Seasonal Variability of Wing Length and Eyespots in Populations of *Erebia ligea* (L.) (Lepidoptera, Satyridae) in the Middle Urals

E. Yu. Zakharova

Institute for Plant and Animal Ecology, Ural Division of Russian Academy of Sciences, Yekaterinburg, Russia

Received May 29, 2008

Abstract—The flight period of *Erebia ligea* (Linnaeus, 1758) in the Sysert District of Sverdlovsk Province lasts from early July until early August only in the odd-numbered years. The species is characterized by protandry, its males emerging 5–7 days before females. *Erebia ligea* manifests sexual and seasonal variation of the wing length and diameter of the eyespots in the Middle Urals. According to the literature and newly obtained data, the relative male/female wing length varies within the species range. The vectors of the seasonal variation of the body size are opposite in the males and females: in early summer the largest males and the smallest females occur, then medium-sized individuals appear, while the smallest males and the largest females fly at the end of the warm period. Although the males of *Erebia ligea* are larger than females, the latter have larger eyespots on the wings. Seasonal variation of the diameters of the forewing eyespots is largely similar in the two sexes: individuals with larger spots appear earlier in the season than those with small spots. The eyespots treated as phenes in this study develop on the wings only if the remaining spots of the wing pattern attain a threshold size. A threshold nature was shown for *P4* spot in M_3–Cu_1 cell of the fore wing and *G2* spot in cell M_1–M_2 of the hind wing. Individuals with and without phene *G2* have opposite vectors of seasonal variation of the sizes of the rest spots of the hind wing pattern. It is apparently the threshold character of variation of these elements of the wing pattern that defines the variation of the proportion of phenetic combinations of the eyespots in the individuals emerging in different periods of the season. Describing phenetic variation at the population level, one should remember that the percentage of the "morphs" or phenetic combinations may vary during a season, especially in the protandric species.

DOI: 10.1134/S0013873810060011

The Arran brown *Erebia ligea* (Linnaeus, 1758) is a broadly distributed Trans-Eurasian species. In the Urals (except for the polar regions) and in Siberia (as far as the Sayan Range) it is represented by the subspecies *E. l. kamensis* Krulikovsky, 1909. The larvae feed on grasses and sedges. The flight period of one generation extends from the end of June to the end of August (Korshunov and Gorbunov, 1995; Korshunov, 2002; Olshvang et al., 2004).

Erebia ligea is a biennial form. The parameters of its flight (year, relative abundance, and the time of emergence) may vary in different parts of its distribution range. According to Dayanov (1984, cited after Ivanov et al., 1987) *E. ligea* in the Southern Urals can be observed only in odd-numbered years. We have observed flight periodicity of this species in the Middle Urals (Sysert District of Sverdlovsk Prov.) since 2001. The butterflies appeared in 2001, 2003, 2005, and 2007, but were not recorded in even years. According to our original and published data, *E. ligea* is

characterized by protandry, its males emerging earlier than the females. The males emerge at the beginning of the flight period of a generation, then the sex ratio becomes even, and the females prevail at the end of the flight (Zakharova, 2006, 2008). In view of this, a question arises as to the nature of phenotypic variation of morphological characters not only in different sexes and generations but also in individuals emerging at different times within the same season.

This communication presents analysis of the metric characters of the wing and eyespots in the wing pattern of *E. ligea* in relation to sex and emergence time during one generation.

MATERIALS AND METHODS

The material was collected near the Biological Station of the Ural State University (Fomino, Sysert District, Sverdlovsk Prov.) in 2005 and 2007. The butterflies were captured daily during the entire flight period of the generation, except for rainy and overcast days
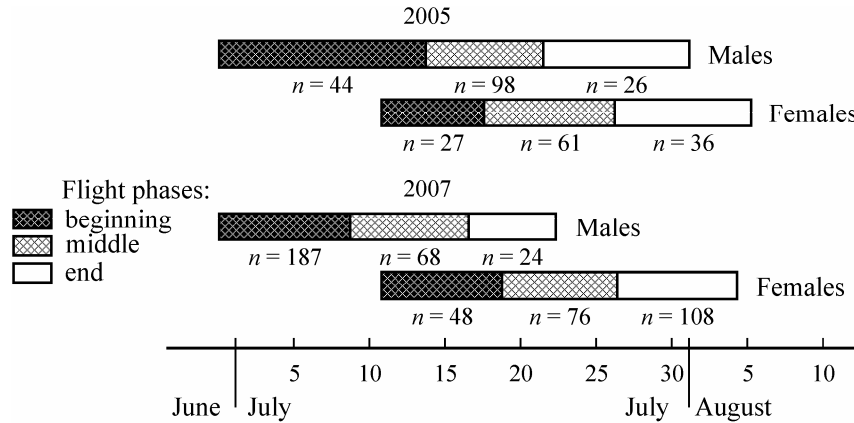
Fig. 1. Timing and relative duration of the flight phases and the size of the corresponding samples (*n*) of *Erebia ligea* (L.) in Sverdlovsk Prov. (2005 and 2007).
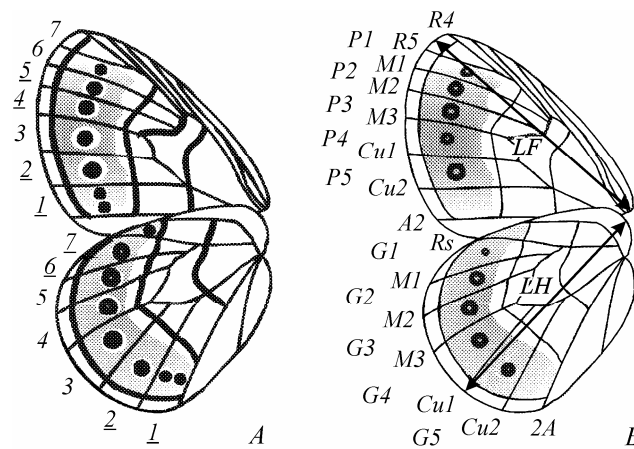

