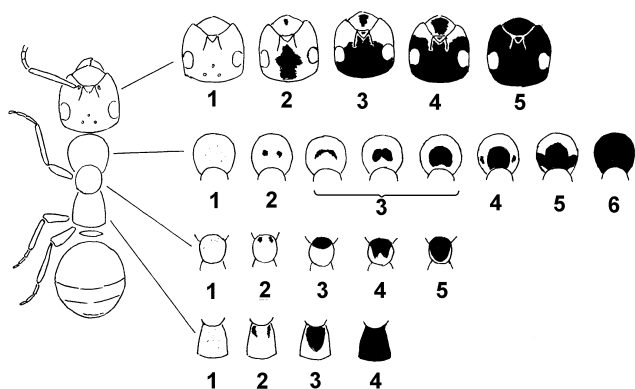


Fig. 1. Head and thorax colour phenotype scheme proposed for *Formica* s. str. (Gilev, 2002).

sky Peninsula, samples were collected from the Pink Rocks location (30–40 individuals from each of 4 nests) and Cape location (3 nests; 40–42 individuals from each nest). The two locations were 1 km from each other (for more information on these locations, see (Markov et al., 2017). The sample sizes are presented in Table 1.

The patterns in the colour on the head and thorax (separately for pronotum, mesonotum and propodeum) of the collected ants are described. The shape of the pattern on each part was visually determined, focusing on a previously proposed scheme of discrete colour variants for red wood ants (Fig. 1). We marked the borders of the dark spots along the border of the palest area. Shades of colour (melanisation) were not analysed. The length of the thorax was used as a measure of body size. All measurements were carried out using a binocular microscope Micromed MC-2 Zoom (40× magnification). The photographs were taken with a Canon EOS 70D digital camera with a macro lens Ultrasonic 100 mm with a flash MT-24EX 58 mm and macro rings (a total ring height of 52 mm).

The correlation of each type of colour with the other types and with the body size was done using Spearman correlation. All calculations were done in Microsoft Excel 2003 and Statistica 6.0 (StatSoft, Inc., 1984–2001).

RESULTS

The colour patterns

In *F. exsecta*, there are 23 variants of the head and thorax colouration. The occurrence of these colour types varied significantly at the different localities studied (Table 1). The Pr2 variant (Table 1), due to the rare occurrence of sub-variants (such as Pr2a, Pr2b, etc., not listed in the table), is presented as one variant. Based on combinatorics, it includes 7 sub-variants depending on the locations of the points and bands fully represented in the diagram (Fig. 2). Of these, 6 sub-variants were recorded in our samples of Pr2a.

The variability in the colouration of *F. exsecta* closely resembles that of *Formica* s. str., which are described by Gilev (2002). However, there are some differences.

The pattern of pigmentation on the head is almost completely consistent with that of red forest ants (Figs 1 and 2), except for H2. On the head, different colour variants are not discrete in the strict sense. The naturally continuous variation is divided into classes according to the location of the pigmented area relative to the compound eyes: pigment

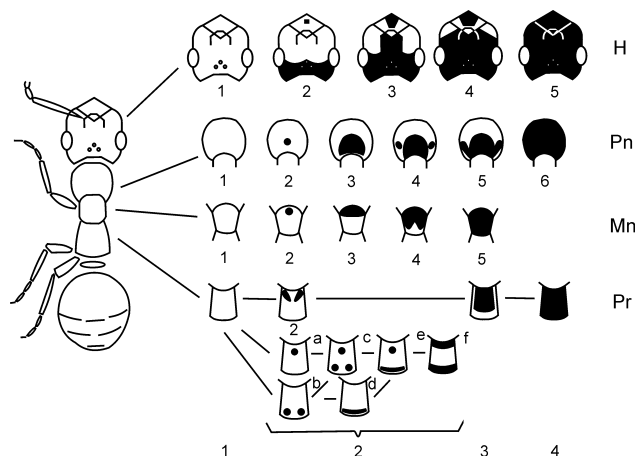


Fig. 2. Head and thorax colour phenotype scheme for *F. exsecta*. The variants H5, Pn2, and Pr2 were not recorded in the samples studied.

is completely absent (H1); pigment is present, covers the back of the head and reaches the eyes only on the upper part (H2); pigment completely covers the frons and the occiput of the head, the genae are a pale colour (H3); pigment extends beyond the lower edges of compound eyes, small areas on the genae are a pale colour (4); completely black head (5). The most numerous is the H3 variant (normal colouring of the head for most bi-coloured *Formica* species) (Table 1). The H5 variant (Fig. 2) was not present in our samples, however, we include it in the figure, by way of comparison with *Formica* s. str. (Fig. 1). In *Formica* s. str., H5 variant is quite rare. However, it is likely it will eventually be detected in *F. exsecta*.

Thoracic colour variants (the pronotum, mesonotum, propodeum) are based on the appearance of new elements (spots and streaks of pigment) and their subsequent merging. Each new element is recorded when a certain level of pigmentation is reached. All of these variants are discrete in the strict sense and correspond to Filippov’s (1961) aberrations or Vasiliev’s (1988) phen compositions.

The pattern of pigmentation on the pronotum almost completely coincides with that of *Formica* s. str. (Figs 1 and 2). The palest type is the complete absence of pigment (Pn1). The next type is one small central spot (Pn2). It does not occur in our samples, but is included in the figure, by way of comparison with *Formica* s. str. The level of pigmentation in Pn3 is greater and there is one large central spot that varies in size. This is the most common variant and the typical pronotum colour of most two-coloured *Formica* ants. The rare infinity-like symbolic form of the central spot, which consists of two merging circles, is thought to have originated by the merging of two separate spots, according to Filippov (1961). Next, small side spots (Pn4) appear in the colour pattern. In the next variant the side spots merge with the central one and form a “three-petal” pattern (Pn5). Finally, the pigment completely covers the pronotum (Pn6).

