ISSN 1995-4255, Contemporary Problems of Ecology, 2017, Vol. 10, No. 5, pp. 488–501. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © E.Yu. Zakharova, A.O. Shkurikhin, T.S. Oslina, 2017, published in Sibirskii Ekologicheskii Zhurnal, 2017, No. 5, pp. 567–582.

Morphological Variation of *Melanargia russiae* (Esper, 1783) (Lepidoptera, Satyridae) from the Main Part of the Range and in Case of Its Expansion to the North under Climate Change Conditions

E. Yu. Zakharova*, A. O. Shkurikhin, and T. S. Oslina

Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, 620144 Russia
**e-mail: zakharova@ipae.uran.ru*

Received November 14, 2016; in final form, March 7, 2017

Abstract—A climate-related shift in the range boundaries of the western–central Eurasian subboreal species *Melanargia russiae* in the Ural region from the northern forest-steppe zone to pine–birch forests was found. Morphological variation of *M. russiae* wings from the boundary northern populations and populations from the main part of the range in the Urals were studied. The results of a complex analysis of the morphological traits (size, wing shape, and wing pattern eyespots) confirm the hypothesis that the local population formed in the south of Sverdlovsk oblast and contradict the hypothesis of the migratory origin of *M. russiae* imago.

Keywords: range expansion, climate change, morphological variation, wing shape, eyespots, the Urals, *Melanargia russiae*

DOI: 10.1134/S1995425517050146

INTRODUCTION

Based long-term climate studies, it has been established that warming is observed throughout Russia. During the period from 1976 to 2014, the average growth rate of the average annual air temperature in the country was 0.42°C for 10 years. Warming mainly occurs in the cold season; this leads to climate mitigation almost everywhere (*Metody otsenki*..., 2012; *Doklad ob osobennostyakh klimata*..., 2015). Analyses of climatic data from the Yekaterinburg meteorological station (http://www.pogodaiklimat.ru/file.htm) shows that the trend of climatic changes in the Urals in general corresponds to the all-Russian trend: there is a gradual warming with a general increase in the amount of precipitation (Table 1). A certain exception is the period from 2010 to 2015, when the increase in average annual temperatures was accompanied by a decrease in the total amount of precipitation and, as a consequence, an increase in the climate aridity degree.

Research into the reactions of living organisms to modern climate changes is an relevent problem in evolutionary ecology. To date, a considerable amount of data has been accumulated and a number of generalizations and forecasts concerning various groups of

Table 1. Climate data for 50 years according to the meteorological station of Yekaterinburg

Five-year period	Average temperature of the air during the period, °C	Total amount of precipitation, mm	De Marton aridity index
1966–1970	1.27	2636	3.90
1971–1975	2.37	2105	2.84
1976–1980	1.92	2421	3.39
1981–1985	2.85	2572	3.34
1986–1990	2.86	2729	3.54
1991–1995	3.11	2614	3.32
1996–2000	2.71	2783	3.65
2001–2005	3.36	2757	3.44
2006–2010	3.44	2727	3.38
2011–2015	3.40	2559	3.18

plants and animals have been made (Parmesan and Yohe, 2003; Parmesan, 2006; Shvartsman et al., 2007; etc.). Insects, as a very significant component of natural communities, are studied from this point of view quite well. Depending on their specific species features, they can respond to climate changes by appropriate shifts in their ranges; irruptions or depressions of populations; and changes in phenology, voltinism, morphology, physiology, and behavior (Bale et al., 2002; Rubtsov and Utkina, 2010; Musolin and Saulich, 2012; etc.). Lepidopterans (Lepidoptera) is a convenient model group for conducting such environmental studies. This is particularly true in accordance to butterflies (Rhopalocera) due to regular faunal works and a good study of a huge number of local and entire regions fauna (Saarinen et al., 2003; Wilson et al., 2005; Sparks et al., 2007; Illan et al., 2012), as well as to various species of phyllophagous lepidopterans because of their economic importance as pests in agriculture and forestry (Battisti, 2008).

In this article, we review the nature of distribution and the principles of the wing morphological variability of one of the butterfly species, *Melanargia russiae* (Esper, 1784) (Lepidoptera, Satyridae), within the Ural region in connection with modern climatic changes. *M. russiae* is a western–central Eurasian subboreal species confined to the steppe and forest-steppe natural zones (Tatarinov and Gorbunov, 2014). On the European part of the range, where by the beginning of the 21st century the population remained quite stable (Van Swaay and Warren, 1999), the distribution of the species has been studied in sufficient detail. *M. russiae* is found on the territory of 13 European states and is a specialist species whose habitats are open biotopes: dry steppes, drylands, alpine and subalpine meadows, wet and forb meadows, forest openings and edges of coniferous and mixed forests, and shrub thickets (Van Swaay et al., 2006).

In some areas, for example, in Hungary, the last finds of *M. russiae* were made about 100 years ago, and the species is considered completely extinct. Possible causes of extinction include the anthropogenic transformation of the territory: drainage works that entailed mesoclimate changes and the intensive use of forest massifs as pastures during World War I (Bálint and Katona, 2013). During studies on the distribution of a species and changing the range boundaries in natural conditions, it is often difficult or impossible to identify the leading factor that influences the disappearance or appearance of a species in any territory. Obviously, both the availability of suitable biotopes and fodder plants and climatic parameters can have a limiting effect on the species distribution.

According to our observations and to literature data, both in the European (Van Swaay et al., 2006) and Asian (Gorbunov and Kosterin, 2007) parts of its range, *M. russiae* is very tolerant of significant anthropogenic transformation of steppe communities and successfully populates old laylands, wastelands, and overgrazed pastures. Modeling of the size of potential range of the species by a combination of several climatic factors, such as the sum of the active temperatures, the water content in the soil upper layers, the total annual temperature, the amount of precipitation, etc., has shown that *M. russiae* is the most sensitive to possible climatic changes (Settele et al., 2008). Therefore, it is logical to expect a shift in the boundaries of the current range of *M. russiae* due to the climate warming that is currently taking place. With the expansion of the species range, rapid morphogenetic rearrangements and formation processes are possible in new population boundaries. They allow us to observe the initial stages of microevolution (Vasil'ev, 2009).

In the Ural region, nominative subspecies *M. russiae* occurs everywhere on the territory of the Republic of Bashkortostan and Orenburg, Chelyabinsk, and Kurgan oblasts on the open steppe landscapes. In the forest-steppe zone, butterflies prefer southern exposure steppe slopes, grass and motley-grass-grasses meadows, laylands, and gullies.

The species range in the north is limited by the subzones of the southern taiga and subboreal forests. The northern boundary of the *M. russiae* range can be drawn more or less clearly along 56° N latitude. Therefore, for the territory of Siberia, the most northern finds of the species are known from settlement of Kaily in Novosibirsk oblast, N 55°17′, E 84°05′ (Korshunov, 1974, cited according: Ivonin et al., 2009), and the Muromtsevskii region of Omsk oblast, N 56°24′, E 75°16′ (Knyazev, 2009). Single specimens of *M. russiae* were recorded by A.G. Tatarinov (2012) in the northern belt of the southern taiga of the northeast of the Russian plain in different years. In a recently published article on the fauna of the Ural Rhopalocera (Tatarinov and Gorbunov, 2014), the two northernmost geographic points for *M. russiae* are mentioned: Perm oblast, the lower reaches of the Sylva River, biological research station Predural'e of Perm State University (N 57°21′, E 57°08′), and the suburbs of the settlement of Kuzino in Sverdlovsk oblast (N 57°01′, E 59°25′). Also, for the beginning and middle of the 20th century, findings of the species in the suburbs of Perm (N 57°58′, E 56°12′) and from settlement of Kurganovo in Sverdlovsk oblast (N 56°37′, E 60°22′) (Gorbunov and Ol'shvang, 1997) are known. One female *M. russiae* was caught in the Visim Nature Reserve (Sverdlovsk oblast, near the settlement of Bolshie Galashki, N 57°28′, E 59°29′) on June 28, 2012 by an employee of the Ural Federal University (UrFU), P.V. Rudoiskatel', and transferred to the Museum of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (Yekaterinburg). According to the publications, this species was not recorded previously or later on the territory of the (well-entomologically studied) Visim Natural Reserve (Ukhova and Ol'shvang, 2014). Thus, all findings of the species north of N 56° are catches of

Table 2. Number of *M. russiae* samples and geographical coordinates of the sampling sites from the territory of the Urals

