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Wing Venation Abnormalities in the Black-Veined White *Aporia crataegi* L. (Lepidoptera, Pieridae): Insight in Terms of Modern Phenetics

I. A. Solonkin[a,*], E. Yu. Zakharova[a], and A. O. Shkurikhin[a]

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, 620144 Russia*

**e-mail: igorsolonkin@yandex.ru*

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Abstract—Patterns of appearance of wing venation abnormalities in the black-veined white *Aporia crataegi* L. were studied. These abnormalities form four types of bilateral compositions and vary quantitatively in the degree of phenotypic expression. This implies that wing venation abnormalities in *A. crataegi* may be regarded as stable states of threshold nonmetric traits with hidden quantitative variability, i.e., "phenes." Different venation abnormalities vary in the pattern of appearance, either showing a trend toward joint occurrence in one individual or occurring independently of each other. Some abnormalities appear mainly asymmetrically, randomly, and independently in different body sides, while others tend to occur symmetrically. Only those abnormalities which appear independently of each other, randomly, and independently in different body sides can be considered random developmental errors and indicators of developmental instability. Such abnormalities were rare, making up less than 20% of the total number.

Keywords: wing venation abnormalities, phenetics, phenotypic variability, phenodeviance, developmental stability, Lepidoptera

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Intraspecific variability of wing venation is a common phenomenon in many actively flying insect species (Martynova, 1948; Sotavalta, 1964; Orlov, 1975; Ross and Robertson, 1990; Padró et al., 2014; Łopuch and Tofilski, 2016; Gülmez, 2019). Patterns of appearance of wing venation abnormalities are well studied in fruit flies of the genus *Drosophila* Fallén, 1823, and among other insects, they have been studied in the natural populations of the honeybee *Apis mellifera* L. (Akahira and Sakagami, 1959; Smith et al., 1997; Porporato et al., 2014; Eligül et al., 2017) and the black-veined white *Aporia crataegi* L. (Solonkin et al., 2017). Wing venation abnormalities in insects are usually regarded as alternative traits, i.e., an abnormality is either present or absent (Smith et al., 1997; Łopuch and Tofilski, 2016; Solonkin et al., 2017; Gülmez, 2019). Discrete variation of such traits often reflects hidden quantitative variation in some morphogenetic parameters, for instance, the level of chemical signals: one of the two alternative states is realized in the phenotype if these parameters reach certain threshold values, and the other state is realized if they remain under the threshold (Vasil'ev, 1988, 2005; Hallgrímsson et al., 2005; Palmer, 2012). In the epigenetic concept of phenetics, going back to the ideas of Waddington (1970) and Shishkin (1984), stable discrete states of nonmetric threshold traits are referred to as phenes (Berry and Searle, 1963; Vasil'ev, 1988, 2005). In this interpretation phenes may be regarded as phenotypic manifestations of alternative morphogenetic pathways. The frequencies of phenes are believed to characterize the potential set of developmental variants of a given morphological structure and their probabilities, i.e., the epigenetic landscape of the population (Vasil'ev, 1988; 2005). A discrete phenotypic trait can be considered a threshold trait based on the following indirect criteria: first, the presence of four types of bilateral compositions: +/+, −/−, +/−, and −/+, with "+" and "–" denoting the alternative states; second, a quantitatively varying level of expression of the phenotypically realized traits (Vasil'ev, 2005).

In phenetic research it is essential to distinguish between stable discrete states of a threshold trait (phenes) and stable combinations of states of different traits (phene compositions) (Vasil'ev, 1988, 2005; Sereno, 2007). In the studies of wing venation abnormalities each vein is commonly considered a trait, and all the abnormalities recorded within that vein are treated equally (Akahira and Sakagami, 1959; Porporato et al., 2014; Solonkin et al., 2017; Eligül et al., 2017). However, wing veins in insects are quite long, and abnormalities may occur in different sections of the same vein. The question arises as to whether such abnormalities should be treated as a single phene or as different phenes. According to the current approaches to defining and delimiting morphological traits (Sereno, 2007; Vasil'ev and Vasil'eva, 2009), if identical or different abnormalities can occur simultaneously in different sections of the same vein, these sections are capable of independent variation and should therefore be regarded as different traits.