The colour pattern of the mesonotum is also very similar to that of *Formica* s. str. (Fig. 1). The palest variant is un-pigmented (Mn1). The next variant has one small spot

Table 2. Number of colour variants recorded for the pronotum (Pn) and mesonotum (Mn) at the different locations.

Location	Pn colour variants	Mn colour variants				
		1	2	3	4	5
Pink Rocks	1	15				
	3	37	13	54	2	1
	4	5	2	1		
	5	2		6		2
	6					2
Cape	1	3				
	3	59	25	43		
	4					
	5		1			
	6					
Varvarovka	1	29	2			
	3	99	69	144	1	
	4	1	2	4		
	5	2	1	27	5	3
	6					

Table 3. Number of colour variants recorded for the mesonotum (Mn) and propodeum (Pr) at the different locations.

Location	Mn colour variants	Pr colour variants			
		1	2	3	4
Pink Rocks	1	59			
	2	14	1		
	3	59	1		1
	4	1		1	
	5		1	1	3
Cape	1	61	1		
	2	24	1	1	
	3	40	1	2	
	4				
	5				
Varvarovka	1	130	1		
	2	73	1		
	3	168	1	6	
	4	4	1	1	
	5		1	2	

on the front edge of the mesonotum (Mn2). Then in Mn3 there is a semi-circular spot on the front edge of the mesonotum (Mn3). Further, with increase in melanisation two backward-extending pigment protrusions form a W-shaped figure (Mn4), and finally, one large spot, which occupies almost the entire mesonotum (Mn5). In fact, the variants Mn2 and Mn3 are the same variant, as both have one central spot and could be combined. We separate them because the Mn2 variant occurs with a frequency, ranging from 15 to 30% in different families (Table 1).

Comparison of the variations in the colour of the mesonotum in *F. exsecta* and *Formica* s. str. (Figs 1 and 2) revealed that the central spot does not become two small spots with decrease in the pigmentation of the cuticle in *F. exsecta*. With decrease in pigmentation, the central spot only decreases in size. However, the Mn2 variant indicates it originated from paired elements consisting of two pigment strips extending posteriorly. In *Formica* s. str., decrease in pigmentation of the cuticle is associated with the central spot becoming two spots (Gilev, 2002). It is likely that such variants might occur occasionally in *F. exsecta*.

Finally, the most complex colour pattern is that on the propodeum, with several patterns, only one of which (Pr2g-Pr3-Pr4) is similar to that of *Formica* s. str. (Gilev, 2002). The palest variant with complete absence of pigment is Pr1. This variant is the most common, it usually occurs in more than 90% of individuals. Then, in Pr2g there are a pair side spots, which form a merging tongue of pigment in (Pr3). Next, the propodeum is completely pigmented (Pr4). Other variants have a single central spot near the mesopropodeal suture (Pr2a) or a pair of back spots (Pr2b), which merge into a pigment strip. Thus, there are several different combinations (Pr2a-f, Table 1, Fig. 2). As all these variants are only recorded for a few individuals, they are combined under the Pr2 variant. The Pr2a variant did not occur in our samples, but it is likely it will be recorded in the future.

Based on the above reasoning, the colour phenotype scheme of the head and thorax in *F. exsecta* includes 26 variants (Fig. 2).

Combinations of colour variants

Since the colour variants are determined by pigmentation of the cuticle, it is likely that they are combined in a non-random way: the palest often with other pale individuals, and the darkest with other dark individuals. However, it is not possible to exclude cases of uneven distribution of pigment, with only one part of the body pigmented. In addition, it is likely that these combinations may occur in different species and populations.

Different colour combinations were recorded on different parts of the body. For example, Table 2 shows the different pronotum and mesonotum colouration combinations and Table 3 those of the mesonotum and propodeum at the locations studied. Tables 2–3 show that, despite the wide range in variation, there are no individuals with the colouration of one part of the body pale and another part dark.

Tables 2–3 also show that at different locations some qualitative and quantitative differences are recorded in the colour combinations. For example, on the Kuzokotsky Peninsula, the Pn1 variant was only recorded in combination with the Mn1 variant, and at Varvarovka only the Mn1 and Mn2 variants. However, it is likely that the number of colour variants recorded will increase with increase in sample size, which facilitates the detection of rarer variants.

The data were ranked based on the percentage of melanisation, where one corresponds to the palest colour (variant one, Table 1) and six the darkest (variant six, Table 1). To determine the relationship between the degree of melanisation of different parts of an ant's body at the locations studied, Spearman's rank correlation was used. Tables 2 and 3 provide examples of the different combinations of colour variants of the pronotum and mesonotum, and the mesonotum and propodeum. Correlation coefficients for the combinations of colour options for all parts of the body are shown in Table 4.

In general, the correlation between the colour and colour intensity of different parts of the body is almost always weak, which indicates relatively independent variation

Table 4. Spearman correlations between the intensity of colour of different parts of the body of ants recorded at the different locations. n – number of individuals. Statistically significant coefficients are in bold italic.