Fig. 2. Eyespots in the wing pattern of the genus *Erebia* Dalman: (*A*) *Protoerebia*: a hypothetical form representing the nymphaloid prototype of wing pattern in the genus *Erebia*. The cells in which eyespots can occur are designated by underlined Arabic numerals (after Schwanwitsch, 1935); (*B*) the maximum possible number of spots on the fore (*P1–P5*) and hind wings (*G1–G5*) of *Erebia ligea* (L.); *LF*, the length of fore wing; *LH*, the length of hind wing.

when the flight activity decreased greatly. The total sample size was 292 ind. in 2005 and 511 ind. in 2007.

In order to analyze the effect of the flight phase on the size parameters, each sample was subdivided into three "subsamples:" "beginning of the flight," "middle of the flight," and "end of the flight," in the same way as we did before when studying other species of Satyridae (Zakharova, 2004, 2008). Since the males started and ceased flying earlier than the females, each flight phase of the males was shifted by 5–10 days relative to the corresponding phase of the females. The relative position and duration of the flight phases and the size of the corresponding samples are shown in Fig. 1.

In the laboratory, the sex of each individual was determined and the length of fore (*LF*) and hind (*LH*) wings was measured. The fore wing was measured

from the base of vein *Sc* to the wing apex, and the hind wing, from the base of vein *Rs* to the end of vein Cu_1.

According to Schwanwitsch (1935), the hypothetical form *Protoerebia*, representing the nymphaloid prototype of wing pattern in the genus *Erebia*, has up to 6 eyespots on the fore wing and up to 7 eyespots on the hind wing (Fig. 2*A*). According to our data, the maximum number of eyespots on the fore wing of *E. ligea* is 5; they are located in cells $R_5–M_1$, $M_1–M_2$, $M_2–M_3$, $M_3–Cu_1$, and $Cu_1–Cu_2$. These eyespots have been designated as *P1*, *P2*, *P3*, *P4*, and *P5*, respectively. The hind wing also has no more than 5 eyespots, located in cells $Rs–M_1$, $M_1–M_3$, $M_2–M_3$, $M_3–Cu_1$, and $Cu_1–Cu_2$, and designated as *G1*, *G2*, *G3*, *G4*, and *G5* (Zakharova, 2008).

Measurements of wing length and eyespot diameters (along the median line of the corresponding cell) were

carried out at 8×0.6 magnification, using an MBS-10 dissecting microscope equipped with an ocular micrometer. The scheme of the eyespots and the corresponding measurements is shown in Fig. 2B. All the measurements were done on the left underside of each specimen.

Statistical data treatment was carried out using the methods of discriminant analysis and three-factor analysis of variance. The significance of sex-related size differences was determined using the t-test. The structure of phenotypic correlations was described by Pearson's coefficients (r); the results were illustrated by cluster analysis. The data were processed using Statistica 5.5 software package.

RESULTS

Protandry and Sex-related Size Dimorphism

The flight of *E. ligea* in Sysert District of Sverdlovsk Province occurs in odd years, from the beginning of July to the beginning of August. The males are the first to emerge, and the first females appear 5–7 days later. This is a typical case of protandry, which is characteristic of many species in the family Satyridae. The males are about 1.2–1.3 times as abundant as the females. A similar sex structure and dynamic sex ratio are also observed in species of the genera *Lopinga*, *Aphantopus*, *Coenonympha*, etc. (Wiklund and Forsberg, 1991; Zakharova, 2004, 2006).

We measured the length of fore and hind wings and the diameters of eyespots in the wing pattern. The mean values of these metric characters for *E. ligea* samples broken down by phases of emergence are given in Table 1. The data obtained demonstrated the presence of sex-related size dimorphism. The wings of males were 0.5–1 mm longer than those of females. These differences are highly significant (2005: $t = 2.69$, $df = 275$, $p < 0.01$; 2007: $t = 9.92$, $df = 494$, $p < 0.0001$).

Various authors reported sex-related differences in the fore wing length within the distribution range of *E. ligea*. For example, in the Russian Far East the length of fore wing varies from 20 to 28 mm in males and from 22 to 29 mm in females (*Key to Insects…*, 2005). There are also published data on the size variation of *E. ligea* in the northeast of European Russia. According to Tatarinov and Kulakova (1999), the females are larger than the males in the upper course of

the Shchugor River, near Syktyvkar and Ukhta, whereas in the Subpolar Urals (the middle course of the Malyi Patok) and the Yany-Pupu-N'er Range the males are larger. The wing length of *E. ligea* from Sweden is 22.3 ± 0.1 mm in males and 22.5 ± 0.2 mm in females. The mean values for continental Europe are 24.1 ± 0.2 mm and 24.5 ± 0.3 mm, respectively (Nylin and Svärd, 1991; Wiklund and Forsberg, 1991).

Although the females in our material were on average smaller than the males, they had larger eyespots in the wing pattern (Table 1). Discriminant analysis of variation of wing length and eyespot diameter (Table 2) demonstrated significant differences between the sexes in the samples taken in different years.

