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Phenotypic Variability of Eye-Spots in Natural Populations of *Coenonympha pamphilus* L. (Lepidoptera, Satyridae)

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Abstract—Variation of eye-spots in the wing pattern of *Coenonympha pamphilus* (Linnaeus, 1758) was studied in the Urals with adjacent territories and in Dzhelal-Abad Province of Kyrgyzstan. Discriminant analysis of the wing length and eye-spot diameter and the phenetic distances calculated by the Hartman's method revealed clinal variation of the characters. The butterflies in the northern parts of the range (Sverdlovsk and Kurgan Provinces) are smaller than those in the south (Orenburg and Chelyabinsk Provinces) and have fewer eye-spots which themselves are smaller. The Kyrgyzstan specimens differ significantly from the Ural ones (the nominotypical subspecies) in both quantitative and qualitative characteristics of the wing pattern, and should probably be assigned to the subspecies *C. pamphilus marginata* Heyne, 1894. As in most Satyridae, females of *C. pamphilus* are larger than males and on the average possess more eye-spots on the wings. The degree of phenotypic variation in natural populations was studied using the method of variation spectra (Kovalenko, 1996a, 1996b, 2007, etc.). The arrangement of phenotypic combinations within the theoretical spectrum (*St*) matrix allows one to describe the actual (*Sr*) and potential (*Sp*) spectra for either sex and for the species as a whole. The actual spectrum was found to be considerably broader in males than in females. With minor variations, *Sr* and *Sp* showed the same general trends in different samples of both sexes. In all the samples, phenetic combinations with one spot on the fore wing (in cell M_1–M_2) were predominant. The hind wing had either no spots (which was typical of males, especially in the northern parts of the range) or the maximum possible number, six (mostly in females, more often in the southern parts of the range). The phenetic combinations with the maximum (4) number of spots on the fore wing and the minimum number (0) on the hind wing are prohibited for *C. pamphilus*.

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The small heath *Coenonympha pamphilus* (Linnaeus, 1758) is a broadly distributed Trans-Eurasian species. In the Crimea and Caucasus this species has 2–3 generations per year, the flight period extending over the entire warm season, from May till October (Nekrutenko, 1985, 1990). In Siberia, according to Yu.P. Korshunov (2002), there is only one generation in the south of the forest zone, emerging in June, whereas in more southern forest-steppe and steppe areas, the species has two generations, the flight occurring in May–June and in late July–August. In the South Urals, the species also usually has two generations (Olshvang et al., 2004). The small heath larvae develop on various grasses (*Poa*, *Anthoxanthum*, *Cynosurus*, *Dactylis*, *Festuca*, *Nardus*, etc.).

Within its entire distribution range *C. pamphilus* shows considerable variation in size, general wing color, and number and diameter of eye-spots in the wing pattern. According to P.Y. Gorbunov (2001), geographic variation has a clinal nature, the butterflies from southern areas being characterized by a wider and more distinct dark grey wing margin; they were described as var. *marginata* Heyne [1894]. The spring and summer generations can be distinguished using the same characters.

In his classical work on the butterflies of the genus *Coenonympha*, D. Davenport (1941) characterized *C. pamphilus* as one of the most variable species of the genus and one of the species posing the greatest problem for a taxonomist, except perhaps *arcania* with its forms. On the one hand, there is extensive information on the species variation within the major part of its range; on the other hand, many subspecies, races, and other forms of unclear taxonomic rank have been described, complicating the nomenclature. In particular, in addition to 5 more or less distinct subspecies, D. Davenport listed 46 races and seasonal forms described by various authors. He doubted the appropriateness of the excessively detailed nomenclature used by R. Verity (1926), who named over 30 races and seasonal forms of *C. pamphilus*. Both authors believed, however, that studies of the variability of this

Table 1. Distribution and morphological specificity of subspecies of *Coenonympha pamphilus* (Linnaeus, 1758) (after Davenport, 1941)