Trait	Location		
	Pink Rocks (n = 142)	Cape (n = 131)	Varvarovka (n = 389)
Head – pronotum	0.462 p = 0.000000	0.235 p = 0.006852	0.385 p = 0.000000
Head – mesonotum	0.301 p = 0.000269	0.346 p = 0.000051	0.408 p = 0.000000
Head – propodeum	0.082 p = 0.329	0.082 p = 0.349	0.277 p = 0.000000
Pronotum – mesonotum	0.327 p = 0.000069	0.142 p = 0.104	0.443 p = 0.000000
Pronotum – propodeum	0.418 p = 0.000000	0.231 p = 0.0078	0.210 p = 0.000029
Mesonotum – propodeum	0.362 p = 0.000009	0.124 p = 0.159	0.198 p = 0.000088

of the melanisation on the head, pronotum, mesonotum and propodeum (Table 4). However, the correlation between the number of colour variants for different parts of the body differs at the locations studied (Table 4): at Varvarovka, all the variants correlate with each other, and at Cape, some variants are not related to one another (more precisely, they are connected only indirectly through correlations with other variants). This highlights the complex interrelationship between the colour patterns on different parts of the body and indicates a population specific variation in the pigmentation of individuals.

The interdependent changes in the colour pattern and body size

Workers of different sizes are pigmented differently, i.e., large individuals are on average less pigmented than small ones. A correlation between colouration and body size is reported by Dlussky (1967) and subsequently repeatedly confirmed. Since the size of workers depends on various external and internal factors, this complicates the use of colour patterns in studies on ants (Gilev, 2002). According to Dlussky's data (1967, p. 31, fig. 15), however, species of red forest ants differ both in the number of colour variants and the relationship between colour and body size. Subsequently, for this species, this relationship is reported to be unstable as in different nests of the same species, worker ants with different dimensional characteristics are coloured differently (Pisarski & Banert, 1982; Czechowski, 1996).

To overcome such difficulties, various methods for standardising sampling were developed, which generally came down to comparing workers of the same size (Dlussky et al., 1998; Gilev, 2002; Seifert, 2004).

The relationship between colour pattern and body size was determined for all the samples. For example, Fig. 3 shows the relationship between the length of the thorax and the colour variant of the pro- and mesonotum. For the pronotum, as mentioned earlier, the Pn3 variant dominates; there is a pronounced tendency to monomorphism. The colour pattern on the mesonotum is more diverse, with the Mn1–Mn3 variants the most frequent. Therefore, the relationship between colour and size is more pronounced for the mesonotum and to a lesser extent for other parts of the body of an ant. Table 5 provides the Spearman correlation coefficients of the percentage of covariation between the number of colour variants and body size at the locations studied. It is clear from Fig. 3 and Table 5 that this connection may be different at different locations. At Pink Rocks the relationship between the number of colour variants and body size is clear, i.e., there are statistically significant correlations for head, pro- and mesonotum colour. However, at Cape, there is no relationship between body size and colour. This is likely because at this location there is little variation in both body size and number of colour variants (Fig. 3). At Varvarovka, the colouring of the thorax correlates with size, but not that of the head, and the correlation is much weaker than at Pink Rocks (Table 5).

The intraspecific phenotypic differentiation

The intraspecific phenotypic differentiation in *F. exsecta* is described using the approach of B. Pisarski (Pisarski & Banert, 1982). The unit of analysis is the ant family, which is characterized by an average thorax length and mean colour score. In our case, to characterize the colouration of the family, instead of the average score, the proportion made up of the most numerous variant of the pronotum Pn3 was used. The relationship between the average size of ants in the family and the proportion of the pronotum variant Pn3 is shown in Fig. 4.

This figure shows that the distribution of points is essentially non-random. The ant families are grouped into four discretely distinct compact groups.

The first two groups in the figure are from nests at Varvarovka and Pink Rocks. One of these groups is characterized by the smallest workers (1.80–1.85 mm). The proportion of the Pn3 variant in this group reaches 0.85–0.90. In the other group, the ants are larger (1.90–1.95 mm) and proportion of variant Pn3 drops to 0.7. It is noteworthy that there is no smooth transition between these groups; their characteristics change abruptly. The reason for this discreteness is unknown. Another nest at Pink Rocks, with the largest workers (about 2.13 mm) and a proportion of the Pn3 variant of about 0.65, forms the third group.

Table 5. The association of the number of colour variants recorded for different parts of the body with the length of the thorax of *F. exsecta* at the different locations. Statistically significant coefficients are in bold italic. L_{th} – length of thorax, mm.

Body part	Pink Rocks (n = 141)			Cape (n = 131)			Varvarovka (n = 387)		
	L_{th}	r_s	p	L_{th}	r_s	p	L_{th}	r_s	p
Head		-0.26	0.002		0.01	0.939		-0.02	0.766
Pronotum		-0.29	0.001		0.03	0.714		-0.14	0.005
Mesonotum	1.94 ± 0.014	-0.53	0.000	2.01 ± 0.010	-0.1	0.441	1.88 ± 0.006	-0.14	0.007
Propodeum		-0.15	0.077		-0.1	0.603		-0.13	0.009