No.	Sampling site	Year	Sample number, ind.		Geographical coordinates		Collectors
			males	females	N	E	
1	Fomino	2012–2015	5	5	56°36′0″	61°3′0″	Listed in the text
2	N. Tukbaevo	2012	73	4	55°40′16″	58°9′0″	E.Yu. Zakharova
3	Uvelka	2008	11	1	54°35′35″	61°6′22″	O.E. Chashchina
4	Zverinogolovskoe	2013, 2014	30	0	54°25′19″	64°45′4″	E.Yu. Zakharova, A.O. Shkurikhin
5	Osipovka	2014	39	1	54°7′19″	61°5′2″	E.Yu. Zakharova, Yu.M. Chibiryak
6	Sukhtelinskii	2012	67	4	53°55′48″	60°0′7″	E.Yu. Zakharova, T.S. Oslina
7	Leonovskie Mountains	2009	78	47	53°55′34″	59°1′52″	″
8	Varshavka	2012	25	3	52°49′52″	60°28′26″	″
9	Elizavetopol'skoe	2012	25	2	52°50′11″	60°36′12″	″
10	Cheka	2010	54	19	52°45′0″	58°52′1″	E.Yu. Zakharova, P.V. Rudoiskatel'
11	Tobol	2013	2	9	52°18′25″	61°38′28″	A.O. Shkurikhin, P.Yu. Gorbunov
12	Mednogorsk	2007	11	6	51°23′56″	57°35′38″	A.O. Shkurikhin, T.K. Tuneva
13	Orsk	2010	16	27	51°13′41″	58°25′26″	P.V. Rudoiskatel'
14	Guberlya	2003	28	0	51°8′20″	57°57′11″	P.Yu. Gorbunov

accidentally species passing through and are extremely rare and unsystematic.

The northernmost point of collection is the surroundings of the village of Fomino, Sysertskii region, Sverdlovsk oblast, where the UrFU biological research station is located. This area belongs to the Sysertskii region of the pine and birch forests subzone of the taiga zone (Kulikov et al., 2013). Here, with the participation of employees and students of the UrFU, since 2001 we have been carrying out phenological and population studies annually on Rhopalocera. *M. russiae* was not recorded in the suburbs of the biological research station in either the 20th century or the first decade of the 21st century (Gorbunov and Ol'shvang, 1997; Tatarinov and Gorbunov, 2014). Since 2012, the species has been caught regularly: 1 ♂ June 25, 2012; 1 ♂ July 3, 2012 (E.Yu. Zakharova); 1 ♀ July 22, 2013 (Yu.M. Chibiryak); 1 ♀ July 16, 2013 (P.V. Rudoiskatel'); 1 ♂ June 23, 2014 (O. Val'keeva); 1 ♀ June 29, 2015 (A.O. Shkurikhin); 1 ♀ July 2, 2015 (M.V. Chibiryak); 1 ♂ July 5, 2015 (Yu.M. Chibiryak); 1 ♂ July 10, 2015 (D. Prokhorov); and 1 ♀ July 22, 2015 (E.Yu. Zakharova). The regular catching of *M. russiae* in this area over the last 4 years indicates an expansion of this species range and a shift in its border to the north.

Two hypotheses about the origin of individuals caught in the taiga zone in the suburb of the village of Fomino could be suggested: on the one hand, they can be the accidental passage of individuals from more southern areas, and, on the other hand, they can be individuals from a small local population that has settled there and successfully survived the winter. An evaluation of the morphological variability degree of a complex of features such as a wing pattern, size, and shape may shed light on this issue.

The aim of this research was to study the patterns of morphological variability of *M. russiae* wings from the main part of the range and the boundary northern populations in the Urals in changing climate conditions.

MATERIALS AND METHODS

Samples from several *M. russiae* habitats in the Southern Urals and adjacent territories were studied (Fig. 1). The geographical coordinates of the sampling sites and the number of sampled material are given in Table 2.

Two geographical sites from all the studies were located in the subzone of the northern forest steppe of the forest-steppe zone, in the vicinity of the village of Nizhnee Tukbaevo (Republic of Bashkortostan) and on the riverbank of the Uvelka River, near the offing of the Sukharysh River (Chelyabinsk oblast). The *M. russiae* population from Nizhnee Tukbaevo lives in the unique plant communities of the azonal Mesyagutovskaya forest steppe—the southern part of the northern Krasnoufimskii forest-steppe island (Krasheninnikov and Vasil'ev, 1949).

Leonovskie Mountains are located on the border of Chelyabinsk oblast and the Republic of Bashkortostan in the southern forest steppe of the Trans-Ural peneplain near the border of the forest-steppe and steppe zones. The vegetation cover of the mountains is typical for the mountain forest steppe of the eastern slope of the Southern Urals (Kulikov, 2005).

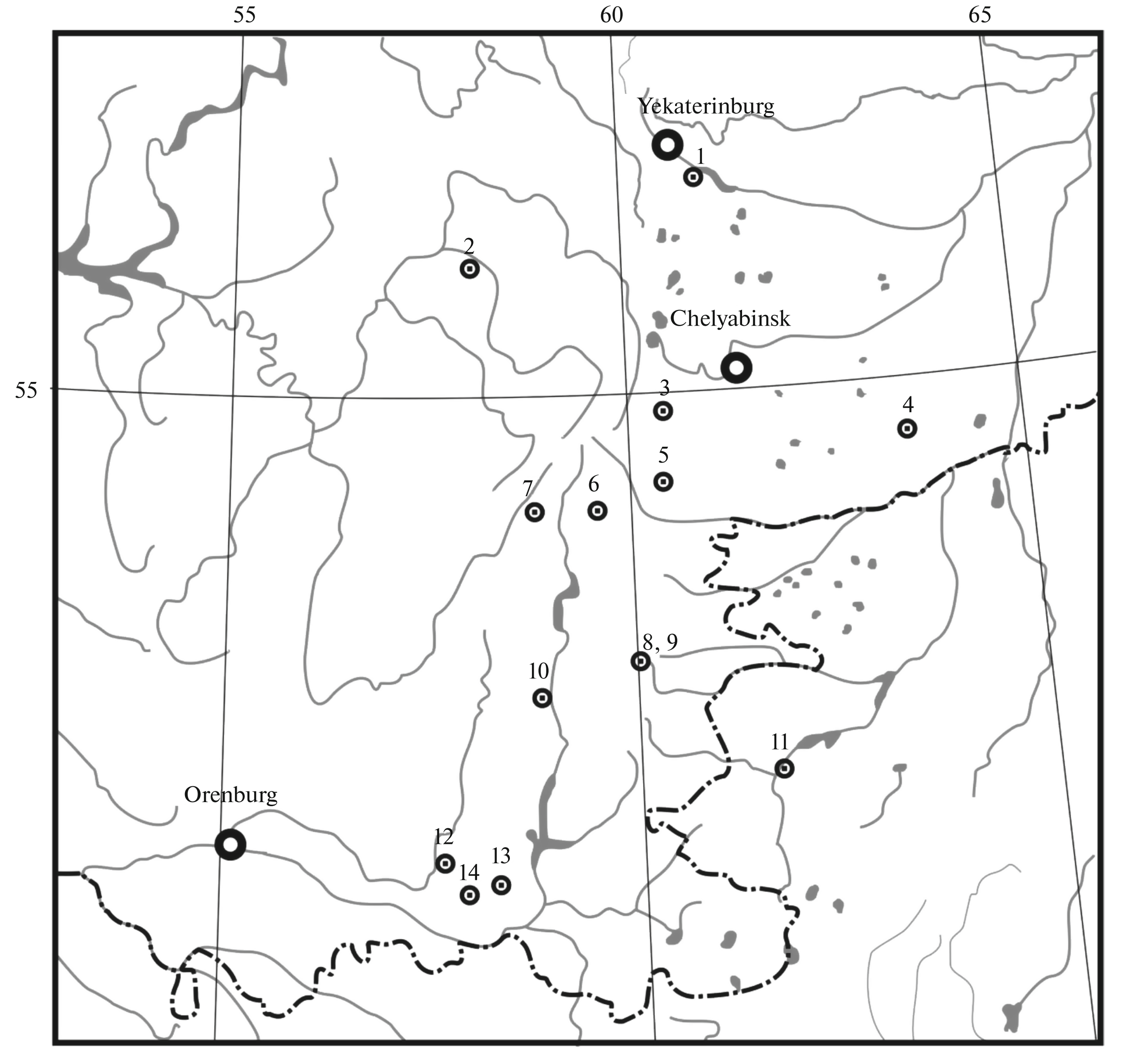

Fig. 1. Research area map. The numbers on the map indicate the sampling sites: 1, Sverdlovsk oblast, Sysertskii region, suburbs of the village of Fomino; 2, Republic of Bashkortostan, Duvansky region, suburbs of the village of Nizhnee Tukbaevo; 3, Chelyabinsk oblast, Emanzhelinskii region, bank of the Uvelka River near the offing of the Sukharysh River; 4, Kurgan oblast, Zverinogolovskii region, suburbs of the settlement of Zverinogolovskoe; 5, Chelyabinsk oblast, Troitskii region, bank of the Ui River, suburbs of the village of Osipovka; 6, Chelyabinsk oblast, Verkhneuralskii region, suburbs of the settlement of Sukhtelinskii, Sheludivye and Ushchel'nye mountains; 7, Chelyabinsk oblast, Verkhneuralskii region, Leonovskie Mountains, Bolshaya Mountain; 8, Chelyabinsk oblast, Bredinskii region, bank of the Karagaily-Ayat River, suburbs of the village of Varshavka; 9, Chelyabinsk oblast, Kartalinskii region, suburbs of the settlement of Elizavetopol'skoe; 10, Chelyabinsk oblast, Kizilskii region, suburbs of the settlement of Zhdanovskii, Mount Cheka; 11, Kazakhstan, Kostanay oblast, Denisovskii region, bank of the Tobol River; 12, Orenburg oblast, suburbs of Mednogorsk; 13, Orenburg oblast, suburbs of Orsk; 14, Orenburg oblast, Gaiskii region, suburbs of the settlement of Guberlya.