The Structure of Phenotypic Correlations between metric Characters in Erebia ligea L.

Many publications devoted to analysis of genetic and phenotypic variation of the lepidopteran wing pattern have appeared since the 1990s. Studies of the wing patterns of such classical research objects as *Bicyclus anynana* (Butler, 1879), *Pararge aegeria* (Linnaeus, 1758) (Satyridae), and *Junonia coenia* Hübner [1822] (Nymphalidae) allowed the authors to describe some aspects of variation of eyespot size (Monteiro et al., 1994; Beldade et al., 2002), shape (Monteiro et al., 1997a), position relative to other elements of the pattern, and the structure of correlations among the various characters (Paulsen and Nijhout, 1993; Monteiro et al., 1997b; Beldade and Brakefield, 2003; Beldade et al., 2005; Breuker et al., 2007, etc.).

Analysis of the structure of phenotypic correlations in the nymphalid *Junonia coenia* (Paulsen and Nijhout, 1993) revealed no significant correlation between different homologous series in the pattern elements, in particular between the parafocal elements and the eyespots. The homologous elements, on the contrary, showed high correlations both in size and in the metric positional parameters. The strongest correlation was observed between the spots located on the upper and lower sides in the same wing cell; between the spots located in adjacent cells, and also between the spots located in homologous cells on the fore and hind wings.

We have analyzed the correlation structure of wing length and eyespot diameters separately for each sex because of the sex-related size dimorphism observed

Table 1. Mean values of wing length and eyespot diameter (mm) in *Erebia ligea* L. related to the time of adult emergence

Emergence	Number of ind.	*LF*	*P2*	*P3*	*P4*	*P5*
2005, males						
Early	44	25.93 ± 0.88	1.69 ± 0.37	1.66 ± 0.35	0.06 ± 0.21	1.00 ± 0.54
Typical	98	25.75 ± 0.97	1.69 ± 0.39	1.69 ± 0.42	0.08 ± 0.22	1.03 ± 0.52
Late	26	25.56 ± 0.96	1.63 ± 0.37	1.69 ± 0.34	0.13 ± 0.30	1.01 ± 0.44
		25.75 ± 0.94	1.67 ± 0.38	1.68 ± 0.37	0.09 ± 0.24	1.02 ± 0.50
2005, females						
Early	27	25.04 ± 1.60	2.00 ± 0.33	1.99 ± 0.36	0.24 ± 0.45	1.41 ± 0.41
Typical	61	25.50 ± 1.01	1.96 ± 0.39	1.92 ± 0.33	0.26 ± 0.46	1.45 ± 0.43
Late	36	25.62 ± 0.84	1.98 ± 0.48	1.96 ± 0.45	0.29 ± 0.44	1.47 ± 0.52
		25.39 ± 1.15	1.98 ± 0.40	1.96 ± 0.38	0.27 ± 0.45	1.44 ± 0.45
2007, males						
Early	187	27.59 ± 0.93	1.89 ± 0.38	1.80 ± 0.42	0.11 ± 0.31	1.22 ± 0.50
Typical	68	27.40 ± 0.91	1.85 ± 0.38	1.79 ± 0.30	0.07 ± 0.21	1.25 ± 0.46
Late	24	27.51 ± 0.83	1.78 ± 0.49	1.68 ± 0.43	0.02 ± 0.12	1.23 ± 0.36
		27.50 ± 0.89	1.84 ± 0.42	1.76 ± 0.39	0.07 ± 0.21	1.23 ± 0.44
2007, females						
Early	48	26.97 ± 0.94	2.15 ± 0.33	2.18 ± 0.33	0.25 ± 0.39	1.64 ± 0.38
Typical	76	26.71 ± 1.02	2.10 ± 0.32	2.05 ± 0.31	0.33 ± 0.47	1.53 ± 0.41
Late	108	26.56 ± 0.95	2.07 ± 0.38	2.01 ± 0.38	0.31 ± 0.47	1.48 ± 0.39
		26.75 ± 0.97	2.10 ± 0.34	2.08 ± 0.34	0.30 ± 0.45	1.55 ± 0.39

Table 1 (Contd.)

Emergence	Number of ind.	*LH*	*G1*	*G2*	*G3*	*G4*	*G5*
2005, males							
Early	44	21.59 ± 0.93	–	0.27 ± 0.38	1.47 ± 0.46	1.04 ± 0.51	1.45 ± 0.38
Typical	98	21.40 ± 0.86	–	0.30 ± 0.40	1.47 ± 0.45	1.10 ± 0.49	1.45 ± 0.46
Late	26	21.13 ± 0.95	–	0.18 ± 0.30	1.29 ± 0.57	1.00 ± 0.45	1.38 ± 0.41
		21.37 ± 0.91	–	0.25 ± 0.36	1.41 ± 0.49	1.04 ± 0.48	1.43 ± 0.42
2005, females							
Early	27	21.13 ± 1.59	–	0.23 ± 0.34	1.54 ± 0.47	1.29 ± 0.44	1.53 ± 0.47
Typical	61	21.31 ± 1.09	–	0.19 ± 0.32	1.50 ± 0.45	1.14 ± 0.54	1.54 ± 0.44
Late	36	21.76 ± 0.86	–	0.19 ± 0.42	1.55 ± 1.23	1.54 ± 1.69	1.55 ± 0.42
		21.40 ± 1.18	–	0.20 ± 0.36	1.53 ± 0.72	1.32 ± 0.89	1.54 ± 0.44
2007, males							
Early	187	22.40 ± 0.85	0.01 ± 0.11	0.23 ± 0.36	1.40 ± 0.47	1.05 ± 0.45	1.43 ± 0.42
Typical	68	22.16 ± 0.87	0.01 ± 0.08	0.30 ± 0.44	1.48 ± 0.52	1.09 ± 0.38	1.51 ± 0.44
Late	24	22.62 ± 0.72		0.33 ± 0.43	1.45 ± 0.54	1.07 ± 0.51	1.39 ± 0.51
		22.39 ± 0.81	0.01 ± 0.06	0.29 ± 0.41	1.44 ± 0.51	1.07 ± 0.44	1.44 ± 0.46
2007, females							
Early	48	22.35 ± 0.88	0.03 ± 0.12	0.31 ± 0.40	1.55 ± 0.52	1.19 ± 0.44	1.68 ± 0.36
Typical	76	22.02 ± 1.01	0.06 ± 0.15	0.31 ± 0.38	1.43 ± 0.56	1.18 ± 0.47	1.55 ± 0.44
Late	108	22.02 ± 0.84	0.07 ± 0.17	0.39 ± 0.41	1.43 ± 0.48	1.13 ± 0.40	1.53 ± 0.37
		22.13 ± 0.91	0.06 ± 0.14	0.34 ± 0.40	1.47 ± 0.52	1.17 ± 0.44	1.58 ± 0.39