Wing venation abnormalities in insects are often regarded as random developmental errors; correspondingly, the frequencies of such abnormalities are used as an additional measure of developmental instability in populations, along with the level of fluctuating asymmetry (Ross and Robertson, 1990; Clarke, 1993; Smith et al., 1997; Padró et al., 2014; Łopuch and Tofilski, 2016). However, the possibility of using the frequencies of rare states of nonmetric traits (phenodeviances) for this purpose remains open to question. To the best of our knowledge, no theoretical justification of this method has been published; moreover, some authors deny the validity of any other measure of developmental instability apart from fluctuating asymmetry (Debat and David, 2001; Willmore et al., 2007; Takahashi, 2019).

Developmental stability is understood as resistance to random morphogenetic abnormalities (ontogenetic noise), i.e., the ability to produce the normal phenotype determined by the given genotype under the same environmental conditions. Developmental stability has attracted the attention of researchers as a potential indicator of the "quality" of individuals in the population, as well as the "quality" of their environment. It was supposed that the fittest individuals had the most stable ontogenesis (Zakharov, 1987; Dongen, 2006; Klingenberg, 2019). The probability of phenotypic realization of a rare state of the nonmetric trait is known to depend on the genotype, the environmental conditions during development, the level of morphogenetic sensitivity to environmental factors, and the level of morphogenetic instability (Debat and David, 2001; Hallgrímsson et al., 2005; Willmore et al., 2007; Takahashi, 2019). Abnormalities of wing venation in insects may be used as markers of developmental instability only if they appear due to random morphogenetic errors and do not depend on the genotype or environmental conditions.

As far as we know, there is no method for direct assessment of the contribution of different factors to phenodeviance development in natural populations. For indirect assessment, the antimeric expression of phenodeviances may be analyzed following the method of Astaurov (1974). If all the individuals have the same genetic or environmentally induced predisposition to a given phenodeviance, the presence of this phenodeviance in particular individuals will depend only on random morphogenetic errors. If the phenodeviance develops independently in two body sides, the probability of its asymmetric manifestation p_{asymm} will be determined by the formula

$$p_{\text{asymm}} = p_a \times (1 - p_b) + p_b \times (1 - p_a), \qquad (1)$$

and the probability of its symmetric manifestation p_{symm} will be

$$p_{\text{symm}} = p_a \times p_b, \qquad (2)$$

where p_a and p_b are the probabilities of the phenodeviance appearing on the right and left body sides, respectively (Astaurov, 1974; Vasil'ev, 2005; Palmer, 2012). Phenodeviances whose observed frequencies do not differ significantly from the values expected in case of their random and independent development in different body sides may be used as indicators of developmental instability in the studied population. This conclusion would be valid only for the specific group in question. Differences between groups in the phenodeviance frequency may be determined not only by different levels of developmental instability but also by genetic or ecological factors; therefore, the levels of developmental instability in different groups should be compared using the rare states of not one but several mutually independent nonmetric traits. Correlated changes in the frequencies of rare states of several such traits would most probably reflect the actual change in the developmental stability level.

In this paper we analyze wing venation abnormalities in the black-veined white *Aporia crataegi*, focusing on the following questions: (1) Do these venation abnormalities constitute threshold traits based on hidden quantitative variation? (2) Can the forks in different sections of the same vein be considered homologous? (3) Do venation abnormalities appear independently of each other? (4) Can the frequencies of wing venation abnormalities be used for estimating the level of developmental instability in insect populations?

MATERIALS AND METHODS

Adults of the black-veined white *Aporia crataegi* L. (3804 males and 3119 females) were collected in 2013–2019 in the environs of Fomino (Sverdlovsk Province, Sysertskii District, 56°36′N, 61°03′E), at the Biological station of the Ural Federal University. The material is kept in the museum of the Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences (Yekaterinburg). Venation abnormalities were detected in detached wings which were examined from the underside under an MBS-10 stereo microscope. Abnormalities were recorded separately in each vein section and in each wing cell, following the scheme shown in Fig. 1. The individuals missing some parts of the wing were excluded from analysis of venation abnormalities in the damaged area, but they were still used in analysis of the other wing parts; correspondingly, the sample size was not the same for different veins (Table 1).

We have analyzed five types of venation abnormalities (Fig. 2): additional forks of veins (I), additional longitudinal veins appearing in wing cells (II), reduction of a vein that is normally present (III), merging of veins that are normally present (IV), and complete splitting of a vein into two parallel veins (V). The technique for recording and classification of venation abnormalities, described in detail in our earlier paper (Solonkin et al., 2017), was used herein with minor modifications; in particular, our analysis included some vein segments (M_2–$M_1 + R_{4+5}$, R_1–R_{2+3}, $M_1 + R_{4+5}$, and M_2 in the forewing) which had not been considered previously.