All the other studied populations of *M. russiae* inhabit the steppe zone. According to the botanical and geographical zoning of Chelyabinsk (Kulikov, 2005), Orenburg (*Geograficheskii atlas*..., 1999), and Kurgan (Naumenko, 2008) oblasts, the collection sites can be grouped as follows: (1) village of Osipovka, settlement of Sukhtelinskii, village of Varshavka, settlement of Elizavetopol'skoe, Cheka Mountain (subzone of feather-grass and grassy northern steppe); (2) city of Mednogorsk, settlement of Zverinogolovskoe (subzone of herb–bunchgrass steppe); and (3) city of Orsk, settlement of Guberlya (subzone of southern wormwood–grass steppe).

Specific features of the structure, formation, and evolution of the wing pattern of species of the genus *Melanargia* Meigen, 1828 are given in the fundamental

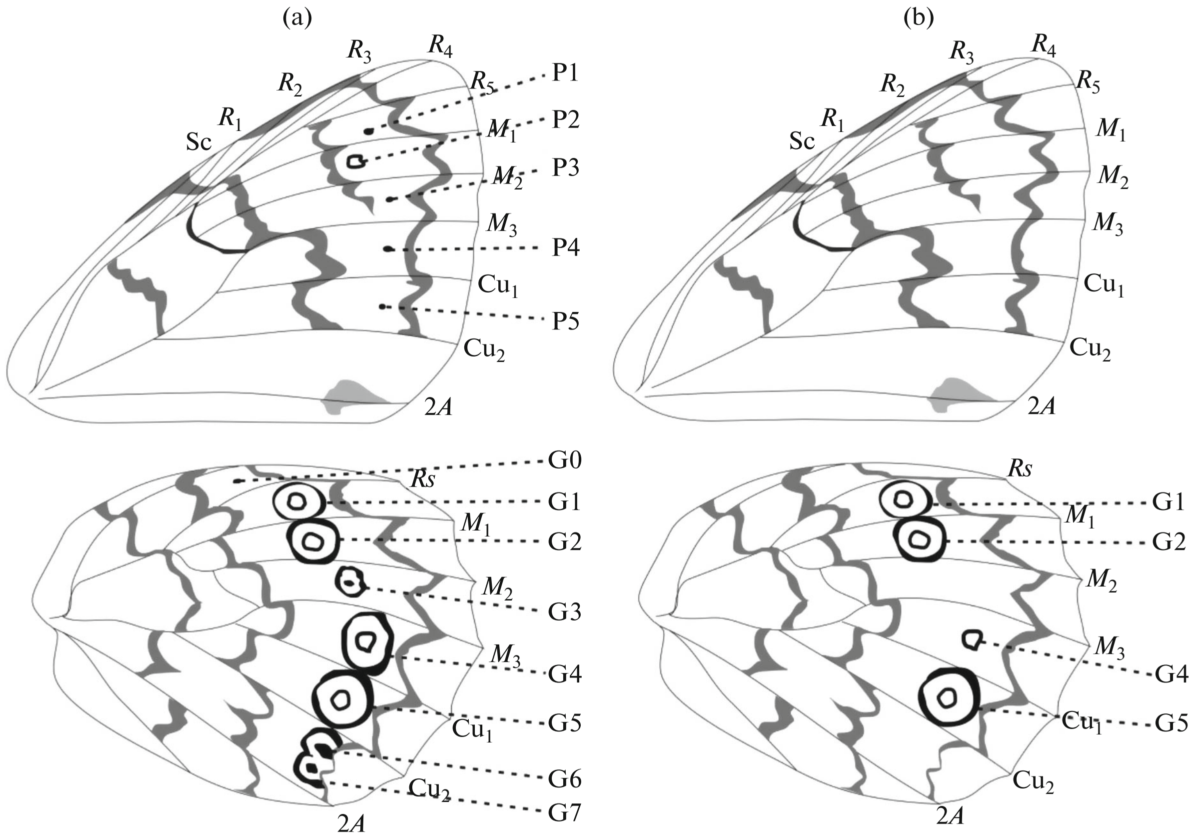

Fig. 2. Eyespots on the wing pattern of *M. russiae*. *A*, maximum possible number of spots on the underside of the forewing P1–P5 and the hindwing G0–G7 (hypothetical situation) and their location in the corresponding wing cells; *B*, minimum number of spots (the detected variant of the wing pattern).

study of Schwanwitsch (1931). We consider the variability of only one type of wing-pattern element: the eyespots. It is well known that eyespots of patches of Satyridae can be absent in the wing pattern and display themselves as a phene, i.e., stable states of threshold features (Vasil'ev, 2005; Zakharova, 2010; etc.). Therefore, during studies of the eyespot variability, they were simultaneously considered nonmetric (phenetic) and metric characters, registering the presence of a spot and its size in a particular cell of the wing of each individual.

M. russiae may have spots in five consecutive cells on the underside of the forewing and in the seven cells of the hindwing. The nomenclature of the wing cells and the designations of the eyespots used in the work are presented in Fig. 2a. We did not find a pattern combination that includes the maximum number of spots on the material; it is hypothetical.

The minimum number of spots was 0 on the forewing and 4 on the hindwing (Fig. 2b). The diameter of the eyespots presented in the figure was measured on the underside of the wings along the median line of the wing cell in which it is located. Measurements were performed on an MBS-10 binocular microscope using an eyepiece micrometer with an increase of 8 × 0.6. Parallel with the diameter measurement, the length of the forewing was measured from the base of the Sc vein to the apex of the wing, and the length of the hindwing was measured from the base of the Rs vein to the apex of the Cu_1 vein. All measurements were made on the left side of the specimen.

The variability of the wing shape was studied using geometric morphometry methods, allowing a quantitative description of the shape of biological objects (Bookstein, 1991; Pavlinov and Mikeshina, 2002; Zelditch et al., 2004; Cardini and Loy, 2013). One important advantage of geometric morphometry methods is the ability to analyze the variability of the actual shape of an object, regardless of its size, and visualize the observed differences.

Wing images were obtained with a Canon Eos 450D digital camera; the height and angle of it were fixed with a tripod. The variability of the shape of the forewing and hindwing on the left side of the specimen was analyzed. In the tpsDig 2.10 software (Rolhf, 2006) nine landmarks were arranged on the images of the forewings (no. 1, 15–22 in Fig. 3a); on the images of hindwings there were ten landmarks (Fig. 3b). In addition, 13 semilandmarks (2–14 in Fig. 3a) were placed on the forewings; they describe the shape of the

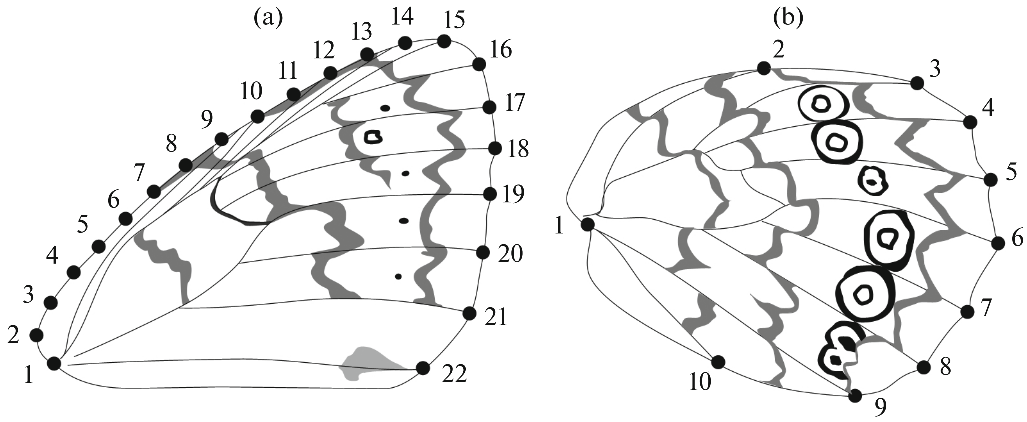

Fig. 3. Arrangement of landmarks and semilandmarks on the fore (a) and hind (b) wings of *M. russiae*.

costal margin of the wing. In geometrical morphometry, various criteria of arrangement are used for landmarks and semilandmarks (Pavlinov and Mikeshina, 2002). The landmarks are placed on structures that are easily homologized for all objects in the structure sample, such as the points of veins branching on the wings of insects. The semilandmarks do not have an exact binding to the structures of the object, but their totality describes the shape of the curve on which they lie.