Subspecies	Range	Morphological specificity
pamphilus Linnaeus, 1758	Northern and central Eurasia between 45° and 66° N (type locality: "Sweden")	Average size of males 27–28 mm; females slightly larger and paler. Upperside ochre-colored with narrow gray edge and pale margin. Underside of fore wing ochre-colored with one apical eye-spot, grayish apex, and pale wavy band behind the eye-spot. Hind wings ash-brown to gray with darker proximal portion, separated from distal one by pale wavy band, which varies in intensity and may be completely absent. Submarginal eye-spots in the form of indistinct brown rings, varying in number. Basal portion of wing more or less pubescent.
scota Verity, 1910	The British Isles (type locality: "north coast of Scotland")	According to the original description, *scota* differs from the continental subspecies in having a well-developed white band on hind wing underside, contrasted by darker background, especially in the basal part which is darker than the rest of the wing. Pale band on underside of fore wing well developed; in some individuals it is very broad and white, surrounding the apical eye-spot.
lyllus Esper, 1805	Western Mediterranean (type locality: "Portugal")	Background of wing upperside intense ochre-yellow. Lateral margin of fore wing with dark edge. Underside of fore wings with eye-spots. Outer margin of hind wings with a row of indistinct white dots inside brown spots. Median spots absent because of larger size of eye-spots. Wing pattern in females less variable.
australis Verity, 1914	Hungary, the northern Balkans, Italy (type locality: "hills of Macerata, m. 300")	Wing upperside yellow, darker and more intense than in *pamphilus*, dark margin marrow, edge shorter and not so distinctly white. White band on underside of fore wings absent or (in females) hardly visible. Hind wings pale gray with dull greenish, sometimes bluish or yellowish tint, uniformly colored from base to outer margin, appear velvety. Eye-spots, when present, surrounded by very obscure circles and separated from wing margin by very indistinct band. Wing band very narrow, diffuse, gray-white or yellowish, noticeable only along costal margin. In some individuals flying in early spring and late autumn, hind wing underside very dark, even blackish with slight blue tint at base and inner margin, with no traces of white band.
marginata Heyne, 1894	Greece, the Aegean region (excluding Crete), in Asia Minor as far as Armenia (type locality: "Kleinasien")	As variable as *lyllus*, differing from the latter only by broad dark wing margin. According to the original description, wing upperside with dilated black-brown outer margin and very pale general coloration. This character is not constant, but more or less distinct bands tend to occur within the entire range of this subspecies, especially in the summer generation.

common and broadly distributed species could provide the most essential data for understanding the evolution of Satyridae.

When describing the morphological specificity of subspecies (Table 1), most of the authors mentioned the variable number of eye-spots on the wing underside, giving no details as to variability of such an important taxonomic character. At the same time, analysis of variation in the number, shape, and size of eye-spots, combined with studies of the general wing pattern and coloration may provide the key to a number of taxonomic problems. Yu.P. Nekrutenko (1985, 1990) proposed a method of phenogeographic analysis for studying geographic variation of *C. pamphilus* in the Crimea and Caucasus. Studies of the clinal variation of *Coenonympha tullia* (Müll.) in Britain carried out by J.R.G. Terner (1963) and K. Porter (1980) may serve as examples of the phenogenetic approach.

This work presents the results of our analysis of geographic variation of *C. pamphilus* in the Urals and adjacent regions, using the phenogenetic approach and the method of variation spectra.

Table 2. Size of *Coenonympha pamphilus* samples collected in different years

Collection locality	Year	Sample size, ind.		Collector
		males	females	
Chelyabinsk Prov., Bredy District, Arkaim Reserve	1999	285	41	E.Yu. Zakharova
Kurgan Prov., Petukhovo District, Petukhovo	1999	38	6	N.A. Utkin
Orenburg Prov., Kuvandyk District, near Kuvandyk	2001	32	5	E.Yu. Zakharova
Orenburg Prov., Totskoe	2001	13	4	E.Yu. Zakharova
Sverdlovsk Prov., Kamensk District, Travyanskoe, August (II generation)	2002	69	5	E.Yu. Zakharova
Kyrgyzstan, Dzhelal-Abad Prov., Toskool-Ata	2003	53	14	E.M. Andreeva
Volgograd Prov., near Frolovo	2005	24	0	P.V. Rudoiskatel

MATERIALS AND METHODS

This work was based on *C. pamphilus* samples collected in 1999–2005 from several localities in Sverdlovsk, Chelyabinsk, Kurgan, Orenburg, and Volgograd Provinces, and also from Dzhelal-Abad Province of Kyrgyzstan (courtesy of E.M. Andreeva, the Botanical Gardens, Ural Division, Russian Academy of Sciences). All the samples together comprised 589 specimens (Table 2).

During material processing we measured the length of fore (*LF*) and hind (*LH*) wings and the diameter of eye-spots in the underside wing pattern (along the median line of the corresponding wing cell). The measurements were carried out at 8 × 0.6 magnification, using an MBS-10 dissecting microscope equipped with an eyepiece micrometer. The forewing length was measured from the base of vein *Sc* to the wing apex, and the hindwing length, from the base of vein *Rs* to the end of vein *Cu1* (Fig. 1). All the measurements were done on the left side of each specimen.