For analysis of variability of the intensity of venation abnormalities and their position along the veins, we selected two most common additional forks, namely those of vein Cu_1 in the forewing and vein M_2 in the hindwing. Altogether, the morphometric parameters of 149 forks of Cu_1 and 350 forks of M_2 were analyzed. Measurements were carried out using an MBS-10 stereo microscope with an ocular micrometer. The scheme of measurements is shown in Fig. 3: parameter *1* describes the total length of the vein from its origin to the terminus of its main branch; parameter *2*, the distance from the additional branching point to the wing margin; parameter *3*, the length of the additional branch (it was not measured if there were two complete branches). The anterior branch of Cu_1 was nearly always complete (in 96% of the cases), while its posterior branch was often weakly developed (in 69% of the cases); therefore, the anterior branch was considered the main one, and the posterior branch, the additional one. By contrast, in the fork of vein M_2 the anterior branch was often weakly developed (in 85% of the cases), while the posterior branch was nearly always complete (in 98% of the cases). Correspondingly, the posterior branch of vein M_2 was considered the main one, and the anterior branch, the additional one.

The position of the abnormality along the vein was characterized by index *1*, calculated as the ratio of parameter *2* / parameter *1*. The degree of development of the additional branch was estimated by index *2*, calculated as the ratio of parameter *3* / parameter *2*. The median values of the indices for different forks were compared using the Mann–Whitney test *U*, and the distributions of indices, using the Kolmogorov–Smirnov test *d*.

Correlations between different venation abnormalities were estimated using Spearman's rank correlation coefficient R_S. Only those abnormalities which were recorded more than 10 times in the pooled sample of specimens of the same sex were used in correlation analysis. As a result, correlations were calculated between 39 abnormalities in males and 33 abnormalities in females. The correlation matrices were processed by metric multidimensional scaling (Principal Coordinates Analysis, PCoA). Then the principal coordinates explaining no less than 2% of total variation each were used in cluster analysis by Ward's method; these were the first 26 coordinates for males and 24 coordinates for females.

Analysis of antimeric occurrence included the wing venation abnormalities which were recorded more than 10 times. In this case, the unit of analysis was the body side: if several identical abnormalities were found in the same vein, they constituted a single record of abnormality in that vein in the corresponding side of the body. As a result, analysis of antimeric patterns included

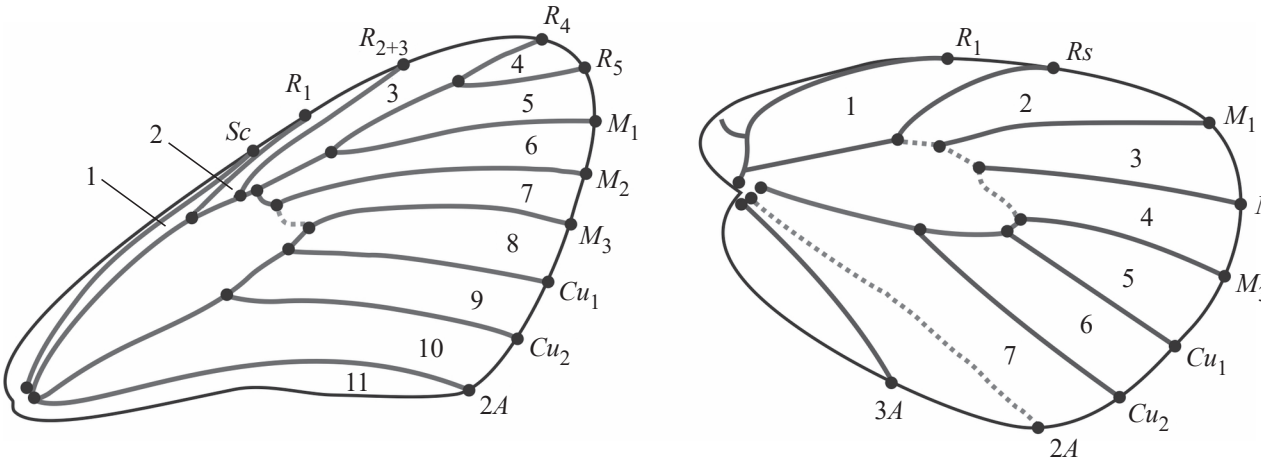

Fig. 1. Scheme of normal wing venation in *Aporia crataegi* L. The boundaries of the vein sections included in analysis are marked with dots; the parts excluded from analysis are shown as dotted lines. The wing cells are numbered.