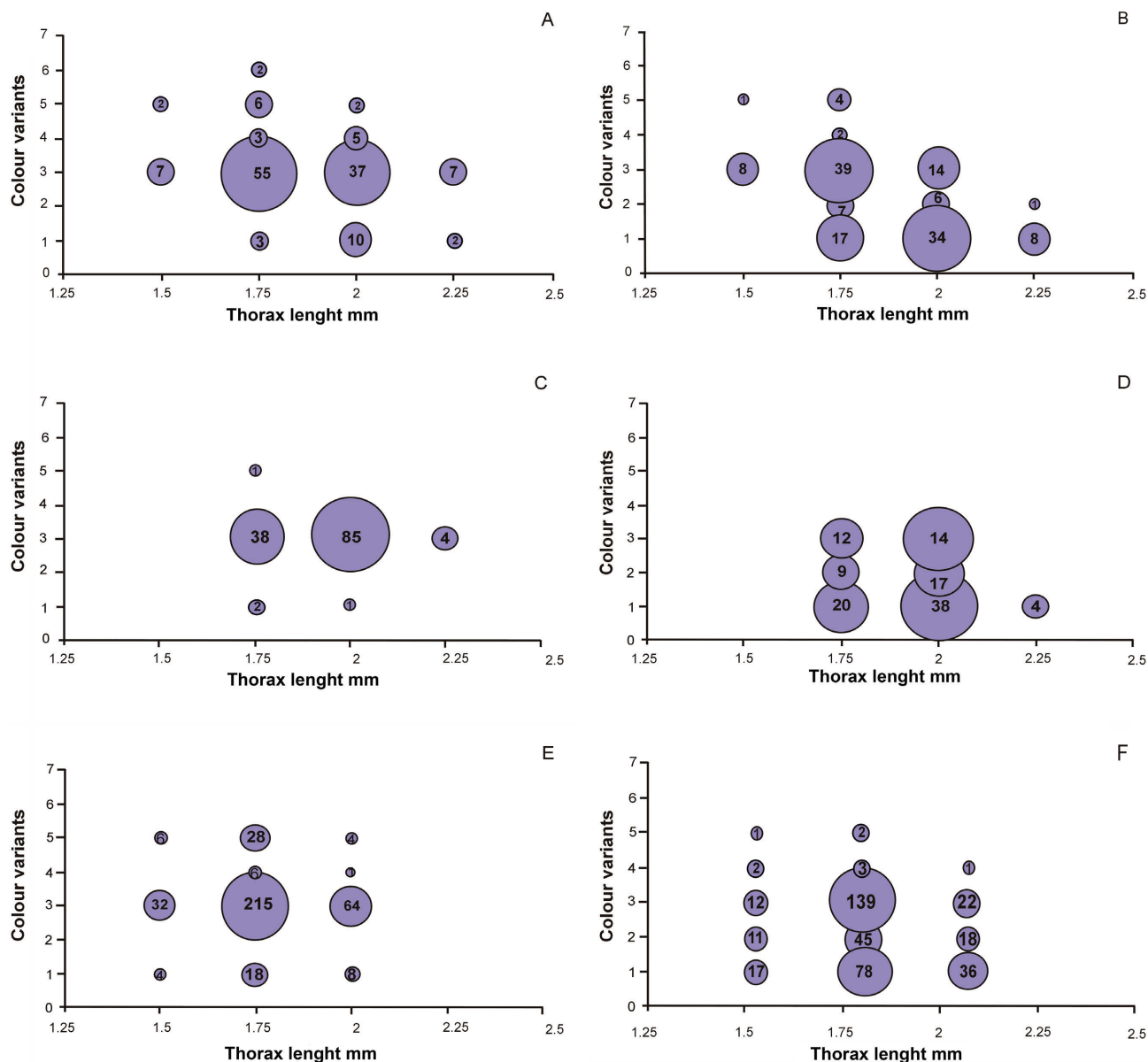


Fig. 3. The association of the different colour variants with the size of the body of *F. exsecta* recorded at the different locations. The size of the circle is proportional to the number of combinations. A, C, E – pronotum; B, D, F – mesonotum; A, B – Pink Rocks, C, D – Cape, E, F – Varvarovka.

If we consider only these three groups, then it could be argued that there is a trade-off between average size and proportion of the Pn3 variant. As the size increases, the Pn3 fraction decreases and then remains approximately constant, not falling below 0.60. At the same time, the characteristics of families do not change smoothly along with this dependence, but there are some stable states, in which most families of ants are located, which manifests itself in the form of discrete, compact groups. It should be noted that this relationship may indeed be true for these two locations, since it is the case for most families at Varvarovka and all families at Pink Rocks.

The fourth group, which includes all families from Cape and one family from Varvarovka, does not fit this trend. This group is characterized by large workers (1.95–2.05 mm) and maximum values for the proportion of Pn3 (up to 0.95–1.00).

It is interesting that in this case there is no difference between the geographically remote locations (Varvarovka and Pink Rocks). In addition, the observed phenotypic differentiation is associated with different habitats at these two locations. As Cape is a windy sea meadow, which is exposed to quite extreme conditions the nests there are segregated. At Varvarovka and Pink Rocks nests are located in a more favourable coniferous forest environment, which is reflected, in particular, in a more complex (compared to a segregated) settlement structure. The phenotypic similarity of the ants in one of the nests at Varvarovka with those from nests at Cape might be resolved by a more detailed study of the micro-habitat conditions at Varvarovka.

DISCUSSION

Different approaches and methods are used to study insect colouration. One of the traditional methods is to give

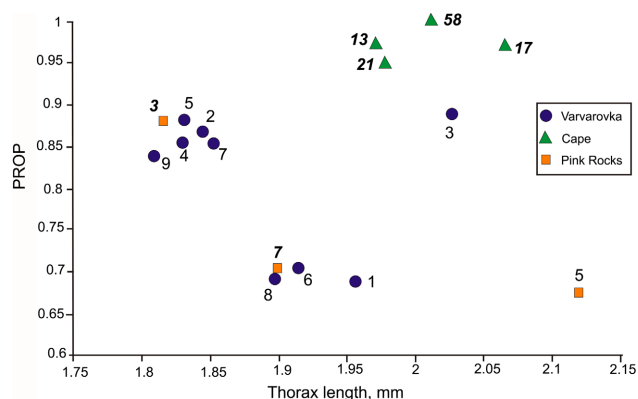


Fig. 4. The association of the proportion of the Pn3 variant with the body size of *F. exsecta* at the different locations. Varvarovka are in regular type, Kuzokotsky Peninsula are in bold italic. Orange – Pink Rocks, green – Cape, Purple – Varvarovka.

the various colour aberrations Latin names. This approach, in particular, was used to describe variability in longhorn beetles (cerambycids) (Plavilshchikov, 1936) and two-spotted lady beetles, one of the model species in studies on polymorphism (Majerus et al., 1994). This approach quickly proved to be unfeasible when the number of known aberrations started to exceed hundreds, making it highly inconvenient and virtually impossible to apply in practice. The situation has not changed, although image acquisition and processing techniques have improved considerably (Miyazaki et al., 2014; Skaldina et al., 2017).