The area of the fore left wing was calculated during analyzing the variability in the size of *M. russiae*; the area was calculated with the software tpsUtil 1.40 (Rohlf, 2008) as the surface area bounded by landmarks and semilandmarks.

The spot indices, calculated as the ratio of the spot diameter to the wing length, were used as metric features in the variability of the eyespots of the wing-pattern analyses. Differences between samples were estimated using canonical discriminant analysis. The statistical significance of the differences between the samples according to the frequency of occurrence of eyespots phenes was estimated using Pearson χ^2 criterion. The statistical significance of the differences in the size of the individuals was assessed by the area of the forewing using a single-factor analysis of variance (ANOVA) and the a posteriori Tukey's test. All statistical calculations were performed with Statistica 8.0 and Past 2.17c software (Hammer et al., 2001). Differences in the wing shape between samples were estimated using canonical discriminant analysis in MorphoJ 1.04a software (Klingenberg, 2011).

Samples of each sex were analyzed separately.

RESULTS

Analysis of the Size Variability of M. russiae Wings

The results of the measurements showed that the length of the forewing of *M. russiae* from the territory of the Urals averages 29.4 ± 0.2 mm for males and 30.3 ± 0.4 mm for females. According to the average size of the forewing, males (354.9 ± 6.7 mm^2) are smaller than females (360.2 ± 10.6 mm^2).

There is no characteristic of a clinal orientation in this part of the range for the geographical variability of the size of the forewing. As can be seen from Fig. 4a, the smallest males of *M. russiae* were found in the northern forest steppe (N. Tukbaevo and Uvelka). One-factor analysis of variance showed ($F = 4.75$, $df = 12$, $p < 0.01$) the presence of statically significant differences between the sample from N. Tukbaevo and two samples from the subzone of feather-grass–grasses of the northern steppes (Cheka and Elizavetopol'skoe) ($p = 0.04$ and $p = 0.01$, respectively). In addition, the northernmost local population from the vicinity of N. Tukbaevo, inhabited the azonal forest steppe, significantly differs from the easternmost point of sampling, Zverinogolovskoe, from the steppes of the Trans-Urals ($p < 0.01$). In general, the area of the forewing of *M. russiae* is very homogeneous in this part of the range. Thus, there were no statistically significant differences between the samples of females according to this feature. It is interesting to note that all the imago, both males and females caught in the vicinity of the village of Fomino in the subzone of the southern taiga, are larger in size than samples from the main part of the species distribution in the Urals (in the forest-steppe and steppe zones). However, due to the small sample number of material from Fomino (Table 2), the observed differences are statistically insignificant.

Analysis of the Eyespot Variability of M. russiae Wing Pattern

The next stage of the work was analyzing the eyespot variability of the wing pattern of *M. russiae*. In all analyzed samples, all the individuals on the underside of the forewing have a spot in the M_1–M_2 cell, specified by us as P2. The only exception out of 592 specimens was found in the form of an asymmetric combi-

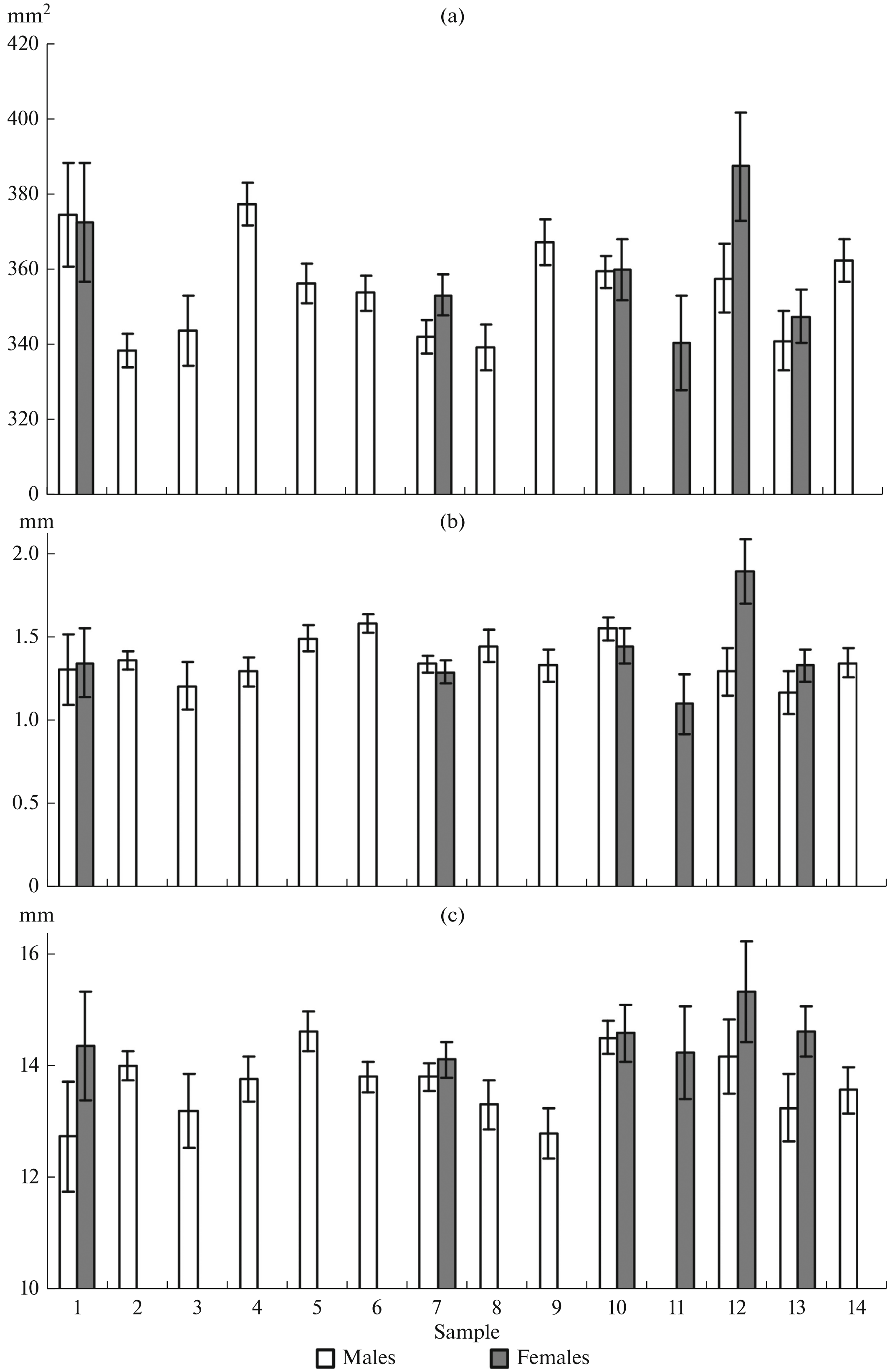

Fig. 4. Variability of the forewing area (a), total diameter of the eyespots on the fore (b) and hind (c) wings in males and females of *M. russiae* from the territory of the Urals. The numbering of samples corresponds to the sampling sites on the map in Fig. 1. Average values and standard errors are given.