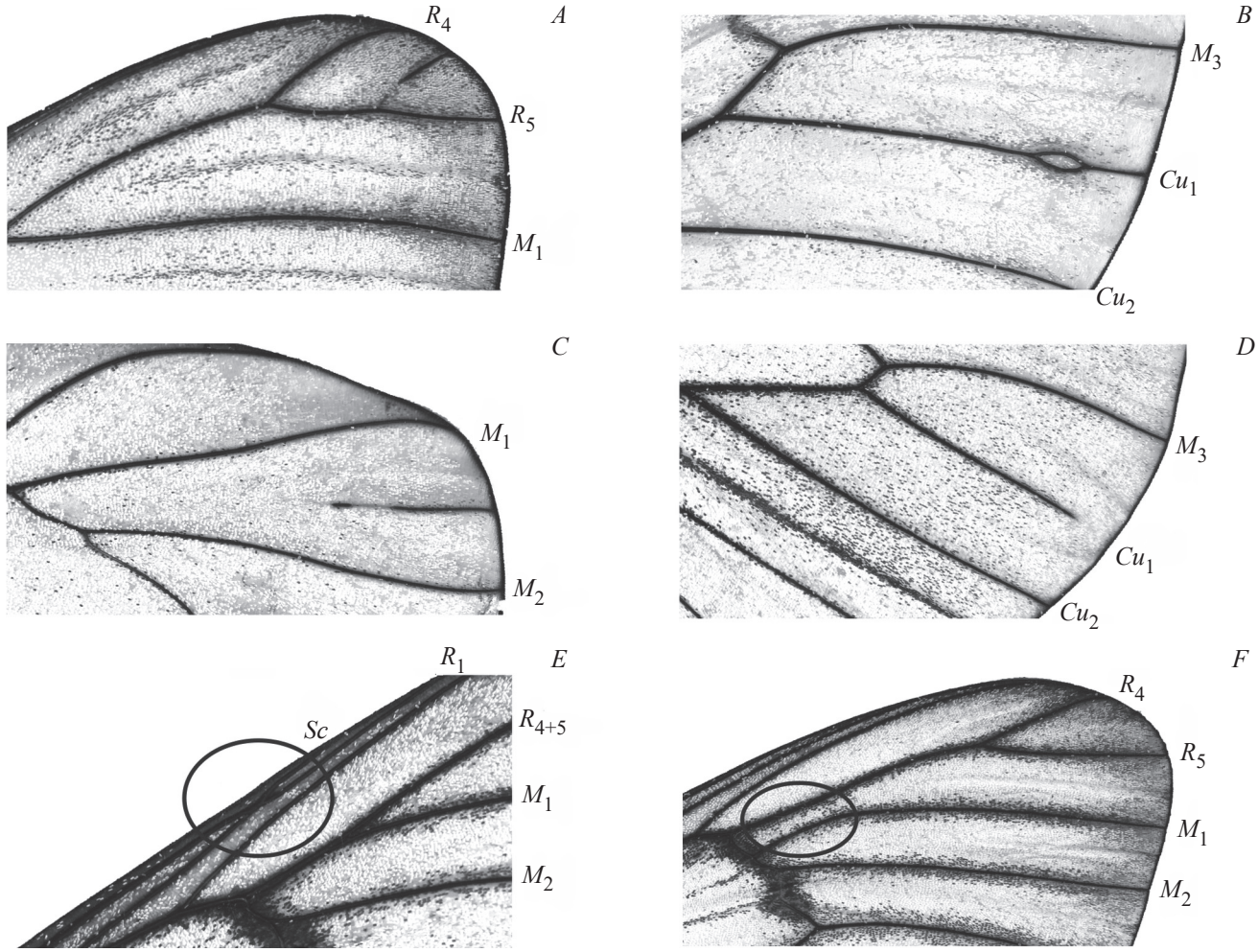

Fig. 2. Types of wing venation abnormalities in *Aporia crataegi* L., analyzed in this work: (*A*, *B*) extra forks of veins (type I); (*C*) an extra vein in the wing cell (type II); (*D*) reduction of a vein (type III); (*E*) merging of veins that are normally present (type IV); (*F*) complete splitting of a vein into two veins (type V). Modified after Solonkin et al., 2017.