Since 2002, a method for describing variability in colour based on uniform colour phenotype schemes has emerged, with the main advantage being that the results are comparable between different studies. This was based on the development of a colour classification scheme for red wood ants (*Formica* s. str.), which describes colour variants in terms of the differences in the degree of melanisation of their cuticle (Gilev, 2002; Skaldina & Sorvari, 2017).

Here, for the first time we describe the variation in colour of *F. exsecta*, carried out a discreteness analysis of this variation and based on this analysis developed a colour pattern scale.

When developing this scale, we used the same approach as that used for *Formica* s. str., which considers the possibility of transitions between different colour variants with increase in melanisation of cuticle in individuals and is based on the combinatorics of the elements of the pattern. This approach, thus, considered the ways to merge spots. The colour pattern is described in terms of its transformation as the overall pigmentation of an insect increases. It is assumed that the individual elements of the colour pattern appear as pigmentation reaches certain thresholds. It should be noted that this does not involve ontogenetic transformations, but are a method of organizing its description. Apparently, one of the first researchers to develop ideas about the transformation of insect patterns was Shvanvich (1949), who devoted three chapters of his capital work “The Course of General Entomology”, to this topic. This problem was further developed by Filippov (1961), who presents a conceptual apparatus for the analysis of ab-

errative variations in colour patterns. In its contemporary form, this approach is widespread in phenetics (Timofeev-Resovsky et al., 1973; Yablokov & Larina, 1985; Grizenko et al., 1998; Korsun, 1994, 2000; Vasilyev, 2005; Benkovskaya & Udalov, 2011).

In this approach, both the ordering of the elements of the colour pattern and reconstruction of some elements of the colour pattern of a sample is important. Thus, a generalised colour phenotype scheme allows one to predict the entire array of possible colour variants, even if they have not yet been found in the populations studied. In the large sample of *F. exsecta* studied (662 individuals), we recorded 23 colour variants, and the colour scale (Fig. 2) includes three more variants that are likely to be recorded in future studies.

Other approaches involve quantitative estimates of the area and degree of pigmentation. This method is successfully used in the study of ants by Dlussky (Dlussky et al., 1998) and more recent studies by Skaldina & Sorvari (2017, 2020) on red wood ants. It should be noted that Skaldina first describes variations in colour using a method similar to ours and then estimates the % degree of pigmentation. In addition, she also uses a modular approach.

Klingenberg’s (2008) modularity concept is an integrative concept aimed at describing the whole organism, which is considered to consist of a set of modules, relatively independent of each other, but within which the individual parts are closely integrated. A module may respond to some environmental factors, while others may not. For example, the colouration of the head and thorax of red wood ants can be considered as separate modules (Skaldina & Sorvari, 2017).

It is likely, however, that the relationship between different modules and within a single module can be complex and ambiguous. The relationship between individual attributes can be non-linear. According to our data (Gilev, 2002), the colouration of different parts of an ants’ thorax also varies relatively independently. In addition, there is a hierarchical relationship between the colouration of the pronotum, mesonotum and propodeum, which is not revealed by simple correlation analysis. The palest variants of one part of the body of *F. exsecta* are more often record for generally pale individuals and vice versa. When the whole body is pale or dark may indicate a relatively synchronous total pigmentation of different parts of the body during ontogenesis. Thus, a certain level of pigmentation of the pronotum is possibly associated with the appearance of a whole spectrum of colour variants of the mesonotum (Gilev, 2002). Such complex and ambiguous interrelations between separate parts of the body indicates that the colouration of the most informative part of the module (not of the whole module) should be considered, and was implemented in our study. In addition, no stable combinations of colour variants were recorded, which indicates the relatively independent variation in colour on different parts of the body. Thus, the colouration on each part of the body of *F. exsecta* may, if necessary, also be considered as a separate phenotypic trait (module).

Furthermore, in *F. exsecta*, a long-known pattern (Dlussky, 1967), according to which large individuals are on average paler than small ones, was revealed. In addition, the correlation between the number of colour variants and body size differs significantly at different locations. This important finding indicates that body size in insects is only one of the factors inducing differences in colouration between individuals. Thus, colouration, which varies relatively independently between body parts, has its own informational value. Therefore, we believe it can be useful for studying intraspecific phenotypic differentiation.

Pigmentation has many adaptive functions, such as (1) physical and immunological protection of the organism; (2) signalling, including camouflage, unpalatability, rival quality and mate choice; and (3) physiological adaptations to temperature, UV radiation and desiccation (Rusina et al., 2007; Tibbets, 2010, 2013; Badejo, 2020). The degree of colouration of *F. exsecta* recorded can be used as a tool to describe the transformations of a given variation. There is every reason to believe that in *F. exsecta* the colour variants are good indicators of adaptive strategies to the spatial and temporal heterogeneity of environmental conditions associated with the habitats occupied by both individual families and their populations (see Fig. 4). Current studies indicate that in ants, colouration is mainly associated with camouflage and thermoregulation (Badejo, 2020).