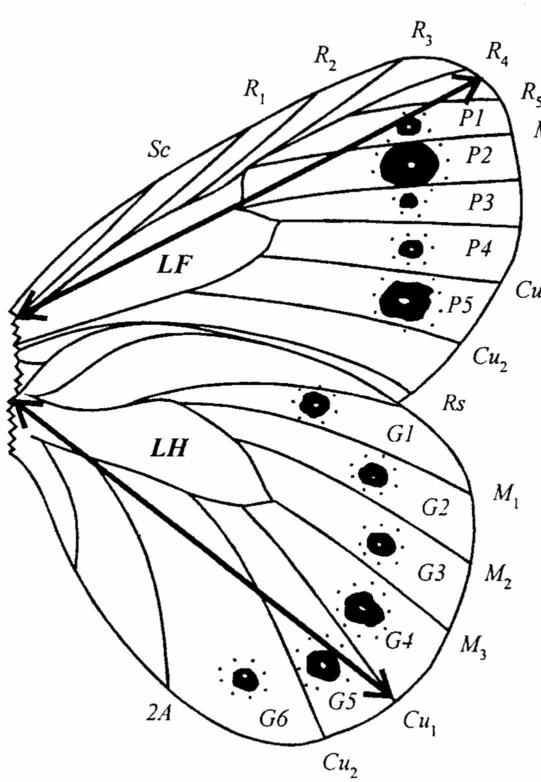

Fig. 1. The maximum number of eye-spots in the wing pattern of *Coenonympha pamphilus*. Spots on the fore wing (*P1–P5*); spots on the hind wing (*G1–G6*); length of fore wing (*LF*) and hind wing (*LH*).

The geographic variation of non-metric (discrete) characters, such as the presence of eye-spots in the wing pattern, was studied by phenetic distance analysis. The phenetic distances, characterized by the mean measures of divergence (MMD) between samples and their mean standard deviations (MSD) were calculated by the formulas proposed by S.E. Hartman (1980). The differences were considered to be statistically significant at MMD > 2MSD ($p < 0.05$). Cluster analysis was then implemented to produce similarity dendrograms by the method of unweighted pairwise group coupling (UPGMA, Vasil'ev et al., 2000). The distances were calculated and the dendrograms were built using the FEN 3.0 software package developed by A.G. Vasil'ev (Institute of Plant and Animal Ecology, Ural Division, Russian Academy of Sciences).

The geographic variation of metric characters (wing length and eye-spot size) was studied using discriminant analysis within Statistica 5.0 software package.

RESULTS

Analysis of Geographic Variation of Metric and Non-Metric Characters Related to the Eye-Spots

We analyzed the geographic variation of the number and size of eye-spots in the wing pattern in *C. pamphilus* populations. The presence of individuals with different number of eye-spots means that at least some of the spots can be regarded as threshold-based non-

Table 3. Mean values of wing length and eye-spot diameter and frequency of individual spots in the wing pattern of *Coenonympha pamphilus*

Sample	Sex	*LF*	*P1*	*P2*	*P3*	*P4*	*P5*	*LH*	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*
Chelyabinsk Prov., Arkaim Reserve	Males (n = 255)	15.79	0.01/1.6	1.82/100	0.02/2.4	0.01/0.4	0.02/4.7	12.61	0.02/7.8	0.02/7.8	0.08/35.3	0.11/51.0	0.13/58.8	0.05/27.5
	Females (n = 30)	17.51	–	1.24/100	0.01/3.3	–	0.01/3.3	14.09	0.06/20.0	0.06/20.0	0.11/43.3	0.13/53.3	0.13/63.3	0.08/40.0
Kurgan Prov., Petukhovo	Males (n = 33)	15.40	–	1.88/100	–	–	–	12.00	0.02/9.1	0.02/9.1	0.09/48.5	0.11/57.6	0.13/66.7	0.06/30.3
	Females (n = 6)	17.07	–	1.93/100	–	–	–	13.70	0.03/16.7	0.06/33.3	0.16/66.7	0.19/83.3	0.19/83.3	0.10/50.0
Orenburg Prov., near Kuvandyk	Males (n = 27)	15.47	0.01/3.7	1.72/100	0.04/3.7	–	–	12.59	–	0.01/3.7	0.07/29.6	0.13/55.6	0.10/44.4	0.06/25.9
	Females (n = 4)	17.06	–	1.85/100	–	–	–	14.21	–	–	0.08/25	0.15/50	0.10/50	0.05/25
Orenburg Prov., Totskoe	Males (n = 12)	15.36	–	1.63/100	–	–	–	12.32	–	0.02/8.33	0.11/50	0.11/50	0.13/58.3	0.06/33.3
	Females (n = 2)	16.41	–	1.88/100	–	–	–	13.62	–	–	0.10/50	0.19/100	0.10/100	0.10/100
Sverdlovsk Prov., Travyanskoe	Males (n = 67)	15.04	–	1.81/100	0.01/1.5	–	0.01/4.5	11.79	0.01/4.5	0.06/19.4	0.22/70.1	0.24/83.6	0.22/91	0.07/35.8
	Females (n = 5)	15.48	–	1.81/100	–	–	–	12.51	0.08/40.0	0.23/60.0	0.27/80.0	0.31/100	0.31/100	0.12/60.0
Volgograd Prov., near Frolovo	Males (n = 22)	16.14	–	1.89/100	0.06/9.1	–	0.05/4.6	13.18	0.06/18.2	0.12/40.9	0.18/68.2	0.17/63.6	0.21/86.4	0.12/59.1
Dzhelal-Abad Prov., Tos-kool-Ata	Males (n = 46)	17.21	0.01/2.2	2.17/100	0.21/15.2	0.05/8.7	0.04/6.5	13.89	0.17/47.8	0.22/60.9	0.35/80.4	0.39/84.8	0.44/87.0	0.30/78.3
	Females (n = 13)	19.03	–	2.37/100	–	0.06/15.4	0.07/15.4	15.86	0.30/69.2	0.34/84.6	0.45/92.3	0.50/100	0.51/100	0.32/84.6