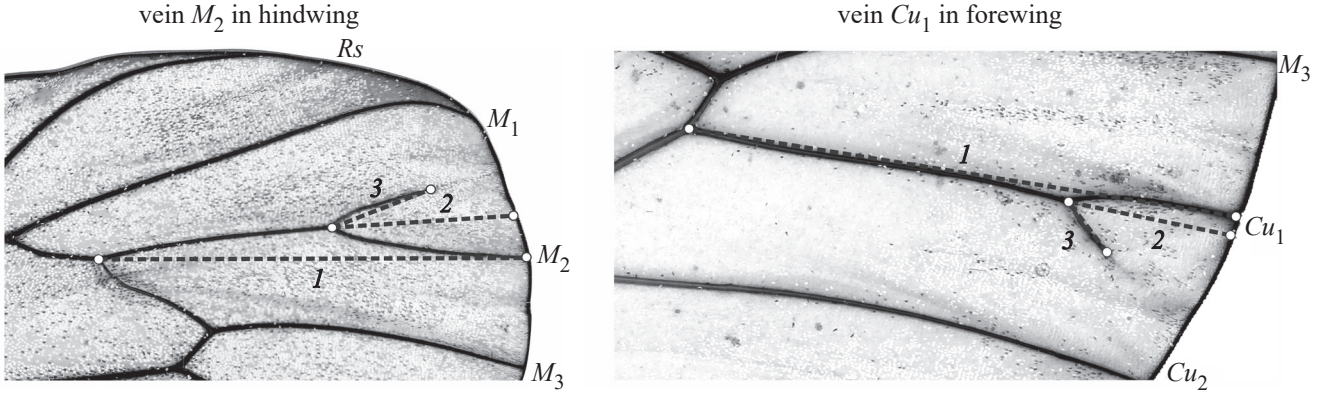

Fig. 3. Scheme of the measured parameters of extra forks of vein M_2 in the hindwing and vein Cu_1 in the forewing of *Aporia crataegi* L. Parameters: *1*, length of the vein from its origin to the terminus of its main branch; *2*, distance from the additional branching point to the wing margin; *3*, length of the additional branch.

39 abnormalities in males and 32, in females. For each venation abnormality we calculated the frequency of its occurrence in the right and the left body side (p_a and p_b, respectively) and the number of specimens with asymmetric and symmetric occurrence of this abnormality. To detect directional asymmetry, the frequencies of each abnormality in the right and the left side were compared using Fisher's exact test. Then the expected frequencies of specimens with asymmetric and symmetric occurrence of each abnormality were calculated using formulas (1) and (2), assuming their random and independent development in the two body sides. The observed and expected frequencies of specimens with asymmetric and symmetric occurrence of each abnormality were compared using Fisher's exact test, and the incidence of asymmetric occurrence was calculated as the number of specimens with asymmetric occurrence related to the total number of specimens showing the particular abnormality. The deviation of the observed frequency of specimens with symmetric abnormality from the expected value was estimated by the difference between the observed and expected frequencies of asymmetric occurrence of this abnormality (D). The variation of D depending on the sex and the abnormality type was analyzed by robust two-way ANOVA. The Benjamini–Hochberg correction was applied in all the cases of multiple comparison. The measure of central tendency was the median (Me). All the procedures of statistical analysis were carried out in PAST 3.26 software (Hammer et al., 2001) and in the R environment (R Core Team, 2019).

RESULTS AND DISCUSSION

The level of expression of the additional branch of vein Cu_1 in the forewing and vein M_2 in the hindwing varied over a wide range, the forks with a weakly developed additional branch being the most common (Fig. 4). The level of expression of the additional branch did not differ between Cu_1 and M_2 either in the median value ($U = 9920$; $p = 0.11$) or in the distribution as a whole ($d = 0.14$; $p = 0.16$). The observed continuous quantitative variation in the forks of Cu_1 and M_2 suggests that discrete variability of the veins reflects quantitative variation of some parameters of wing morphogenesis, i.e., that venation abnormalities appear if these parameters exceed (or fall below) a certain threshold (Vasil'ev, 2005; Hallgrímsson et al., 2005). According to our observations, other wing venation abnormalities (forks, extra veins, and reduced veins) also displayed continuous variation, similar to the forks of veins Cu_1 and M_2 analyzed herein. Besides, they formed four types of bilateral compositions: +/+, −/−, +/−, and −/+. Therefore, venation abnormalities may be regarded as stable states of threshold nonmetric traits with hidden quantitative variability, i.e., phenes (Vasil'ev, 2005). The exact nature of the factors that vary in a continuous quantitative manner and induce venation abnormalities through a threshold mechanism remains to be determined. We may suppose that the frequencies of different venation abnormalities reflect the spectrum of potential pathways of vein morphogenesis and the probabilities of their realization, i.e., they characterize the epigenetic landscape of the population.