The factors affecting the variation in colour require further study. However, this study indicates that discrete (and semi-discrete) colour variants can be used for studying time-space phenotypic differentiation, adaptation to microhabitats and other aspects of the biology of *F. exsecta*. It should be noted that the variation in pigmentation in ants of the subgenus *Formica* s. str. are similar and form homologous series of variations. It can be expected that, as in the case of *Formica* s. str., our generalised colour scale will be suitable for describing the colour variants in other species of the subgenus *Coptoformica*.

ACKNOWLEDGEMENTS. We are grateful to an anonymous referee for careful reading this paper and helpful comments, and to A. Markov, Jr., for help in collecting the ants and for proofreading the manuscript. This research was carried out as part of the Scientific Project of the State Order of the Government of Russian Federation to Lomonosov Moscow State University 04-1-21 No121031600198-2.

REFERENCES

- ALPATOV V.V. 1924: Variation and the lower systematic categories. — *Russ. Zool. Zh.* **4**: 227–244 [in Russian].
- BADEJO O., SKALDINA O., GILEV A. & SORVARI J. 2020: Benefits of insect colours: a review from social insect studies. — *Oecologia* **194**: 27–40.
- BELSKII E.A. & GILEV A.V. 2007: Accumulation of heavy metals in ants in the vicinity of the Middle Ural copper plant. — *Problems Biogeochem. Geochem. Ecol.* **2**: 92–97 [in Russian].
- BENKOVSKAYA G.V. & UDALOV M.B. 2011: *Colorado Potato Beetles Investigations in the South Urals*. Nova Science, New York, 60 pp.
- BENKOVSKAYA G.V., LEONT'eva T.L. & UDALOV M.B. 2008: Colorado potato beetle resistance to insecticides in the Southern Urals. — *Agrokhimiya* **8**: 55–59 [in Russian].

- BERMAN D.I., ALFIMOV A.V., ZHIGULSKAYA Z.A. & LEIRIKH A.N. 2007: *Overwintering and Cold Hardiness of Ants in North-East of Asia*. KMK Scientific Press, Moscow, 261 pp.
- BERNSTEIN R.A. 1976: The adaptive value of polymorphism in an alpine ant, *Formica neorufibarbis gelida* Wheeler. — *Psyche* **83**: 180–184.
- BOITEAU G. 1994: Genetics of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). In Jolivet P.H., Cox M.L. & Petitpierre E. (eds): *Novel Aspects of the Biology of Chrysomelidae. Series Entomologica, Vol. 50*. Springer, Dordrecht, pp. 231–236.
- BRAKEFIELD P.M. & FRANKINO W.A. 2009: Polyphenisms in *Lepidoptera*: Multidisciplinary approaches to studies of evolution. In Whitman D.W. & Ananthakrishnan T.N. (eds): *Phenotypic Plasticity of Insects, Mechanisms and Consequences*. Science Publishers, Enfield, pp. 337–368.
- CAIN A.J. & SHEPPARD P.M. 1954: The theory of adaptive polymorphism. — *Am. Nat.* **88**: 321–326.
- CUTHILL I.C., ALLEN W.L., ARBUCKLE K. & CASPERS B. 2017: The biology of color. — *Science* **357**(6350): eaan0221, 7 pp.
- CHETVERIKOV S.S. 1926: On certain aspects of the evolutionary process from the standpoint of modern genetics. — *J. Exp. Biol.* **2**: 3–54 [in Russian]. English translation (1961) *Proc. Am. Phil. Soc.* **105**: 167–195.
- CZECHOWSKI W. 1996: Colonies of hybrids and mixed colonies: interspecific nest takeover in wood ants (Hymenoptera, Formicidae). — *Memorab. Zool.* **50**: 1–116.
- DLUSSKY G.M. 1967: *Ants of the Genus Formica (Hymenoptera, Formicidae)*. Nauka, Moscow, 236 pp. [in Russian].
- DLUSSKY G.M., FEDOSEEVA E.B. & THOMPSON L.C. 1998: Variability of the ants *Solenopsis richteri* and *S. wagneri* (Hymenoptera, Formicidae): Statistical analyses of morphometric characters. — *Uspekhi Sovrem. Biol.* **118**: 283–298 [in Russian, English abstract].
- DOBZHANSKY T. 1937: *Genetics and the Origin of Species*. Columbia University Press, New York, 364 pp.
- FILIPPOV N.N. 1961: Regularities of aberrative variability of elytra pattern in Coleoptera. — *Russ. J. Zool.* **40**: 372–385 [in Russian with English abstr.].
- FORD E.B. 1965: *Genetic Polymorphism*. Faber & Faber, London, 101 pp.
- FUZEAU-BRAESCH S. 1972: Pigments and color changes. — *Annu. Rev. Entomol.* **17**: 403–424.
- GILEV A.V. 2002: Discrete variations of coloration and some patterns of pigmentation variability in workers of wood ants of the subgenus *Formica* (Hymenoptera, Formicidae). — *Entomol. Rev.* **82**: 481–486.
- GILEV A.V. 2003: The population pattern of the ant *Formica aquilonia* (Hymenoptera, Formicidae) in the Middle Urals. — *Biol. Bull. Rev.* **123**: 223–228 [in Russian, English abstract].
- GILEV A.V., MERSHCHIEV A.V. & MALYSHEV D.S. 2015: Interpretation of postglacial recolonization of *Formica aquilonia* (Hymenoptera, Formicidae) in Fennoscandia according to workers' color variation. — *Entomol. Rev.* **95**: 941–946.
- GRINKOV V.G., PALKO I.V. & HELMUT S. 2019: Character displacement within the breeding area questions reinforcement in *Ficedula* flycatchers. — *Bio Rxiv* 515916; doi: <https://doi.org/10.1101/515916>.
- GRIZENKO V.V., GLOTOV N.V. & ORLINSKY D.B. 1998: Ecological and genetic analysis of central elements variation in *Leptinotarsa decemlineata* (Coleoptera) foreback pattern. — *Russ. J. Zool.* **77**: 278–284 [in Russian, English abstract].
- GORYUNOV D.N. 2007: Forms of settlement organization in *Formica exsecta* ants. — *Uspekhi Sovrem. Biol.* **127**: 203–208 [in Russian, English abstract].