Note: The diameter of eye-spots (mm) is shown in the numerator, and their frequency (%), in the denominator; the length of wings is given in mm.

metric (discrete) characters, or phenes (Zakharova, 2002).

The frequencies of occurrence of different eye-spots and their mean diameters in the populations are shown in Table 3. The only eye-spot that is always present is *P2*, located on the forewing, in cell M_1–M_2. This spot is the largest and usually consists of a central white focus surrounded by a black circle and a yellow peripheral ring. All the other eye-spots in the wing pattern are small and may appear as silver or whitish scales visible only under magnification.

The results of discriminant analysis of the geographic variation of wing length and eye-spot diameter in *C. pamphilus* are shown in Table 4. The samples of females from Kurgan, Orenburg, and Sverdlovsk Provinces were excluded from statistical processing because of their small size. All the samples, except two from Orenburg Province (Totskoe and Kuvandyk: males), were found to be significantly different in the complex of metric characters (*F* criterion, $p < 0.05$). These results indicate the presence of sex dimorphism with respect to wing length and eye-spot size (the females are always larger and have larger eye-spots) and

Table 4. Generalized Mahalanobis distances between *Coenonympha pamphilus* samples, based on the complex of metric characters

No.	1	2	3	4	5	6	7	8	9
	Arkaim (males)	Arkaim (females)	Petukhovo (males)	Kuvandyk (males)	Totskoe (males)	Travyanskoe (males)	Frolovo (males)	Toskool-Ata (males)	Toskool-Ata (females)
1	0.00	**7.59**	**1.40**	**1.84**	**4.06**	**4.69**	**4.65**	**12.45**	**48.99**
2		0.00	**12.82**	**11.42**	**15.16**	**20.82**	**10.13**	**9.46**	**27.39**
3			0.00	**4.52**	**6.56**	**3.35**	**8.53**	**16.27**	**58.86**
4				0.00	**2.16**	**5.49**	**5.75**	**16.38**	**53.72**
5					0.00	**7.87**	**10.01**	**21.46**	**60.33**
6						0.00	**8.69**	**17.83**	**62.93**
7							0.00	**8.06**	**35.49**
8								0.00	**17.87**
9									0.00

Note: The statistically significant differences (*F* criterion, $p < 0.05$) are shown in bold.

Table 5. Phenetic distances between *Coenonympha pamphilus* samples

	1	2	3	4	5	6	7	8	9
	Arkaim (males)	Arkaim (females)	Petukhovo (males)	Kuvandyk (males)	Totskoe (males)	Travyanskoe (males)	Frolovo (males)	Toskool-Ata (males)	Toskool-Ata (females)
1	–	0.006	–0.014	–0.003	–0.057	**0.164**	**0.179**	**0.572**	**0.943**
2	0.012	–	–0.021	0.043	–0.053	**0.125**	0.036	**0.308**	**0.623**
3	0.011	0.020	–	0.005	–0.082	**0.760**	**0.102**	**0.448**	**0.887**
4	0.013	0.022	0.021	–	–0.080	**0.222**	**0.246**	**0.681**	**1.005**
5	0.027	0.036	0.035	0.037	–	**0.071**	**0.076**	**0.451**	**0.775**
6	0.006	0.015	0.014	0.016	0.030	–	**0.047**	**0.306**	**1.155**
7	0.015	0.024	0.023	0.026	0.039	0.019	–	**0.054**	**0.641**
8	0.008	0.017	0.016	0.018	0.032	0.012	0.021	–	**0.604**
9	0.025	0.034	0.033	0.035	0.049	0.028	0.038	0.030	–

Note. The phenetic distances (MMD) are shown in the *upper right* part of the matrix, and the mean standard deviations (MSD), in the *lower left* part. The statistically significant differences (χ^2 criterion, $p < 0.05$) are shown in bold.

the clinal pattern of geographic variation: the butterflies are smaller in northern parts of the range (Sverdlovsk, Kurgan, and Chelyabinsk Prov.) than in southern ones (Volgograd and Dzhelal-Abad Prov.).