Table 2. Generalized Mahalanobis distances between samples of *Erebia ligea* (L.) based on the complex of metric characters (the pairs with $p < 0.0001$ are marked with ***)

	Males, 2005	Females, 2005	Males, 2007	Females, 2007
Males, 2005	–	2.053***	4.434***	2.712***
Females, 2005		–	7.627***	2.461***
Males, 2007			–	2.238***
Females, 2007				–

in our material. The coefficients of correlation calculated for males and females of *E. ligea* are shown in Fig. 3. Both sexes revealed considerable similarity in the general structure and arrangement of the clusters. As can be seen from the dendrograms, all the metric characters form 2 isolated clusters, one of them uniting the lengths of the fore and hind wings (*LF* and *LH*), and the other, the diameters of the eyespots. The structure of the latter cluster reflects the correlation among
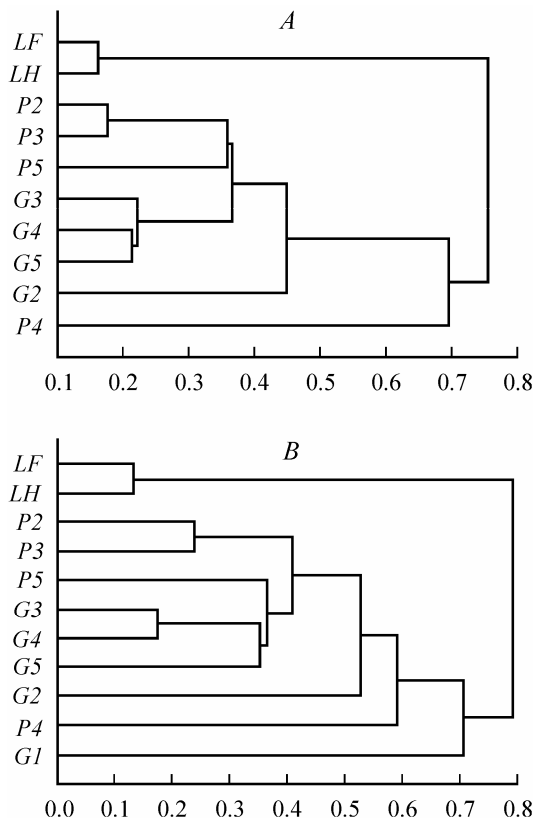
the stable and discrete elements of the wing pattern. The most closely correlated by size are spots *P2*, *P3*, *P5*, *G3*, *G4*, *G5*, the values of the corresponding coefficients (*r*) varying from 0.55 to 0.85; these spots are also the largest and the most stable as concerns their manifestation in the pattern (Table 1). The size of spots *P4* and *G2* is weakly correlated with that of the remaining spots. The frequency of occurrence of these spots varies from 12.3 to 41.8%. Spot *G1* may be regarded as rare; it occurs in about 6% of the females and is usually absent in the males, therefore it is not shown in the dendrogram (Fig. 3*A*). Spot *P1* is the rarest: it was observed in only one female from the sample of 2007. The frequency of this spot was therefore about 0.1%, and the values of *r* between its diameter and diameters of the remaining spots were not calculated (Zakharova, 2008).

Our results largely agree with those obtained by other authors (Paulsen and Nijhout, 1993; Breuker et al., 2007, etc.). The lengths of fore and hind wings are strongly correlated (*r* varying from 0.80 to 0.85); at the same time, the size of the eyespots in the wing pattern only weakly depends on the size of the wing on which they occur. The strongest correlation is observed between the spots located in adjacent cells on the same wing (e.g., spots *P2* and *P3*; *G3* and *G4*). Considerable correlations occur between the spots located in homologous cells on the fore and hind wings.

The general pattern of correlations is determined by the stable eyespots, which are always present. The size of the rare spots (*P4*, *G1*, and *G2*) varies almost independently of that of other spots in the wing pattern.

Fig. 3. Similarity dendrogram of wing length and eyespot size in *Erebia ligea* (L.): males (*A*) and females (*B*). Abscissa: coefficients of correlation (1–*r*). The characters are designated as in Fig. 2*B*.