Table 3. Frequency of occurrence (%) of eyespots phenes in *M. russiae* wing pattern

No.	Sample	Sex	P1	P3	P4	P5	G0	G3	G6	G7
1	Fomino	Males	0.0	0.0	0.0	0.0	0.0	20.0	100.0	80.0
		Females	0.0	20.0	20.0	0.0	0.0	25.0	100.0	100.0
2	N. Tukbaevo	Males	8.2	8.2	11.0	2.7	4.2	29.2	100.0	88.9
3	Uvelka	Males	0.0	0.0	9.1	0.0	0.0	0.0	100.0	100.0
4	Zverinogolovskoe	Males	13.3	10.0	3.3	3.3	0.0	16.7	100.0	80.0
5	Osipovka	Males	35.1	35.1	5.4	0.0	0.0	23.1	100.0	92.3
6	Sukhtelinskii	Males	36.9	54.5	6.1	0.0	0.0	41.8	98.5	89.1
7	Leonovskie mountains	Males	7.7	21.8	5.1	1.3	1.3	41.0	100.0	87.0
		Females	25.5	34.0	2.1	0.0	0.0	27.7	100.0	93.6
8	Varshavka	Males	12.0	40.0	0.0	0.0	4.0	32.0	100.0	80.0
9	Elizavetopol'skoe	Males	29.2	33.3	0.0	0.0	4.0	32.0	96.0	79.2
10	Cheka	Males	38.9	37.0	7.4	0.0	7.4	25.9	98.1	83.0
		Females	36.8	36.8	0.0	0.0	0.0	15.8	100.0	100.0
11	Tobol	Females	14.3	14.3	0.0	0.0	0.0	37.5	100.0	100.0
12	Mednogorsk	Males	18.2	45.5	0.0	0.0	0.0	36.4	100.0	90.9
		Females	16.7	33.3	16.7	0.0	0.0	16.7	100.0	100.0
13	Orsk	Males	6.3	6.3	0.0	0.0	0.0	13.3	100.0	61.5
		Females	14.8	18.5	7.4	0.0	3.7	34.6	100.0	92.0
14	Guberlya	Males	10.7	35.7	0.0	3.6	3.6	42.9	100.0	78.6

nation with a complete absence of eyespots on the left side of the forewing (♂, June 9, 2012, near the settlement of Sukhtelinskii). This structure variant is illustrated in Fig. 2b. On the right side of this specimen, the P2 spot is presented. The remaining spots on the forewing (P1, P3, P4, and P5) were discrete in their display, phenes, and often absent in the pattern. In the hindwing pattern, four spots (G1, G2, G4, and G5) were found in all individuals in the sample (Fig. 2), and the spots G0, G3, G6, and G7 were discrete (phenetic). The frequencies of occurrence of eyespots phenes of the wing pattern for all the studied samples are shown in Table 3.

To determine the statistical significance of the differences between the samples for the frequency of eyespot occurrence on the *M. russiae* wing pattern, we calculated the values of the Pearson χ^2 test (Table 4). There are usually no significant differences between samples from the close-located geographical points, for example, Varshavka and Elizavetopol'skoe (about 10 km) and Mednogorsk and Guberlya (about 40 km). Between the outlying local populations, the differences in the frequencies of occurrence of phenes are, as a rule, significant (Table 4). The samples from the marginal northern populations, N. Tukbaevo and Uvelka, differ by a certain phenetic distinctness. The males from the vicinity of the village of Fomino do not have phenes P1, P3, P4, P5, and G0 in the wing pattern; females do not have P1, P5, and G0 phenes (Table 3). Obviously, the probability of finding an individual with a rare sign depends on the total sample size, so our material allows only to assume that a small local *M. russiae* population was formed in the vicinity of the village of Fomino. This population is characterized by its own unique phenotype with the reduction of additional spots in the wing pattern.

The variability of the size of the eyespots in the *M. russiae* wing pattern is illustrated in Fig. 4. For clarity, we summarized all the average values of the spot diameters separately for the forewing (Fig. 4b) and hindwing (Fig. 4b) within each sample. Like other species of the family, the eyespots of females are larger than of males. The differences between the samples of males according to the total diameter of the pattern spots are statistically significant both in the forewings ($F = 2.28$, $df = 12$; $p < 0.001$) and in the hindwings ($F = 1.79$; $df = 12$; $p < 0.05$) based on the ANOVA results. Between the samples of females, the differences are insignificant.

Based on the spot size indices calculated as the ratio of the spot diameter to the wing length, we estimated the differences between the samples using canonical discriminant analysis (Fig. 5). For samples of male Wilks $\lambda = 0.49$, $F = 1.97$; $df\ 1 = 156$; $df\ 2 = 3682$; $p < 0.01$. For samples of females Wilkes $\lambda = 0.43$, $F = 1.44$; $df\ 1 = 55$; $df\ 2 = 397$; $p < 0.05$, which indicates the existence of significant differences between the comparative samples according to the size of the spots of the wing pattern. As can be seen from Fig. 5a, the sampling of males from Fomino has a significant

Table 4. Pearson χ^2 values for samples of male (upper triangular matrix) and female (lower triangular matrix) *M. russiae* according to the frequency of occurrence of phenes of eyespots of the wing pattern

No.	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Fomino	–	**30.8**	**31.1**	**28.70**	**62.00**	**326.8**	**36.2**	**49.9**	**59.3**	**75.2**		**54.0**	**15.9**	**51.2**
2	N. Tukbaevo		–	**49.7**	12.2	**40.5**	**54.4**	12.0	**35.6**	**40.6**	**39.6**		**45.8**	**20.1**	**31.1**
3	Uvelka			–	**47.6**	**83.4**	**114.0**	**69.4**	**93.5**	**103.3**	**98.4**		**98.7**	**43.0**	**100.8**
4	Zverinogolovskoe				–	**21.9**	**40.4**	**16.1**	**30.3**	**29.9**	**33.4**		**30.6**	9.2	**28.8**
5	Osipovka					–	7.7	**27.3**	**22.5**	12.0	8.6		**15.1**	**33.6**	**32.2**
6	Sukhtelinskii						–	**27.8**	**21.2**	**13.4**	**13.6**		**11.6**	**51.9**	**28.0**
7	Leonovskie Mountains	**43.4**						–	**15.5**	**24.2**	**32.6**		**19.5**	**22.2**	11.5
8	Varshavka								–	7.8	**22.0**		5.7	**27.2**	5.4
9	Elizavetopol'skoe									–	9.8		9.1	**32.4**	**13.9**
10	Cheka	**62.9**						7.7			–		**25.2**	**44.7**	**31.8**
11	Tobol	**37.7**						**14.6**			**27.8**	–			
12	Mednogorsk	**21.4**						**16.1**			**24.4**	**32.0**	–	**30.9**	11.2
13	Orsk	**26.2**						**14.6**			**33.4**	12.1	**18.0**	–	**31.1**
14	Guberlya														–

χ^2 indices with $p < 0.01$ are marked in semibold.

morphological distinctness and differs significantly from samples from some steppe habitats (for example, Osipovka, Cheka, and Elizavetopol'skoe). It turned out that *M. russiae* males caught in the vicinity of the village of Fomino were characterized not only by the absence of five phenetic eyespots in the wing pattern, but also by a certain size ratio of the existing spots (Fig. 4). With a relatively large whole size of the individual, a single large P2 spot develops on the forewing and, on the hindwing, small G1–G7 spots. However, females from Fomino do not differ in spot size from the females from steppe habitats. The *M. russiae* boundary northern population from N. Tukbaevo has a specific background habitus (Fig. 5a). Here the males have relatively small wings with large spots on the wing pattern (Fig. 4).

Analysis of Variability in Shape of M. russiae Wings

According to the results of the canonical discriminant analysis of procrustean excesses characterizing the variability of the male forewing forms, the morphological distinctness of the sample from N. Tukbaevo is exhibited on canonical axis 1, which accounts for 24.8% of intergroup variance, and the distinctness of Fomino sample is on canonical axis 5 (8.7% intergroup variance) (Fig. 6a). Results of an analysis of variability in female forewing forms showed that differences in Fomino imago emerged from the other in the space of canonical axes 3 and 5, which account for 19.0 and 7.7% of intergroup variance, respectively (Fig. 6b).

According to the shape of the forewing, males from N. Tukbaevo statistically significantly differ from all others, except for the samples from Orsk and Fomino. Due to the fact that only five *M. russiae* imago of each sex were caught in the vicinity of the village of Fomino, it is impossible to assess the statistical significance of the differences in the shape of the forewing of these individuals from the others. However, it is possible to directly visualize differences in the shape of the wing between groups that are discriminated by canonical axes.

The forewings of *M. russiae* males from N. Tukbaevo are characterized by an almost noncurved extraneous margin and a more rounded apex, unlike males from other habitats (Fig. 7a). For males from Fomino, forewings with extraneous and anal margin convergence at a blunter tornal angle than in males from other geographical points are specific (Fig. 7b). In females from Fomino, the forewings are more elongated, with a pointed apex, and also with a blunter tornal angle than in individuals from other habitats (Fig. 7b). In general, the shape of the *M. russiae* forewing is heterogeneous in most of the studied range, which is confirmed by the results of the canonical discriminant analysis of samples of males (Wilkes λ = 0.075, $F = 1.81$, $df1 = 528$, $df2 = 3800$, $p < 0.01$) and females (Wilkes λ = 0.025, $F = 1.39$, $df1 = 220$, $df2 = 279$, $p < 0.01$). However, significant differences were not found between all groups. There were no clinal regularities in the geographical variability of the shape of the forewing of *M. russiae* on the studied part of the range.