As can be seen from Table 3, all the spots (except *P2*) have different frequencies in the wing pattern; therefore they can be used as non-metric characters in analysis of the geographic variation. In order to estimate the degree of phenogenetic differentiation of natural populations, we calculated phenetic distances between the samples (Table 5).

Cluster analysis shows some samples to be the most similar as to their phenetics. The dendrogram (Fig. 2) includes 2 large clusters and a separate pair of samples, namely those of males and females from Dzhelal-Abad Province. One cluster comprises the samples from Orenburg, Kurgan, and Chelyabinsk Provinces with small and mostly non-significant phenetic distances (Table 5). The other cluster unites samples from quite remote localities: Sverdlovsk and Volgograd Provinces. The phenetic distance between these samples is small though statistically significant (0.047), indicating considerable phenetic similarity of the corresponding populations.

Analysis of the Actual Phenotypic Variation in Natural Populations

A specimen of *C. pamphilus* can have up to 5 eye-spots on the fore wing and up to 6 eye-spots on the hind wing (Fig. 1). However, the total number of the pattern elements usually does not reach the maximum value owing to the discrete nature of individual spots. The variability of the eye-spot number in Satyridae is a well-known phenomenon, which was analyzed in detail in the middle of the last century by the example of European populations of *Maniola jurtina* L. and some related species (McWhirter, 1957, 1969; Creed et al., 1962; Ford, 1965; Beaufoy et al., 1970, etc.). The cited authors used the number of eye-spots on the hindwing underside as a measure of population variability, but the position of the spots was not taken into account. Such an approach made the results difficult to interpret, because the same total number of spots could be obtained by different combinations. For example, the wing pattern of *M. jurtina* characterized by 2 spots on the hind wing could be represented by two different combinations, the spots being located either in cells Rs–M_1 and M_1–M_2 or in cells M_1–M_2 and Cu_1–Cu_2 (Zakharova, 1998, 2002). This ambiguity was not considered during a detailed analysis of natural populations of this species in Western Europe, on the British Isles, and on adjacent small isolated islands.

We propose to estimate the variability in the number of eye-spots and the degree of its realization using the approach based on variation spectra, i.e., assuming analysis of phenotypic combinations rather than individual characters. For clearness, the different categories of characters may be arranged in a table, showing the corresponding combinations in the cells. This approach has already been implemented in a number of zoological studies. It was used, in particular, to describe morphotypes of some vole species based on plication and the presence of closed areas on the surface of their molars (Bol'shakov et al., 1980), and also to characterize the polymorphism of pronotal and elytral patterns in leaf beetles (Mikhailov, 1998, 1990). The technique for building variation spectra was developed by E.E. Kovalenko by the example of skeletal structures of Anura (Kovalenko, 1996a, 1996b, 2003, 2007; Kovalenko and Popov, 1997; Kovalenko and Danilov, 2006a, 2006b). We used technique to build the variation spectrum of each of the examined populations of *C. pamphilus* (separately for either sex) as well as the total variation spectrum for the part of the species' distribution range covered by our study.

Analysis of phenotypic variation and the degree of its realization in *C. pamphilus* included several stages. First, the limits of variation in the number of spots on the fore and hind wings were determined. It was found that the fore wing could simultaneously have from 1 to 4 spots, and the hind wing, from 0 to 6 spots. The same total number of spots could result from different combinations. All the combinations observed are listed in Table 6.

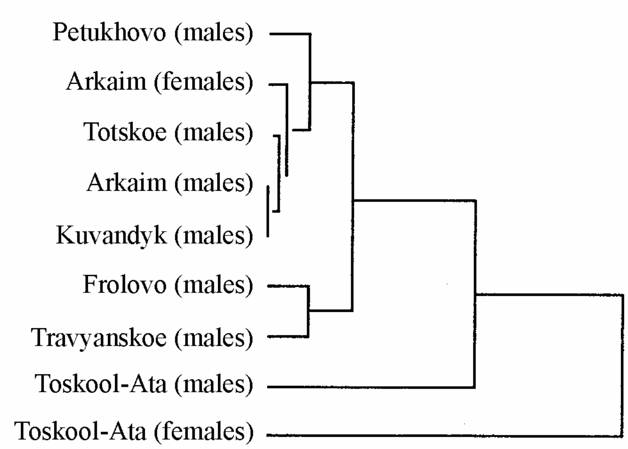

Fig. 2. Similarity dendrogram of *Coenonympha pamphilus* samples based on non-metric characters of the wing pattern.