- GORYUNOV D.N. 2010: Territorial strategies in ants. — *Entomol. Rev.* **91**: 212–220.
- GORYUNOV D.N. 2015: Nest-building in ants *Formica exsecta* (Hymenoptera, Formicidae). — *Entomol. Rev.* **95**: 953–958.
- HOLLOWAY G.J., BRAKEFIELD P.M., DE JONG P.W., OTTENHEIM M.M., DE VOS H., KESBEKE F. & PEYNEBURG L. 1995: A quantitative genetic analysis of an aposematic colour pattern and its ecological implications. — *Phil. Trans. R. Soc. Lon. (B)* **348**: 373–379.
- ILYINA T.A. & IVANKINA E.V. 2001: Seasonal variation of winging activity and relative effect of the advertising behaviour of males with different plumage colour in the Pied Flycatcher *Ficedula hypoleuca*. — *Acta Ornithol.* **36**: 85–89.
- KERIMOV A.B., GRINKOV V.G., IVANKINA E.V., ILYINA T.A. & BUSHUEV A.V. 2014: The influence of spring temperature on the intensity of advertising behavior and basal metabolic rate in bright and pale pied flycatcher (*Ficedula hypoleuca*) males. — *Russ. J. Zool.* **93**: 1288–1302 [in Russian, English abstract].
- KINGSOLVER J.K., DIAMOND S.E., SEITER S.A. & HIGGINS J.K. 2012: Direct and indirect phenotypic selection on developmental trajectories in *Manduca sexta*. — *Funct. Ecol.* **26**: 598–607.
- KLINGENBERG C.P. 2008: Morphological integration and developmental modularity. — *Annu. Rev. Ecol. Syst.* **39**: 115–132.
- KOROCHKINA N.I., KONOPLEVA E.E. & ZRYANINA T.A. 2014: Population structure of *Formica aquilonia* (Hymenoptera, Formicidae) at the boundary of boreal and subboreal landscapes in the Volga river region. — *Entomol. Rev.* **94**: 664–674.
- KORSUN O. 1994: Variability and population structure of *Hoplia aureola* Pall. (Coleoptera, Scarabaeidae). — *Ecology* **25**: 372–379.
- KORSUN O. 2000: Phenotypic appearance of the natural populations of *Adalia bipunctata* L. (Coleoptera, Coccinellidae). — *Siber. J. Ecol.* **6**: 723–726.
- MAJERUS M.E.N. 1994: *Ladybirds*. London, Harper Collins, 367 pp.
- MARKOV A.V. & IVNITSKY S.B. 2016: Evolutionary role of phenotypic plasticity. — *Vestn. Mosk. Univ.* **16**: 3–11 [in Russian, English abstract].
- MARKOV A., GILEV A. & PUTYATINA T. 2017: Structure of mound-building ant settlements (genus *Formica*) in Kuzokotsky Peninsula (northern Karelia). — *Biol. Commun.* **62**: 93–102.
- MIYAZAKI S., OKADA Y., MIYAKAWA H., TOKUDA G., CORNETTE R., KISHIKAWA S., MAEKAWA K. & MIURA T. 2014: Sexually dimorphic body colour is regulated by sex-specific expression of yellow gene in ponerine ant, *Diacamma* sp. — *PLoS ONE* **9**(3): e92875, 8 pp.
- MOCZEK A.P. 2008: On the origins of novelty in development and evolution. — *BioEssays* **30**: 432–447.
- MOCZEK A.P. 2012: The nature of nurture and the future of Evodevo: toward a theory of developmental evolution. — *Integr. Comp. Biol.* **52**: 108–119.
- NISHIKAWA H., IJIMA T., KAJITANI R., YAMAGUCHI J., ANDO T., SUZUKI Y., SUGANO S., FUJIYAMA A., KOSUGI S., HIRAKAWA H. ET AL. 2015: A genetic mechanism for female-limited Batesian mimicry in *Papilio butterfly*. — *Nature Genet.* **47**: 405–409.
- OMS C.S., CERDÁ X. & BOULAY R. 2017: Is phenotypic plasticity a key mechanism for responding to thermal stress in ants? — *Sci. Nat.* **104**: 42, 7 pp.
- PISARSKI B. & BANERT T. 1982: Influence de la structure sociale sur la morphologie de *Formica (Coptoformica) exsecta* Nyl. — *Mem. Zool.* **38**: 137–162.
- PLAVILSHCHIKOV N.N. 1936: Woodcutter beetles (Cerambycidae). In: *Fauna of the USSR. Coleoptera. V. XXI. Part 1*. Academy of Sciences of the USSR, Moscow, Leningrad, 612 pp.
- QVARNSTROM A.A., RICE M. & ELLEGREN H. 2010: Speciation in *Ficedula* flycatchers. — *Philos. Trans. R. Soc. Lond. (B)* **365**: 1841–1852.
- ROULIN A. 2014: Melanin-based colour polymorphism responding to climate change. — *Glob. Change Biol.* **20**: 3344–3350.
- ROULIN A. 2016: Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. — *Biol. Rev.* **91**: 328–348.
- RUSINA L.Y., GILEV A.V., SKOROKHOD O.V., FILIMONOVA N.B. & FIRMAN L.A. 2007: Relationship between coloration of the wasp (*Polistes dominulus*) and spatial and ethological structure of its population in the Lower Dnieper Basin. — *Biol. Bull. Rev.* **127**: 157–165.
- RUSINA L.Y., GILYOV A.V., FIRMAN L.A., PEKANOVA I.A. & ORLOVA E.S. 2011: Dynamic stability of phenotypic variation in *Polistes* wasps (Hymenoptera: Vespidae). — *Russ. Entomol. J.* **20**: 321–326.
- RUZSKY M.D. 1905: *The Ants of Russia. Part 1*. Kazan, 798 pp.
- SACCHERI I.J., FRANÇOIS R., PHILLIP C.W., PAUL M.B. & LAURENCE M.C. 2008: Selection and gene flow on a diminishing cline of melanic peppered moths. — *Proc. Natn. Acad. Sci. USA* **105**: 16212–16217.
- SEIFERT B. 2004: The “Black Bog Ant” *Formica picea* Nylander, 1846 – a species different from *Formica candida* Smith, 1878 (Hymenoptera: Formicidae). — *Myrmecol. Nachr.* **6**: 29–38.
- SEIFERT B. 2007: *Die Ameisen Mittel- und Nordeuropas*. Lutra Verlags und Vertriebsgesellschaft, Tauer, 368 pp.
- SHVANVICH B.N. 1949: *The Course of General Entomology*. — Sov. Science, Moskow, 900 pp. [in Russian].
- SIMPSON S.J., SWORD G.A. & LO N. 2011: Polyphenism in insects. — *Curr. Biol.* **21**: 738–749.
- SKALDINA O. & SORVARI J. 2017: Not simply red: Colouration of red wood ant *Formica rufa* (Hymenoptera: Formicidae) is polymorphic, modular and size-dependent. — *Eur. J. Entomol.* **114**: 317–324.
- SKALDINA O. & SORVARI J. 2020: Phenotypic diversity in red wood ants (Hymenoptera: Formicidae): Is kinship involved? — *Eur. J. Entomol.* **117**: 27–33.
- SKALDINA O., PIRANIEMI S. & SORVARI J. 2018: Ants and their nests as indicators for industrial heavy metal contamination. — *Environ. Pollut.* **240**: 574–581.
- STOEHR A.M. 2016: Costly melanin ornaments: the importance of taxon? — *Funct. Ecol.* **20**: 276–281.
- TIBBETS E.A. 2010: The condition dependence and heritability of signaling and nonsignal color traits in a paper wasp. — *Am. Nat.* **175**: 495–503.
- TIBBETS E.A. 2013: The function, development, and evolution stability of conventional signals of fighting ability. — *Adv. Study Behav.* **45**: 49–80.
- TIMOFEEV-RESOVSKIY N.W., TIMOFEEV-RESOVSKAYA E.A. & ZIMMER K.G. 1965: Experimental and systematic analysis of geographic variability and morphogenesis in *Epilachna chrysomelina*. In: *Proceedings of the Institute of Biology of the Ural Branch of the Academy of Sciences of the USSR*. Ekaterinburg, pp. 27–63.
- TIMOFEEV-RESOVSKY N.V., YABLOKOV A.V. & GLOTOV N.V. 1973: *Essay of the Theory on Population*. Nauka, Moscow, 278 pp. [in Russian].
- VAN’T HOF A.E., CAMPAGNE P., RIGDEN D.J., YUNG C.J., LINGLEY J., QUAIL M.A., HALL N., DARBY A.C. & SACCHERI I.J. 2016: The industrial melanism mutation in British peppered moths is a transposable element. — *Nature* **534**: 102–105.
- VASILYEV A.G. 2005: *Epigenetic Patterns of Phenetics: On the Way to Population Meroonomy*. Akademkniga, Yekaterinburg, 640 pp. [in Russian].