According to the results of the canonical discriminant analysis of the shape of the forewing, the morphological uniqueness of the sample of males from N. Tukbaevo was exhibited by canonical axis 1 (22.9% of the intergroup dispersion) and, of the samples from

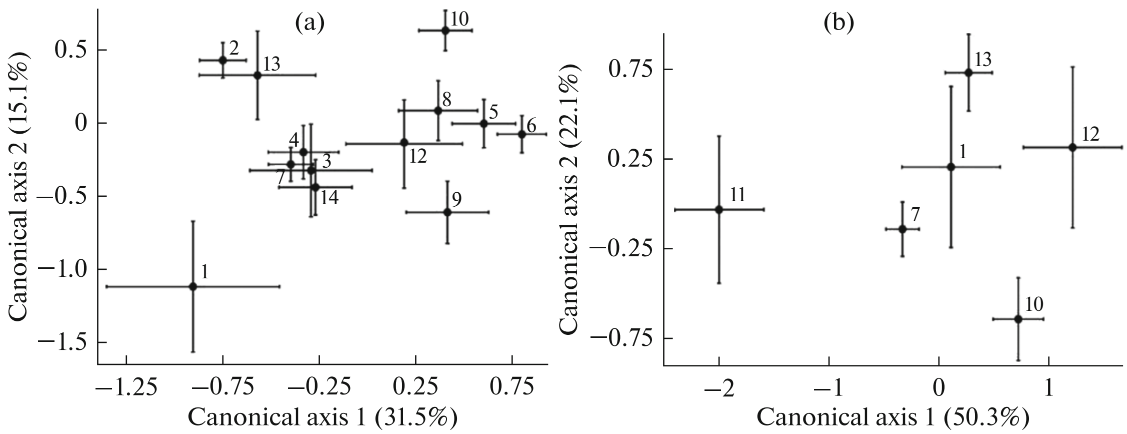

Fig. 5. Results of the canonical discriminant analysis of the geographic variability in the wing spots sizes of *M. russiae* male (a) and female (b) from the territory of the Urals. The numbering of samples corresponds to the sampling sites on the map in Fig. 1. Centroids of samples are given taking into account the values of standard errors.

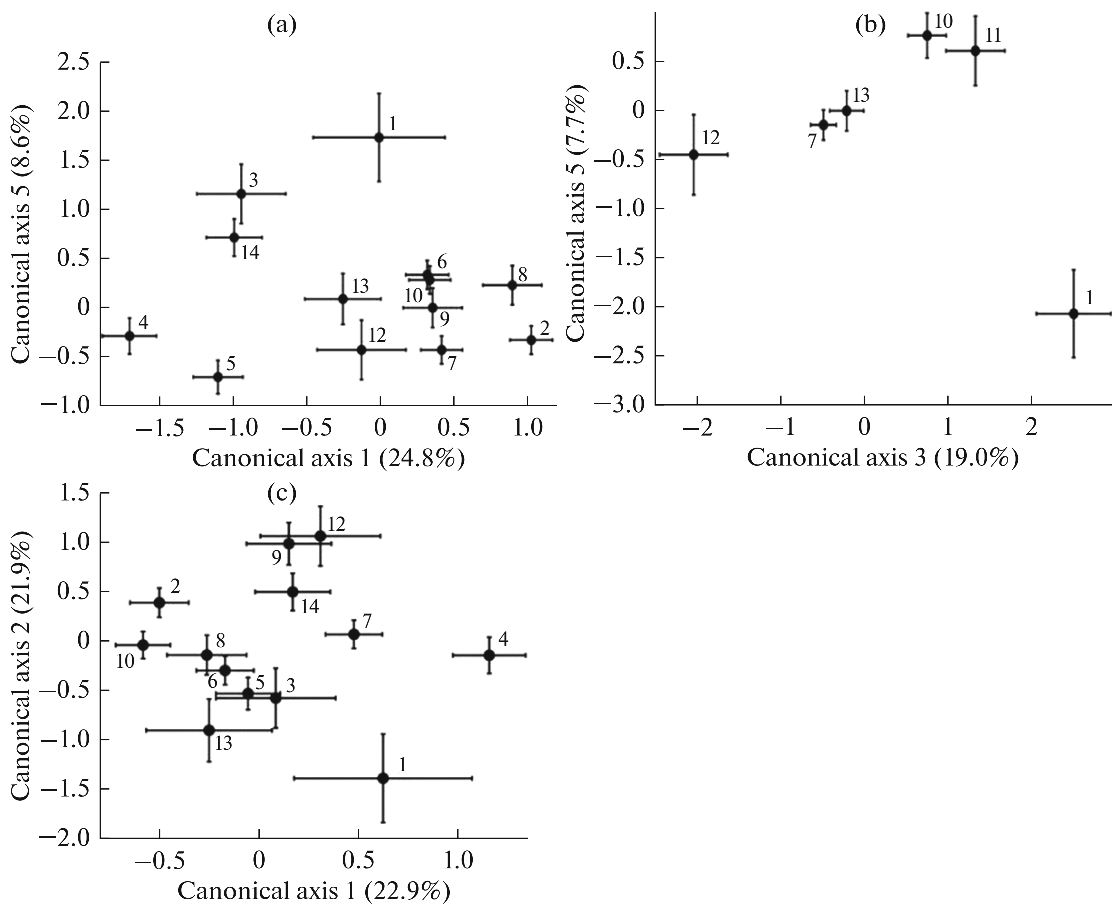

Fig. 6. Results of the canonical discriminant analysis of the geographical variability in the shape of the forewing of *M. russiae* males (a), females (b), and the hindwing of males (c) from the territory of the Urals. The numbering of samples corresponds to the sampling sites on the map in Fig. 1. The sample centroids are given taking into account standard errors

Fomino, by canonical axis 2 (21.9% of the intergroup dispersion) (Fig. 6b). Samples of females do not differ from each other by the shape of the hindwing.

Wider and shorter hindwings are specific for males from N. Tukbaevo in comparison with other analyzed groups (Fig. 7d). It is interesting to note that the males from Zverinogolovskoe, the easternmost of all the studied geographical points, mostly differed in the shape of the hindwing from the males from the azonal Mesyagutovskaya forest steppe. These imagoes are