All the observed variants of spot combinations on the fore wing can be arranged in the first column of the table, and those found on the hind wing, in the first row. The resulting matrix corresponds to the theoretical variation spectrum (*St*), in the terminology used by the author of the method. If the spots combined randomly, then the theoretical variation spectrum of *C. pamphilus*, determined by combinatorial calculus, would comprise 1984 cells. Indeed, the fore wing may have from 1 to 4 spots (31 combinations), and the hind wing, from 0 to 6 (64 combinations), therefore the total number of combinations is $31 \times 64 = 1984$. However, only 10 combinations were actually observed in the fore wing, and only 23, in the hind wing (Table 6). Therefore the theoretical variation spectrum is only $10 \times 23 = 230$ cells.

Thus, the theoretical variation spectrum determines the smallest and the greatest number of spots on the fore and hind wings, and describes the combinations of phenes producing a particular number of spots.

The theoretical variation spectrum was filled with frequencies of phenetic combinations observed in each sample (separately for the two sexes), producing the actual variation spectrum (*Sr*). The actual variation spectrum is a subset of the theoretical one; it shows which particular combinations of phenes were observed in males and females and with what frequencies. The distribution of frequencies determines the shape of the potential variation spectrum (*Sp*): its

Table 6. Realized variants of eye-spot combinations in *Coenonympha pamphilus*

Number of spots						
0	1	2	3	4	5	6
			Fore wing			
	– P2– – – –	*P1P2– – –*	*P1P2– – P5*	*– P2P3P4P5*		
		– P2P3– –	*– P2P3P4–*			
		– P2– P4–	*– P2– P4P5*			
		– P2– – P5	*– P2P3– P5*			
			Hind wing			
– – – – – –	*– – G3– – –*	*– – G3G4– –*	*– G2G3G4– –*	*G1– – G4G5G6*	*G1G2G3G4G5–*	*G1G2G3G4G5G6*
	– – – G4– –	*– G2– – G5–*	*– – G3G4G5–*	*– G2G3G4G5–*	*G1– G3G4G5G6*	
	– – – – G5–	*– G2– G4– –*	*– – – G4G5G6*	*– G2G3– G5G6*	*– G2G3G4G5G6*	
		– – G3– G5–		*– G2– G4G5G6*		
		– – – G4G5–		*– – G3G4G5G6*		
		– – – – G5G6		*G1– G3G4G5–*		

Note: The spots are designated as in Fig. 1. The absent spots are designated with dashes.

Table 7. Variation spectra of males and females of *Coenonympha pamphilus* and their contribution into the total variation spectrum of the species

Sex	*St*	*Sp*	*Sr*	*Sr/Sp** 100%	*Sr/St** 100%	*Sp/St** 100%
Males	230	169	47	27.8	20.4	73.5
Females	230	70	13	18.6	5.7	30.4
Total spectrum	230	169	50	29.6	21.7	73.5

boundaries correspond to the extreme values of the actual spectrum (Fig. 3).

For example, let us consider the variation spectra for samples of *C. pamphilus* males from Chelyabinsk Prov. (Arkaim Reserve) and from Kyrgyzstan (Toskool-Ata). As can be seen from Fig. 3, the actual and potential variation spectra for the first sample are 30 and 122 combinations, and for the second sample, 17 and 34 combinations, respectively. This means that males of *C. pamphilus* occurring in the South Urals are nearly twice as variable as those from Kyrgyzstan, and show quite different frequencies of phenotypic combinations. The Chelyabinsk sample is dominated by males with a single spot on the fore wing and no spots on the hind wing (38.4%), and the Kyrgyzstan sample, by males with one spot on the fore wing and the greatest possible number of spots (6) on the hind wing (23.9%).

Finally, the variation spectra of all the samples were combined (separately for the two sexes) into the general variation spectrum, characterizing the entire territory covered by our study (Fig. 4). The two spectra obtained for males and females may be combined into a single spectrum of the species; this step is quite obvious and will not be considered here for brevity. The sex-related differences in the wing pattern variation of *C. pamphilus* can be assessed by comparing the two spectra in Fig. 4.

The actual variation spectrum of males is 3.5 times as broad as that of females (Table 7). Three phenotypic combinations are specific to females, 37 have been found only in males, and 10 can be observed in both sexes. In males, the most frequent variant is one spot on the fore wing and no spots on the hind wing. In females, two combinations can be considered prevalent: both have a single eye-spot on the fore wing, while the hind wing has no spots in one variant (as in males) and six spots, or the greatest possible number, in the other variant. In general, the wing pattern of females is more conservative and characterized by the number of spots on the fore and hind wings increasing simultaneously. The males are more variable and more often have a small number of spots on both wings.