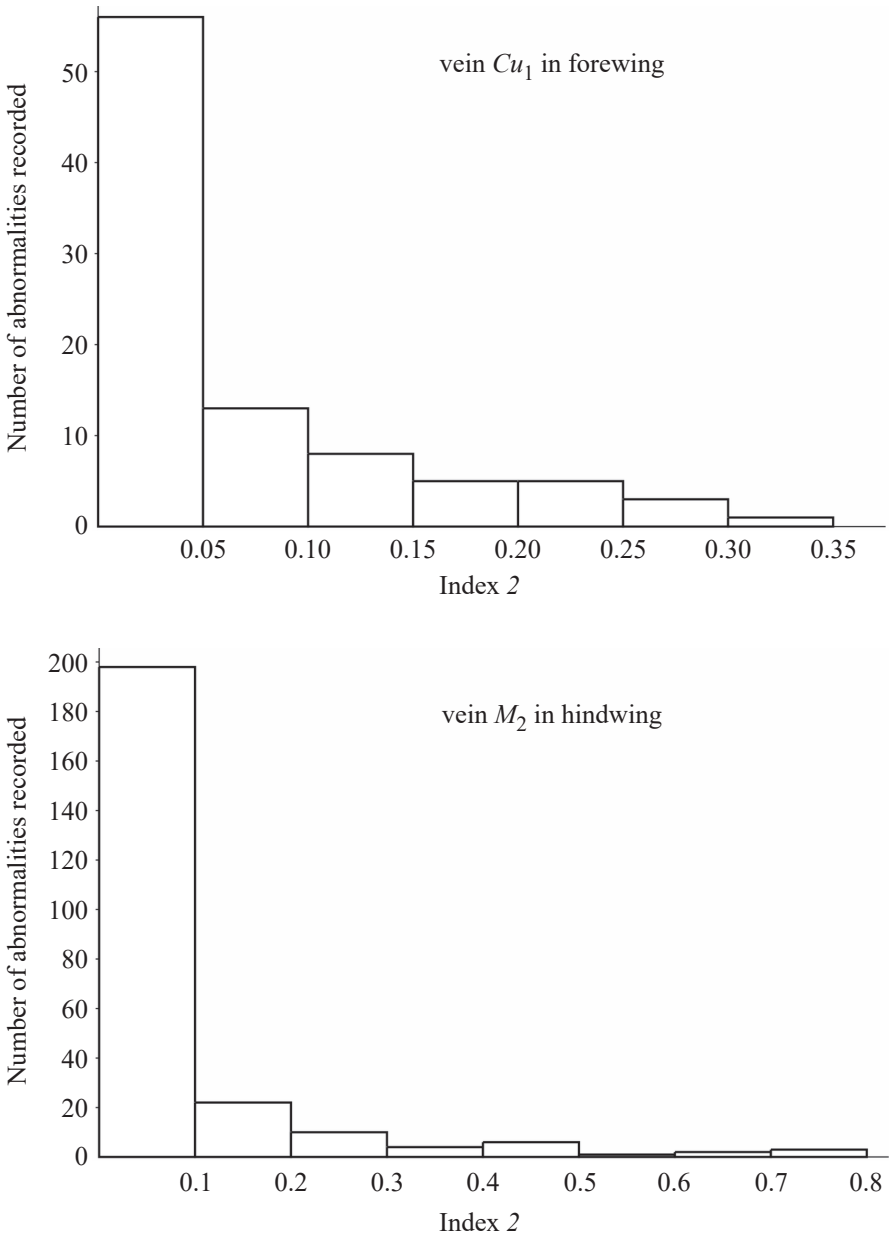

Fig. 4. Distribution of extra forks of vein Cu_1 in the forewing and vein M_2 in the hindwing of *Aporia crataegi* L. by the level of expression of the additional branch.