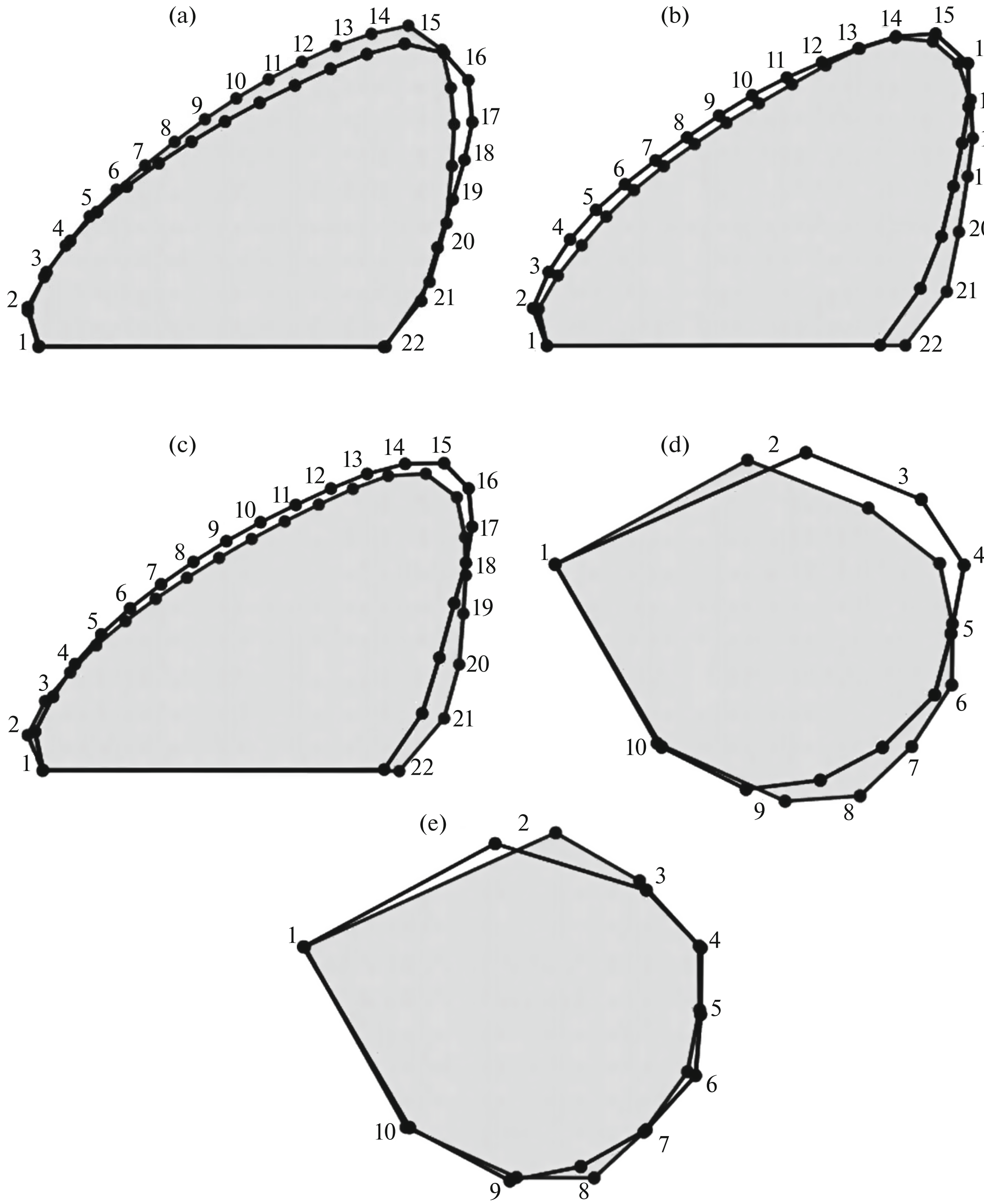

Fig. 7. *M. russiae* wings configurations, characterizing the differences in shape between different populations. The black outline denotes (a, d) forewings and hindwings of males from N. Tukbaevo; (b, e) fore and hindwings of males from Fomino; and (c) forewing of females from Fomino. The gray outline denotes generalized configurations of the wings of individuals from all other studied habitats.

characterized by the narrowest and elongated hind wings. The males from Fomino have hindwings with an angular extraneous margin, while males from other habitats are characterized by more rounded wings (Fig. 7e). In the shape of the hindwing, *M. russiae* males are heterogeneous in the studied part of the range (Wilkes $\lambda = 0.336$, $F = 1.69$; $df1 = 240$; $df2 = 3649$; $p < 0.01$). Nevertheless, we have not revealed any regularity in the nature of the geographic variability of the shape of the hindwing or of the fore one.

DISCUSSION

In the last 10–15 years, the climate in the south of the Middle Urals has become warmer and drier. These changes are accompanied by the response of the insect populations. We managed to directly observe the expansion to the north of the range boundaries of the west–central Eurasian subboreal Lepidoptera species, *M. russiae*, sensitive to changes in factors such as temperature and humidity. During a long period of entomological observations and student practices conducted on the basis of the biology research station of the Ural Federal University (near the village of Fomino in Sverdlovsk oblast), *M. russiae* was not registered. However, since 2012, we have caught ten individuals of this species, which allowed us to make an assumption about the formation of a new local population in this area. An alternative hypothesis was that all *M. russiae* representatives were migrants to the pre-forest-steppe pine–birch forest subzone from the northern forest steppe.

In insect ecology, findings of new local populations and shifts in range boundaries due to climate change are frequent and have been repeatedly described (Wilson et al., 2005; Illan et al., 2012; etc.). For some species of the genus *Melanargia* (*M. galathea* (Linnaeus, 1758) and *M. lachesis* (Hübner, 1790)), the ways of post-glacial distribution in Europe (Habel et al., 2005; 2011) and features of migration activity and spatial structure of populations (Baguette et al., 2000) have been studied. However, there are few studies on the regularities of morphological variability under the range boundaries changes. For example, an attempt to compare a number of morphological characters (mass of the breast, abdomen, and shape and size of the wings) was made for two subspecies of *Pararge aegeria* (Linnaeus, 1758) under the widening of the range and the repopulation of the territories. The authors (Hill et al., 1999) believed that the morphological differences in the structure of the wing and in the indices of the thorax muscle mass may indicate a different load on the wing and, as a consequence, different flight-behavior strategies. The morphological variability of the imago in combination with the subspecies features of the preimago stages has an important evolutionary significance in the further distribution and territorial dissociation of subspecies.

We analyzed the variability of the complex of morphological traits of the wings (size, shape, and elements of the wing pattern) to test the hypothesis of the formation of *M. russiae* local population in the vicinity of the village of Fomino. Unfortunately, the amount of available material was small (5 ♂♂, 5 ♀♀), which affects the statistical significance of the differences. However, even now we can draw some conclusions. All captured individuals, both males and females, were characterized by large wings, the absence of some phenic eyespots on the wing pattern, certain proportions of sizes of the existing spots, and some peculiarity of the shape of the forewings and hindwings. All the observed morphological features indicate the similarity of the imago from Fomino to each other and their differences from the imago of the forest-steppe and steppe habitats of the Urals. The result speaks in favor of the hypothesis of a new local population of *M. russiae* formation in the subzone of pre-forest-steppe pine–birch forests of the taiga zone of the Middle Urals.

M. russiae population from the vicinity of the village Nizhnee Tukbaevo inhabiting the unique plant communities of the Mesyagutovskaya forest steppe is of no less interest. Here, the area suitable for *M. russiae* habitats is limited by the island of the azonal forest steppe, as well as by the significant anthropogenic transformation of the lands due to plowing and overgrazing. Thus, *M. russiae* of the Mesyagutovskaya forest steppe is isolated to some extent from other populations from the southern forest-steppe and steppe territories. Partial isolation and existence at the edge of the range determined the formation of a peculiar phenotype: imagoes have wide, short, and small wings with relatively large eyespots. This population can be considered as "forpost" (Vasil'ev, 2009; 2012) characterized by rapid morphogeny processes.

The results of a comparison of morphological features of *M. russiae* populations from Fomino and N. Tukbaevo indicate the presence of statistically significant differences between them in terms of the set of studied features. Imagoes from Fomino were more similar, despite their peculiarity, to the imago of Uvelka habitats in the subzone of the northern forest steppe of Chelyabinsk oblast. Apparently, the *M. russiae* local population of pre-forest-steppe pine–birch forests in the south of Sverdlovsk oblast was formed by individuals migrating from the southeast, from the Trans-Ural part of the range, but not from Bashkortostan. Further observations of the state of populations and the studies on the morphological variability of *M. russiae* in the Urals would make it possible to establish the probable ways it migrates to the north under the conditions of the current climate warming in this region.

ACKNOWLEDGMENTS

We are grateful to our entomologist colleagues P.Yu. Gorbunov, P.V. Rudoiskatel', T.K. Tuneva, K.I. Fadeev, O.E. Chaschina, M.V. Chibiryak, and Yu.M. Chibiryak, as well as to the students of the Ural Federative University for their help in material samplings.

This work was supported by "Wild Life" Program no. 12 of the Ural Branch of the Russian Academy of Sciences, project no. 15-12-4-25 and by the Russian Foundation for Basic Research, project no. 16-04-01831a.

REFERENCES

Baguette, M., Petit, S., and Quéva, F., Population spatial structure and migration of three butterfly species within the same habitat network: consequences for conservation, *J. Appl. Ecol.*, 2000, vol. 37, no. 1, pp. 100–108.

Bale, J.S., Masters, G.J., Hodkinson, I.D., et al., Herbivory in global climate change research: direct effects of rising temperature on insect herbivores, *Global Change Biol.*, 2002, vol. 8, pp. 1–16.

Bálint, Z. and Katona, G., Notes on the Hungarian populations of *Melanargia russiae* (Esper, 1783) extinct since a hundred years (Lepidoptera: Nymphalidae, Satyrinae), *Ann. Hist.-Nat. Mus. Natl. Hung.*, 2013, vol. 105, pp. 179–198.

Battisti, A., Forests and climate change—lessons from insects, *iForest–Biogeosci. For.*, 2008, no. 1, pp. 1–5.

Bookstein, F.L., *Morphometric Tools for Landmark Data: Geometry and Biology*, New York: Cambridge Univ. Press, 1991.

Cardini, A. and Loy, A., On growth and form in the "computer era": from geometric to biological morphometrics, *Hystrix*, 2013, vol. 24, no. 1, pp. 1–5.

Doklad ob osobennostyakh klimata na territorii Rossiiskoi Federatsii za 2014 g. (A Report on Specific Climate in Russian Federation in 2014), Moscow, 2015.

Geograficheskii atlas Orenburgskoi oblasti (Geographical Atlas of Orenburg Oblast), Chibilev, A.A., Ed., Moscow: DIK, 1999.

Gorbunov, P. and Kosterin, O., *The Butterflies (Hesperioidea and Papilionoidea) of North Asia (Asian part of Russia) in Nature*, Moscow: Rodina & Fodio, 2007, vol. 2.

Gorbunov, P.Yu. and Ol'shvang, V.N., Fauna of butterflies (Lepidoptera, Rhopalocera) from Southern, Central, and Northern Ural, in *Uspekhi entomologii na Urale (Advances in Entomology of Ural)*, Yekaterinburg: Ural. Otd., Russ. Entomol. O-va, 1997, pp. 88–97.