The potential variation spectra of the two sexes also differ in size (Table 7) but have the same shape and position within the theoretical spectrum (Fig. 4). The

A

4																							
3				*Sr* = 30								0.4											
				Sp = 122														0.4					
												0.8	0.4										
2	0.8											0.8		0.4	0.4			0.4					
	0.8								0.4			0.4						0.4					
																							0.4
1	38.4			7.8	0.8			0.4	7.8	0.8	0.4	2.0	7.6		0.4			1.2	0.4	0.4	1.2	0.8	6.0
	0	*1*			2	*2*					*3*			*4*						*5*			*6*

B

4																							
3				*Sr* = 17																			
				Sp = 34																			
																						2.2	
																							2.2
2																							
																							2.2
																							8.7
1	8.7	2.2	2.2						4.3	2.2				2.2	2.2	2.2		1.9		2.2	4.3	13.4	23.9
	0	*1*			*2*						*3*			*4*						*5*			*6*

Fig. 3. Variation spectra for samples of *Coenonympha pamphilus* males from Arkaim Reserve (Chelyabinsk Prov.) (*A*) and Toskool-Ata (Dzhelal-Abad Prov., Kyrgyzstan) (*B*). The combinations of spots on the fore wing (from 0 to 4) are shown in the *first column*, and those on the hind wing (from 0 to 6), in the *last row*. The frequencies of phenotypic combinations are shown in the cells of the table. The combinations included into the potential variation spectrum are outlined in bold. The combinations with frequencies exceeding 5% are regarded as common.

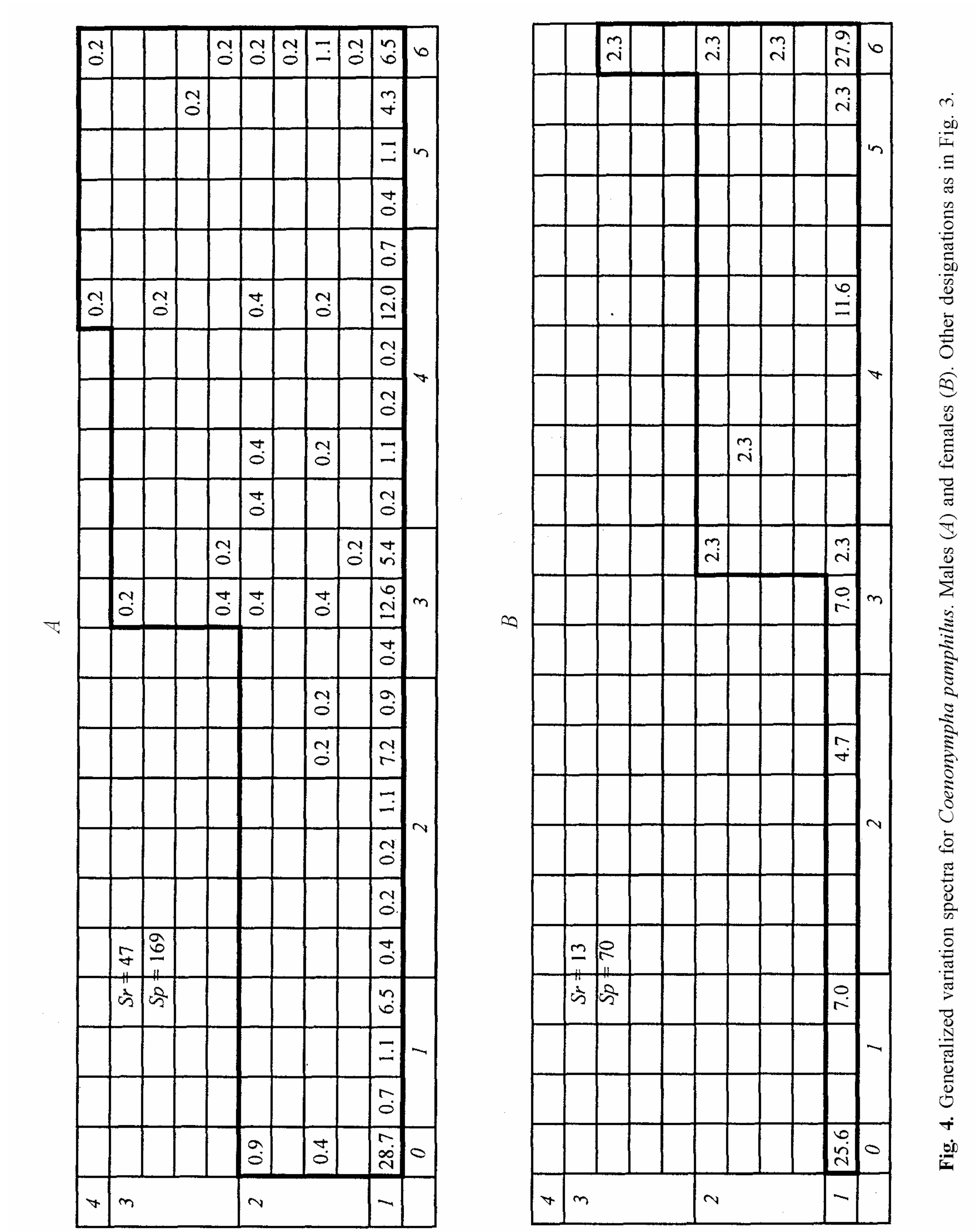

Fig. 4. Generalized variation spectra for *Coenonympha pamphilus*. Males (*A*) and females (*B*). Other designations as in Fig. 3.