It remains unknown whether identical abnormalities occurring in different sections of the same vein should be treated as one phene or different phenes. Our results concerning this question have been inconsistent. Identical venation abnormalities co-occurred in vein Cu_1 of the same specimen in 5% of the cases, and in vein M_2 in 3% of the cases. The observed frequency of co-occurrence of forks in Cu_1 corresponded to the value expected in case of their random and independent combination ($p = 0.07$), whereas joint occurrence of forks in vein M_2 were observed significantly less frequently than it would be expected assuming their random and independent combination ($p = 0.006$). The simultaneous presence of identical forks in different sections of the same vein indicates that these sections can vary independently of each other; therefore, they should be regarded as different traits, while abnormalities occurring in different vein sections should be treated as different phenes

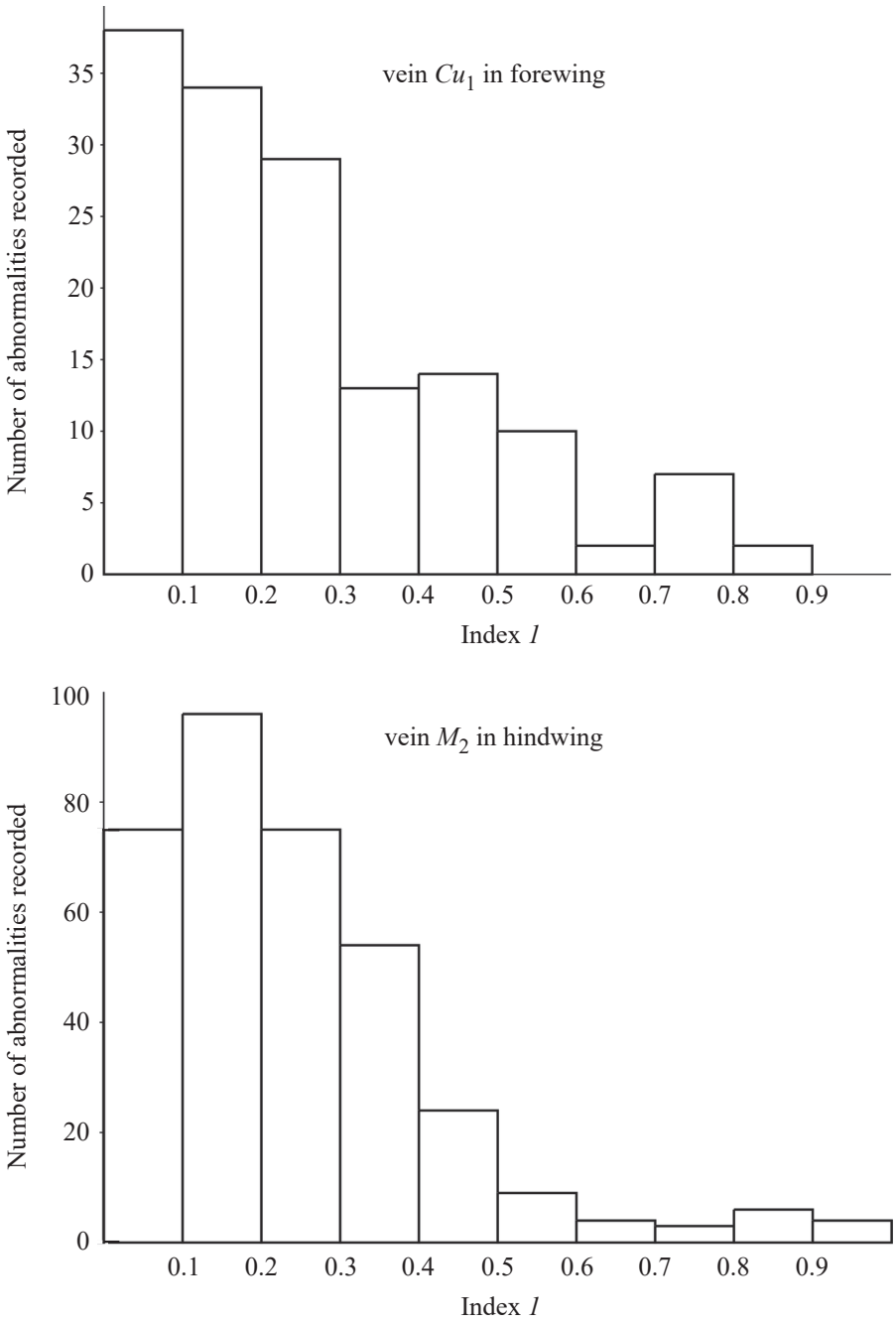

Fig. 5. Distribution of extra forks of vein Cu_1 in the forewing and vein M_2 in the hindwing of *Aporia crataegi* L. by their position along the vein.

(Sereno, 2007; Vasil'ev and Vasil'eva, 2009). On the other hand, as revealed by the distribution of index *I* in Fig. 5, forks could appear along the entire length of veins Cu_1 and M_2 rather than in any specific sections. The probability of forking was the lowest at the bases of both veins and increased gradually toward the distal wing margin (Fig. 5). The position of forks along veins Cu_1 and M_2 did not differ either in the median value ($U = 26064$; $p = 0.99$) or in the distribution as a whole ($d = 0.10$; $p = 0.20$). Thus, veins Cu_1 and M_2 did not include any identifiable sections which could be analyzed as separate traits.