- VILCINSKAS A. & VOGEL H. 2016: Seasonal phenotype-specific transcriptional reprogramming during metamorphosis in the European map butterfly *Araschnia levana*. — *Ecol. Evol.* **6**: 3476–3485.
- WEST-EBERHARD M.J. 2003: *Developmental Plasticity and Evolution*. Oxford University Press, New York, 816 pp.
- WHITMAN D.W. & AGRAWAL A.A. 2009: What is phenotypic plasticity and why is it important? In Whitman D.W. & Ananthakrishnan T.N. (eds): *Phenotypic Plasticity of Insects, Mechanisms and Consequences*. Science Publishers, Enfield, pp. 1–63.
- YABLOKOV A.V. & LARINA N.I. 1985: *Introduction in Population Phenetics: The New Method to Investigation of Natural Populations*. Vyshaya Shkola, Moscow, 159 pp.
- ZAKHAROV A.A. 2015: *Ants of Forest Communities, their Life and their Role in Forest*. KMK Scientific Press, Moscow, 404 pp. [in Russian].
- ZHAN S., ZHANG W., NIITEPÖLD K., HSU J., HAEGER J.F., ZALUCKI M.P., ALTIZER S., DE ROODE J.C., REPERT S.M. & KRONFORST M.R. 2014: The genetics of monarch butterfly migration and warning colouration. — *Nature* **514**: 317–321.

Received February 22, 2021; revised and accepted November 30, 2021
Published online January 18, 2022