phenotypic combinations not included into the potential variation spectrum are unlikely or prohibited combinations. According to E.E. Kovalenko and I.Yu. Popov (1997), such combinations provide important information on the variability of the characters in question. Indeed, the upper left corner of *St* spectrum contains combinations with many spots on the fore wing and a reduced number of spots on the hind wing; however, such individuals were never found in natural populations of *C. pamphilus*, and this variant can be regarded as uncharacteristic of the species.

DISCUSSION

Analysis of morphological variation of *Coenonympha pamphilus* (Linnaeus, 1758) with respect to the size and number of eye-spots has shown the clinal nature of variation in the samples taken from some natural populations of the Urals and adjacent territories. The butterflies from the northern parts of the range (Sverdlovsk and Kurgan Prov.) are smaller than the individuals from more southern territories (Orenburg and Chelyabinsk Prov.). Correspondingly, the wing pattern of "northern" specimens comprises fewer eye-spots, and the spots themselves are relatively smaller. The butterflies occurring in the Urals are commonly considered to belong to the nominotypical subspecies (Higgins and Riley, 1993; Korshunov and Gorbunov, 1995; Korshunov, 2002; Olshvang et al., 2004). All the populations from the Middle and Southern Urals, Cis- and Transural territories, and the Southern Volga Basin appear to belong to this subspecies as well. Discriminant analysis of the wing length and eye-spot diameter, and also calculation of the phenetic distances revealed the greatest similarity between the samples from Orenburg, Chelyabinsk, and Kurgan Provinces. A similar wing pattern, with respect to the size and occurrence of eye-spots, was observed in the geographically remote populations of southern Sverdlovsk (Travyanskoe) and southern Volgograd Provinces (Frolovo). This similarity may be accounted for by the fact that the first sample was collected in early August, i.e., during the flight of the second generation, therefore its phenology could have been affected by seasonal variation. Individuals of the second generation are usually smaller and have smaller wing spots, which is related to the limited period of development (Wiklund and Forsberg, 1991; Wiklund et al., 1991).

The Kyrgyzstan sample differs from the rest of the samples examined in the complex of metric and nonmetric characters of the wing pattern. It includes large butterflies (with wings 2 mm longer than in the Ural specimens) with a significantly greater number of eye-spots in the wing pattern; the spots themselves are larger. The calculated Mahalanobis and phenetic distances indicate a subspecies-level difference between the Kyrgyzstan and Ural populations of *C. pamphilus*. The sample from Dzhelal-Abad should probably be assigned to the subspecies *marginata* Heyne, 1894.

As in most Satyridae, females of this species are larger than males (Verity, 1926; Davenport, 1941; Nekrutenko, 1985, 1990; Wiklund and Forsberg, 1991; Wiklund et al., 1991; Zakharova, 2002, 2004, etc.). In addition, the wing pattern of females includes on the average more eye-spots, and the spots are larger than those of males. There are also qualitative sex-related differences in the eye-spot variation, which can be revealed by comparing the variation spectra. Arrangement of the observed phenotypic combinations within the matrix of the theoretical variation spectrum allowed us to describe the actual and potential variation spectra for both sexes and for the species in general. The actual variation spectrum proved to be much broader in males than in females.

Despite the quantitative differences, the *Sr* and *Sp* spectra show more or less similar trends in different samples for both sexes. The phenotypic combinations prevalent in all the samples include 1 spot on the fore wing (in cell M_1–M_2). The hind wing in this case may have either no spots at all, which it typical of males, especially from the northern parts of the range, or the greatest number of spots (6), which is most often observed in females, especially from the southern parts of the range.

The shape and position of the potential variation spectrum within the theoretical one describes the possible and unlikely phenotypic combinations in the wing pattern of *C. pamphilus*. The possible variants are: (1) a simultaneous reduction in the number of eye-spots on the fore and hind wings; (2) a simultaneous increase in the number of eye-spots; (3) an increase in the number of eye-spots on the hind wing accompanied by a decrease of that on the fore wing. The phenotypic combinations with the maximum number of spots on the fore wing and the minimum number of spots on the hind wing were found to be prohibited for this species.

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