Variability of Metric Characters in Relation to the Phase of Emergence

Discriminant analysis of wing lengths and diameters of spots in the wing pattern revealed significant differences between the sexes and generations (Table 2). It was not clear, however, whether the metric parameters are uniform in individuals of the same sex emerging at different time during the same generation. In order to assess this seasonal variation, we used the implementation of analysis of variance suitable for studying variability of qualitative characters (Gritsenko et al., 1998). The factors "sex" and "phase of adult emergence" were considered to be independent in our analysis. In addition, the presence of one of the spots in the wing pattern was also regarded as an

Table 3. Results of analysis of variance for wing lengths (*LF*, *LH*) and diameters of eyespots on the fore wing (*P2*, *P3*, *P5*)

Parameter	λ	F	df1	df2	p
2005					
Sex	**0.876**	**9.16**	**4**	**258**	**0.000001**
Emergence phase	0.979	0.69	8	516	0.696539
Presence of spot *P4*	**0.888**	**8.17**	**4**	**258**	**0.000003**
Sex × emergence phase	**0.937**	**2.14**	**8**	**516**	**0.030372**
Sex × presence of spot *P4*	0.996	0.24	4	258	0.914006
Emergence phase × presence of *P4*	0.976	0.78	8	516	0.620114
Sex × emergence phase × presence of *P4*	0.979	0.70	8	516	0.691493
2007					
Sex	**0.895**	**9.95**	**5**	**423**	**0.000001**
Emergence phase	**0.954**	**2.00**	**10**	**846**	**0.030500**
Presence of spot *P4*	**0.961**	**3.41**	**5**	**423**	**0.004939**
Sex × emergence phase	0.991	0.38	10	846	0.954904
Sex × presence of spot *P4*	0.985	1.32	5	423	0.253712
Emergence phase × presence of *P4*	**0.954**	**2.00**	**10**	**846**	**0.030671**
Sex × emergence phase × presence of *P4*	0.963	1.62	10	846	0.095015
2005 and 2007					
Sex	**0.884**	**17.52**	**5**	**669**	**< 0.000000**
Emergence phase	**0.969**	**2.16**	**10**	**1338**	**0.018129**
Presence of spot *P4*	**0.900**	**14.90**	**5**	**669**	**< 0.000000**
Sex × emergence phase	**0.965**	**2.41**	**10**	**1338**	**0.007814**
Sex × presence of spot *P4*	0.996	0.58	5	669	0.714330
Emergence phase × presence of *P4*	0.985	1.02	10	1338	0.424614
Sex × emergence phase × presence of *P4*	0.986	0.91	10	1338	0.520522

independent factor. Since the frequency of occurrence of different spots was not the same, we treated the spots showing a discrete variant of manifestation as phenes (spots *P1*, *P4*, and *P5* on the fore wing and spots *G1*, *G2*, *G3*, and *G4* on the hind wing) (Zakharova, 2008). Analysis of the structure of phenotypic correlations showed that the diameters of spots *P4*, *G1*, and *G2* were weakly correlated with those of other spots in the wing pattern. Therefore, we regarded the presence of spot *P4* as the third independent factor for estimating the seasonal variability of characters of the fore wing by analysis of variance. The presence of spot *G2* was selected to assess the characters of the hind wing in the same way.

The results of three-factor analysis of variance are presented in Tables 3 and 4 and illustrated in Fig. 4. The influence of sex and the presence of the corresponding spot (*P4* or *G2*) were significant for all the samples. Therefore we may conclude that spot *P4*

appears on the fore wing (spot *G2*, on the hind wing) on which other spots have reached a certain threshold size. In other words, if spots *P2*, *P3, P5* (or *G3*, *G4*, *G5*) do not reach the threshold size, then spots *P4* (*G2*) will be absent in the wing pattern of this individual. The results obtained suggest that the spots with a discrete variant of manifestation can be regarded as phenes, i.e., stable states of threshold-based characters, as proposed by Vasil'ev (1988, 1996, 2005, etc.).

The influence of the phase of emergence on the characters in question was not always significant; however, significant interactions with this factor were observed (Tables 3, 4).

Analysis of seasonal variation in the wing length revealed not only sex-related differences (males being larger than females), but also significant differences between individuals of the same sex captured in different time. During the flight period of one generation,

Table 4. Results of analysis of variance for wing lengths (*LF*, *LH*) and diameters of eyespots on the hind wing (*G3*, *G4*, *G5*)

Parameter	λ	F	df1	df2	p
			2005		
Sex	**0.874**	**6.21**	**5**	**215**	**0.000021**
Emergence phase	0.938	1.40	10	430	0.176540
Presence of spot *G2*	**0.785**	**11.75**	**5**	**215**	**0.000001**
Sex × emergence phase	**0.876**	**2.94**	**10**	**430**	**0.001392**
Sex × presence of spot *G2*	**0.931**	**3.18**	**5**	**215**	**0.008662**
Emergence phase × presence of *G2*	**0.889**	**2.62**	**10**	**430**	**0.004265**
Sex × emergence phase × presence of *G2*	**0.917**	**1.91**	**10**	**430**	**0.041621**
			2007		
Sex	**0.823**	**16.36**	**5**	**381**	**< 0.000000**
Emergence phase	0.962	1.48	10	762	0.140742
Presence of spot *G2*	**0.828**	**15.83**	**5**	**381**	**< 0.000000**
Sex × emergence phase	0.961	1.54	10	762	0.121186
Sex × presence of spot *G2*	0.995	0.39	5	381	0.852335
Emergence phase × presence of *G2*	0.989	0.44	10	762	0.929023
Sex × emergence phase × presence of *G2*	0.992	0.30	10	762	0.981279
			2005 and 2007		
Sex	**0.912**	**11.76**	**5**	**612**	**< 0.000000**
Emergence phase	**0.958**	**2.67**	**10**	**1224**	**0.003132**
Presence of spot *G2*	**0.828**	**25.45**	**5**	**612**	**< 0.000000**
Sex × emergence phase	**0.970**	**1.87**	**10**	**1224**	**0.044960**
Sex × presence of spot *G2*	0.990	1.28	5	612	0.271130
Emergence phase × presence of *G2*	0.983	1.03	10	1224	0.415699
Sex × emergence phase × presence of *G2*	0.994	0.36	10	1224	0.964745