Habel, J.C., Schmitt, T., and Müller, P., The fourth paradigm pattern of post-glacial range expansion of European terrestrial species: the phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera), *J. Biogeogr.*, 2005, vol. 32, pp. 1489–1497.

Habel, J.C., Lens, L., Rödder, D., and Schmit, T., From Africa to Europe and back: refugia and range shifts cause high genetic differentiation in the Marbled White butterfly *Melanargia galathea, BMC Evol. Biol.*, 2011, vol. 11, no. 215. doi doi 10.1186/1471-2148-11-215

Hammer, O., Harper, D.A.T., and Ryan, P.D., PAST: paleontological statistics software package for education and data analysis, *Palaeontol. Electron.*, 2001, vol. 4, no. 1.

Hill, J.K., Thomas, C.D., and Blakeley, D.S., Evolution of flight morphology in a butterfly that has recently expanded its geographic range, *Oecologia*, 1999, vol. 121, pp. 165–170.

Illan, J.G., Gutiérrez, D., Diez, S., and Wilson, R., Elevational trends in butterfly phenology: implications for species responses to climate change, *Ecol. Entomol.*, 2012, vol. 37, pp. 134–144.

Ivonin, V.V., Kosterin, O.E., Nikolaev, S.L., Butterflies (Lepidoptera, Diurna) of Novosibirsk oblast, Russia. 1. Hesperiidae, Papilionidae, Pieridae, *Evraziat. Entomol. Zh.*, 2009, vol. 8, no. 1, pp. 85–104.

Klingenberg, C.P., MorphoJ: an integrated software package for geometric morphometrics, *Mol. Ecol. Res.*, 2011, vol. 11, pp. 353–357.

Knyazev, S.A., Butterflies (Lepidoptera, Diurna) of Omsk oblast, Russia, *Evraziat. Entomol. Zh.*, 2009, vol. 8, no. 4, pp. 441–461.

Korshunov, Yu.P., Fauna and biotope location of Rhopalocera insects in Northern Baraba, in *Fauna i ekologiya nasekomykh Sibiri* (Fauna and Ecology of Insects in Siberia), Novosibirsk: Nauka, 1974, pp. 32–39.

Krasheninnikov, I.M. and Vasil'ev, Ya.Ya., Forest-steppe of the western slope of Southern Ural, *Tr. Pochv. Inst. im. V.V. Dokuchaeva, Akad. Nauk SSSR*, 1949, vol. 30, pp. 143–178.

Kulikov, P.V., *Konspekt flory Chelyabinskoi oblasti (sosudistye rasteniya)* (Catalogue of Flora of Chelyabinsk Oblast: Vascular Plants), Yekaterinburg: Geotur, 2005.

Kulikov, P.V., Zolotareva, N.V., and Podgaevskaya, E.N., *Endemichnye rasteniya Urala vo flore Sverdlovskoi oblasti* (Endemic Plants of Ural in Flora of Sverdlovsk Oblast), Yekaterinburg: Goshchitskii, 2013.

Metody otsenki posledstvii izmeneniya klimata dlya fizicheskikh i biologicheskikh sistem (Evaluation of Climate Change Consequences for Physiological and Biological Systems), Moscow: Rosgidromet, 2012.

Musolin, D.L. and Saulich, A.Kh., Responses of insects to the current climate changes: from physiology and behavior to range shifts, *Entomol. Rev.*, 2012, vol. 92, no. 7, pp. 715–740.

Naumenko, N.I., *Flora i rastitel'nost' yuzhnogo Zaural'ya* (Flora and Vegetation of Southern Trans-Ural Region), Kurgan: Kurgansk. Gos. Univ., 2008.

Parmesan, C., Ecological and evolutionary responses to climate changes, *Annu. Rev. Ecol. Evol. Syst.*, 2006, vol. 37, pp. 637–669.

Parmesan, C. and Yohe, G., A globally coherent fingerprint of climate change impacts across natural systems, *Nature*, 2003, vol. 421, pp. 37–42.

Pavlinov, I.Ya. and Mikeshina, N.G., Principles and methods of geometric morphometry, *Zh. Obshch. Biol.*, 2002, vol. 63, pp. 473–493.

Rohlf, F.J., *TpsDig Version 2.10. Ecology & Evolution: Program*, New York: Suny at Stony, 2006.

Rohlf, F.J., *TpsUtil Version 1.40. Ecology & Evolution: Program* New York: Suny at Stony, 2008.

Rubtsov, V.V. and Utkina, I.A., Phyllophages of forest ecosystems affected by climate change, *Vestn. Mariisk. Gos. Tekh. Univ.*, 2010, no. 3, pp. 3–15.

Saarinen, K., Lanti, T., and Marttila, O., Population trends of Finnish butterflies (Lepidoptera: Hesperioidea, Papilionoidea) in 1991–2000, *Biodiversity Conserv.*, 2003, vol. 12, pp. 2147–2159.

Schwanwitsch, B.N., Evolution of the wing-pattern in palaearctic Satyridae. II. Genus *Melanargia, Z. Morphol. Ökol. Tiere*, 1931, no. 21, pp. 316–408.

Settele, J., Kudrna, O., Harpke, A., et al., Climatic risk atlas of European butterflies, *BioRisk*, 2008, vol. 1, pp. 1–712.

Shvartsman, Yu.G., Bolotov, I.N., and Iglovskii, S.A., Impact of climate change on environment of European North, *Materialy nauchno-prakticheskoi konferentsii "Global'noe izmenenie klimata: naychnye dannye, problemy i reshsniya. Regional'nyi aspekt"* (Proc. Sci.-Pract. Conf. "Global Climate Change: Scientific Data, Problems and Solutions. Regional Aspects"), Arkhangelsk, 2007, pp. 14–21.

Sparks, T., Dennis, R., Croxton, Ph., and Cade, M., Increased migration of Lepidoptera linked to climate change, *Eur. J. Entomol.*, 2007, vol. 104, pp. 139–143.

Tatarinov, A.G., Landscape-zonal distribution of butterflies (Lepidoptera, Papilionoidea, Hesperioidea) in the northeast of the Russian Plain, *Entomol. Rev.*, 2013, vol. 93, no. 1, pp. 56–68.

Tatarinov, A.G. and Gorbunov, P.Yu., The structure and spatial organization of the butterfly fauna (Lepidoptera, Rhopalocera) of the Ural Mountains, *Entomol. Rev.*, 2014, vol. 94, no. 4, pp. 541–561.

Ukhova, N.L. and Ol'shvang, V.N., *Bespozvonochnye zhivotnye Visimskogo zapovednika. Annotirovannyi spisok vidov* (Invertebrates of Visimskiy Nature Reserve. Annotated List of Species), Yekaterinburg: Raritet, 2014.

Van Swaay, C.A.M. and Warren, M.S., Red Data Book of European butterflies (Rhopalocera), in *Nature and Environment No. 99*, Strasbourg: Council of Europe, 1999.

Van Swaay, C., Warren, M., and Loïs, G., Biotope use and trends of European butterflies, *J. Insect Conserv.*, 2006, vol. 10, pp. 189–209.

Vasil'ev, A.G., *Epigeneticheskie osnovy fenetiki: na puti k populyatsionnoi meronomii* (Epigenetic Principles of Phenetics: Towards Meronomy), Yekaterinburg: Akademkniga, 2005.

Vasil'ev, A.G., Fast epigenetic transformations of populations as one of the probable mechanisms of the global biocenotic crisis, *Biosfera*, 2009, vol. 1, no. 2, pp. 166–177.

Vasil'ev, A.G., The problem of stability of forpost populations and communities: from theory to evaluation methods, *Materialy IV nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem "Biologicheskie sistemy: ustoichivost', printsypy i mekhanizmy funktsionirovaniya"* (Proc. IV Sci.-Pract. Conf. with Int. Participation "Biological Systems: Resistance, Principles, and Mechanisms"), Nizhny Tagil: Nizhetagilsk. Gos. Sots.-Pedagog. Inst., 2012, part 1, pp. 76–80.

Weather and climate. http://www.pogodaiklimat.ru/file.htm

Wilson, R.J., Gutiérrez, D., Gutiérrez, J., et al., Changes to the elevational limits and extent of species ranges associated with climate change, *Ecol. Lett.*, 2005, vol. 8, pp. 1138–1146.

Zakharova, E.Yu., Seasonal variability of wing length and eyespots in populations of *Erebia ligea* (L.) (Lepidoptera, Satyridae) in the Middle Urals, *Entomol. Rev.*, 2010, vol. 90, no. 6, pp 669–678.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., et al., *Geometric Morphometrics for Biologist: A Primer*, New York: Elsevier, 2004.

Translated by T. Kuznetsova