the largest males are the first to emerge; they are followed by middle-sized males, and the smallest ones appear later. The females showed the opposite trend: the smallest individuals were the first to emerge, and the largest ones appeared at the end of the flight, but these differences were not always significant (Fig. 4*A*). The males lacking spot *P4* in the wing pattern were larger than those possessing this spot. The females did not reveal a significant difference in wing length depending on the presence of spot *P4*.

Although the females were generally smaller than the males, they had larger spots in the wing pattern. The trend of seasonal variation of diameters of fore wing spots (Table 3; Fig. 4*B*) was the same in both sexes. Adults emerging at the beginning of the flight period had larger spots. For example, in the males possessing spot *P4* and emerging at the beginning of the flight period, the mean diameter of spot *P5* was 1.7 mm, whereas in the last males to emerge, this was

1.2 mm, i.e., the difference was 0.5 mm. The males lacking spot *P4* had small and not insignificant seasonal differences in the size of spot *P5* (1.14 mm in the "early" males and 1.11 mm in the "late" ones).

The pattern of seasonal variation in the size of spots *G3*, *G4*, and *G5* in individuals lacking phene *G2* was the same in both sexes: the largest spots were observed in the adults emerging in the middle of the flight period, and the smallest ones, in those emerging at the end (Fig. 4*C*). Examination of the samples of butterflies possessing phene *G2* showed that the males emerging in the middle of the flight period had the largest spots, and those emerging at the end of the flight period had the smallest spots. The females possessing spot *G2* and emerging at the beginning of the flight period had small spots *G3* and *G4*, while those emerging at the end had the largest spots. The diameter of spot *G5* in the females showed the same variation trends as in the males.
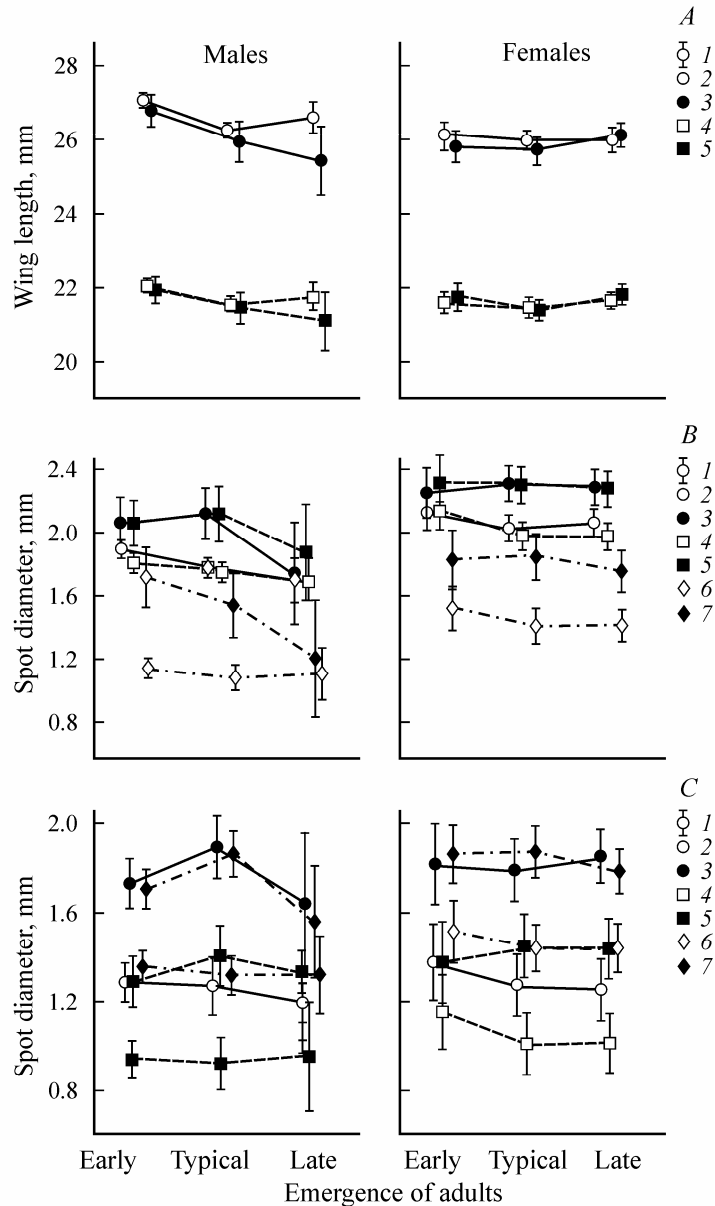
Fig. 4. Variability of wing length (*A*) and diameters of eyespots on the fore (*B*) and hind (*C*) wings of *Erebia ligea* (L.), as related to sex, phase of emergence, and manifestation of phenes *P4* and *G2*. *A*: mean ± standard deviation (*1*), length of fore wing in the absence (*2*) and presence (*3*) of spot *P4*, and length of hind wing in the absence (*4*) and presence (*5*) of spot *P4*). *B*: mean ± standard deviation (*1*), diameter of *P2* in the absence (*2*) and presence (*3*) of spot *P4*, diameter of *P3* in the absence (*4*) and presence (*5*) of spot *P4*, and diameter of *P5* in the absence (*6*) and presence (*7*) of spot *P4*. *C*: mean ± standard deviation (*1*), diameter of *G3* in the absence (*2*) and presence (*3*) of spot *G2*, diameter of *G4* in the absence (*4*) and presence (*5*) of spot *G2*, and diameter of *G5* in the absence (*6*) and presence (*7*) of spot *G2*.

DISCUSSION

The Arran brown *Erebia ligea* in the Middle Urals reveals sex-related and seasonal variations in the wing length and eyespot diameter. According to the previously published data and the results obtained in this study, in some parts of the distribution range the males are larger than the females, whereas in other areas the females are larger. Thus, the value of the "sex dimor-phism index" (ratio of the mean wing lengths of males and females), proposed by some authors (Wiklund and Forsberg, 1991; Nylin et al., 1993), shows geographic variability within the range from 0.99 (Sweden) to 1.02 (Middle Urals).

The species in question is biennial over most of its range (Ivanov et al., 1987) and is characterized by protandry. In the south of Sverdlovsk Province the

adults appear only in odd-numbered years, the males emerging 5–7 days earlier than the females. The individuals emerging at different times during the flight period differ in size. The trends of seasonal size variation in the males and females from our material were differently directed: the largest males and the smallest females appeared at the beginning of the flight period and were followed by medium-sized adults of both sexes, whereas the smallest males and the largest females appeared at the end. Although this trend has been repeatedly described in the literature, in particular for *Pararge aegeria* (Linnaeus, 1758) (Satyridae) and *Lycaena tityrus* (Poda, 1761) (Lycaenidae) (Fischer and Fiedler, 2001), its adaptive significance has not yet been explained. It was assumed that individuals of different sexes might respond differently to selective pressure, for example, to changes in the thermal regime of larval development.

The size of individual eyespots in the wing pattern is only weakly correlated with the size of the wing itself. Even though the males are larger than the females, the females possess larger eyespots. The seasonal variation in the diameters of forewing spots is largely the same in both sexes of *E. ligea*: adults with large spots emerge early, while those with small spots appear late.

Assessment of seasonal variation in the eyespot size and stability of their appearance in the pattern, performed by analysis of variance, has demonstrated a threshold mode of eyespot manifestation. The eyespots treated as phenes in this study appear on the wings only if other spots in the wing pattern have reached a certain threshold size (Brakefield and van Noordwijk, 1985; Zakharova, 2002; Vasil'ev, 2005). The vector of seasonal variation in the eyespot size has been found to change depending on the presence of phene *G2* (spot in cell M_1–M_2 of the hind wing). Spots *G3*, *G4*, and *G5* are the largest in the females lacking phene *G2* and emerging at the beginning of the warm season, and the smallest in those emerging at the end. Among the females possessing spot *G2*, those emerging at the beginning of the flight period have small spots *G3* and *G4*, while those emerging at the end have the largest spots. The opposite trends of seasonal variation in metric characters, observed in the samples of individuals possessing marker phenes (e.g., *P4* and *G2*) and lacking them may suggest an epigenetic explanation: different ontogenetic responses to environmental influences at the population level. Since analysis of variation of the eyespots deals with stable discrete states of threshold-based characters, it should be borne in mind that changes in the size of some spots are correlated with manifestation of certain phenes in the wing pattern. The varying ratio of phenetic eyespot combinations in individuals emerging at different times during the flight period appears to be determined by the threshold nature of their variation. Thus, when describing phenotypic variation at the population level, one should remember that the relative abundance of "morphs" or phenetic combinations may vary during the flight period of a generation, especially in protandric species.

ACKNOWLEDGMENTS

REFERENCES

1. Beldade, P. and Brakefield, P.M., "Concerted Evolution and Developmental Integration in Modular Butterfly Wing Patterns," Evol. Dev. **5**, 169–179 (2003).

2. Beldade, P., Brakefield, P.M., Long, A.D., "Generating Phenotypic Variation: Prospects from 'Evo-Devo' Research on *Bicyclus anynana* Wing Patterns," Evol. Dev. **7**, 101–107 (2005).

3. Brakefield, P.M. and van Noordwijk, A.J., "The Genetics of Spot Pattern Characters in the Meadow Brown Butterfly *Maniola jurtina* (Lepidoptera: Satyrinae)," Heredity **54**, 275–284 (1985).

4. Breuker, C.J., Gibbs, M., Van Dyck, H., et al., "Integration of Wings and Their Eyespots in the Speckled Wood Butterfly *Pararge aegeria*," J. Exp. Zool. (Mol. Dev. Evol.) **308B**, 454–463 (2007).

5. Fischer, K. and Fiedler, K., "Sexual Differences in Life-History Traits in the Butterfly *Lycaena tityrus*: a Comparison between Direct and Diapause Development," Ent. Exp. Appl. **100**, 325–330 (2001).

6. Gritsenko, V.V., Glotov, N.V., Orlinskii, D.B., "Eco-Genetic Analysis of Variability of the Central Pronotal Pattern in the Colorado Potato Beetle (*Leptinotarsa decemlineata*)," Zool. Zh. **77** (3), 278–284 (1998).

7. Ivanov, A.I., Lukhtanov, V.A., Prasolov, V.N., and Sokolov, B.M., "Flight Periodicity of *Erebia ligea* L., *E. embla* Thunb., and *Oeneis jutta* Hbn. (Lepidoptera,

Satyridae) in Leningrad Province," Vestnik Len. Gos. Univ. Ser. 3, No. 2, 8–12 (1987).

8. *Key to Insects of the Russian Far East. Vol. 5. Trichoptera, Lepidoptera. Part 5* (Dal'nauka, Vladivostok, 2005) [in Russian].

9. Korshunov, Yu.P., *Rhopalocera of Northern Asia* (KMK Press, Moscow, 2002) [in Russian].

10. Korshunov, Yu.P. and Gorbunov, P.Yu., *Diurnal Butterflies of the Asian Part of Russia* (Ural Univ., Yekaterinburg, 1995) [in Russian].

11. Monteiro, A.F., Brakefield, P.M., and French, V., "The Evolutionary Genetics and Developmental Basis of Wing Pattern Variation in the Butterfly *Bicyclus anynana*," Evolution **48** (4), 1147–1157 (1994).

12. Monteiro, A.F., Brakefield, P.M., and French, V., "The Relationship between Eyespot Shape and Wing Shape in the Butterfly *Bicyclus anynana*: A Genetic and Morphometrical Approach," J. Evol. Biol. **10**, 787–802 (1997a).

13. Monteiro, A.F., Brakefield, P.M., and French, V., "Butterfly Eyespots: the Genetics and Development of the Color Rings," Evolution **51** (4), 1207–1216 (1997b).

14. Morbey, Y. and Ydenberg, R., "Protandrous Arrival Timing to Breeding Areas: a Review," Ecol. Letters **4**, 663–673 (2001).

15. Nylin, S. and Svärd, L., "Latitudinal Patterns in the Size of European Butterflies," Holarct. Ecol. **14**, 192–202 (1991).

16. Nylin, S., Wiklund, C., Wickman, P., and Garcia-Barros, E., "Absence of Trade-offs between Sexual Size Dimorphism and Early Male Emergence in a Butterfly," Ecology **74** (5), 1414–1427 (1993).

17. Olshvang, V.N., Nupponen, K.T., Lagunov, A.V., and Gorbunov, P.Yu., *Lepidoptera of the Ilmen Nature Reserve* (IGZ RAS, Yekaterinburg, 2004) [in Russian].

18. Paulsen, S.N. and Nijhout, H.F., "Phenotypic Correlation Structure among Elements of the Color Pattern in *Precis coenia* (Lepidoptera: Nymphalidae)," Evolution **47** (2), 593–618 (1993).

19. Schwanwitsch, B.N., "Evolution of the Wing-Pattern in Palaearctic Satyridae III. *Pararge* and Five Other Genera," Acta Zool. **16**, 143–281 (1935).

20. Tatarinov, A.G. and Kulakova, O.I., "On the Variability of *Erebia ligea* (Lepidoptera, Satyridae) in Northeastern European Russia," in *Biodiversity of Epigeic and Edaphic Invertebrates in the North: Abstracts of Papers of Int. Conf.* (Syktyvkar, 1999), pp. 190–191.

21. Vasil'ev, A.G., "Epigenetic Variability: Non-Metric Threshold Characters, Phenes, and Their Compositions," in *Phenetics of Natural Populations* (Moscow, 1988), pp. 158–169 [in Russian].

22. Vasil'ev, A.G., *Phenetic Analysis of Biodiversity at the Population Level*. Doctoral Dissertation in Biology (Yekaterinburg, 1996).

23. Vasil'ev, A.G., *The Epigenetic Basis of Phenetics: Development of Population Meronomy* (Akademkniga, Yekaterinburg, 2005) [in Russian].

24. Wiklund, C. and Forsberg, J., "Sexual Size Dimorphism in Relation to Female Polygamy and Protandry in Butterflies: a Comparative Study of Swedish Pieridae and Satyridae," Oikos **60**, 373–381 (1991).

25. Wiklund, C., Nylin, S., and Forsberg, J., "Sex-Related Variation in Growth Rate as a Result of Selection for Large Size and Protandry in a Bivoltine Butterfly, *Pieris napi*," Oikos **60**, 241–250 (1991).

26. Zakharova, E.Yu., *Analysis of Phenotypic Variation of Eye-Spots in Browns (Lepidoptera: Nymphalidae: Satyrinae)*, Candidate's Dissertation in Biology (Yekaterinburg, 2002).

27. Zakharova, E.Yu., "Protandry and Size Variations in Populations of Univoltine Species of Browns (Lepidoptera: Satyridae)," Evraziat. Entomol. Zh. **3** (1), 59–65 (2004).

28. Zakharova, E.Yu., "Sex Structure and Protandry in the Populations of Some Uni- and Bivoltine Species of Satyridae in the Southern and Middle Urals," in *Biodiversity of Steppe Communities. Proc. of Int. Conf.* (Kostanai, 2006), pp. 74–78.

29. Zakharova, E.Yu., "Seasonal Variability of the Wing Pattern in *Erebia ligea* L. (Lepidoptera: Satyridae) in the Middle Urals," in *Modern Problems of Evolution. A.A. Lyubishchev Memorial Lectures, Issue XXII, Vol. 2: Section of Ecology and Biology* (Ulyanovsk, 2008), pp. 30–38 [in Russian].