It is remarkable that the same forking variants appeared in specific parts of the same vein in different individuals. Earlier we demonstrated that certain abnormalities were realized consistently in different veins of *A. crataegi* (Solonkin et al., 2017). In view of these data, we may conclude that the patterns of occurrence of forks along the studied veins confirm the morphogenetic unity of each vein and the existence of morphogenetic differences between different veins. At the present state of knowledge, venation abnormalities may be considered either as phenes or as compositions of phenes pertaining to different traits.

The independence of wing venation abnormalities was assessed by calculating Spearman's rank correlation coefficients R_S between different abnormalities. In cluster analysis, all the venation abnormalities formed two distinct clusters (Fig. 6). Cluster 1 in males comprised additional veins in cells 3, 8, and 9 of the forewing and cell 1 of the hindwing. In females this cluster comprised the same set of traits and also additional veins in cells 4–6 of the forewing. All the remaining venation abnormalities were united in cluster 2 (see Fig. 6), which was clearly subdivided into two major subclusters in both sexes. Each subcluster included the venation abnormalities belonging to different types and occurring in different vein stems on both the forewings and the hindwings; thus, we could not identify the trends underlying the formation of these two subclusters.

The venation abnormalities included in cluster 2 were mostly manifested independently of each other, with the coefficients of correlation varying from −0.16 to 0.19 (Me = −0.02) in females and from −0.11 to 0.18 (Me = −0.01) in males. In addition, in males the coefficients of correlation between different abnormalities of the same veins were significantly greater than those between abnormalities in different veins ($Me_1 = 0.08$; $Me_2 = -0.01$; Mann–Whitney test: $U = 1185$, $p < 0.001$). Most abnormalities of various types occurring in the same veins in males were clustered together (Fig. 6); thus, if more than one abnormality was present in the wing of a male, the abnormalities were more likely to occur on the same vein than on different veins.

The traits composing cluster 1, as compared with the abnormalities from cluster 2, more often co-occurred in the same individual, the coefficients of correlation varying from 0.11 to 0.55 (Me = 0.31, $p < 0.05$) in females and from 0.12 to 0.24 (Me = 0.17, $p < 0.05$) in males. Since the venation abnormalities included in cluster 2 appeared independently of each other, the correlated expression of abnormalities from cluster 1 could hardly have resulted from coordination of morphogenetic processes in different wing cells. It is much more probable that co-occurrence of abnormalities forming cluster 1 was determined by a certain factor (for instance, genetic or environmental one) increasing the predisposition to development of extra veins in some wing cells. Thus, wing venation abnormalities in *A. crataegi* can be united in two groups. The first group is composed of the abnormalities included in cluster 1, which relatively often co-occur in the same individual. The second group includes the abnormalities from cluster 2, which appear independently of each other.

No directional asymmetry was revealed in the wing venation abnormalities (Table 1). The deviation of the observed incidence of asymmetric occurrence from the expected value (D) for different abnormalities varied from 0 to 35% (Me = 7.2%) in males and from 0 to 55% (Me = 13.6%) in females. This deviation was significant in 38% of cases in males and in 44% of cases in females (see Table 1). Thus, antimeric occurrence of different venation abnormalities in *A. crataegi* showed different patterns: some abnormalities appeared randomly and independently on different body sides, while others revealed a more or less distinct trend toward symmetry. No deviation toward asymmetric occurrence (i.e., antisymmetry) was observed. This result is consistent with the literature data, according to which phenodeviances either occur randomly and independently of each other in different sides of the body (Astaurov, 1974; Vasil'ev, 2005) or tend to occur symmetrically (Palmer, 2012).

According to the robust ANOVA results, deviation from the expected frequency of asymmetric occurrence (D) considerably depended on the group of abnormality ($p = 0.001$) and to a lesser extent depended on the sex ($p = 0.026$). On the whole, venation abnormalities were more symmetric in females than in males (Fig. 7). Abnormalities of the first group were more symmetric than other abnormalities in both sexes (Fig. 7). Thus, venation abnormalities of the first group relatively often occurred simultaneously and symmetrically in the same individual. It should be noted that all the abnormalities of the first group are extra veins, i.e., the product of excessive and ectopic differentiation of the cells forming the wing membrane into those forming the veins. These abnormalities may result from realization of the

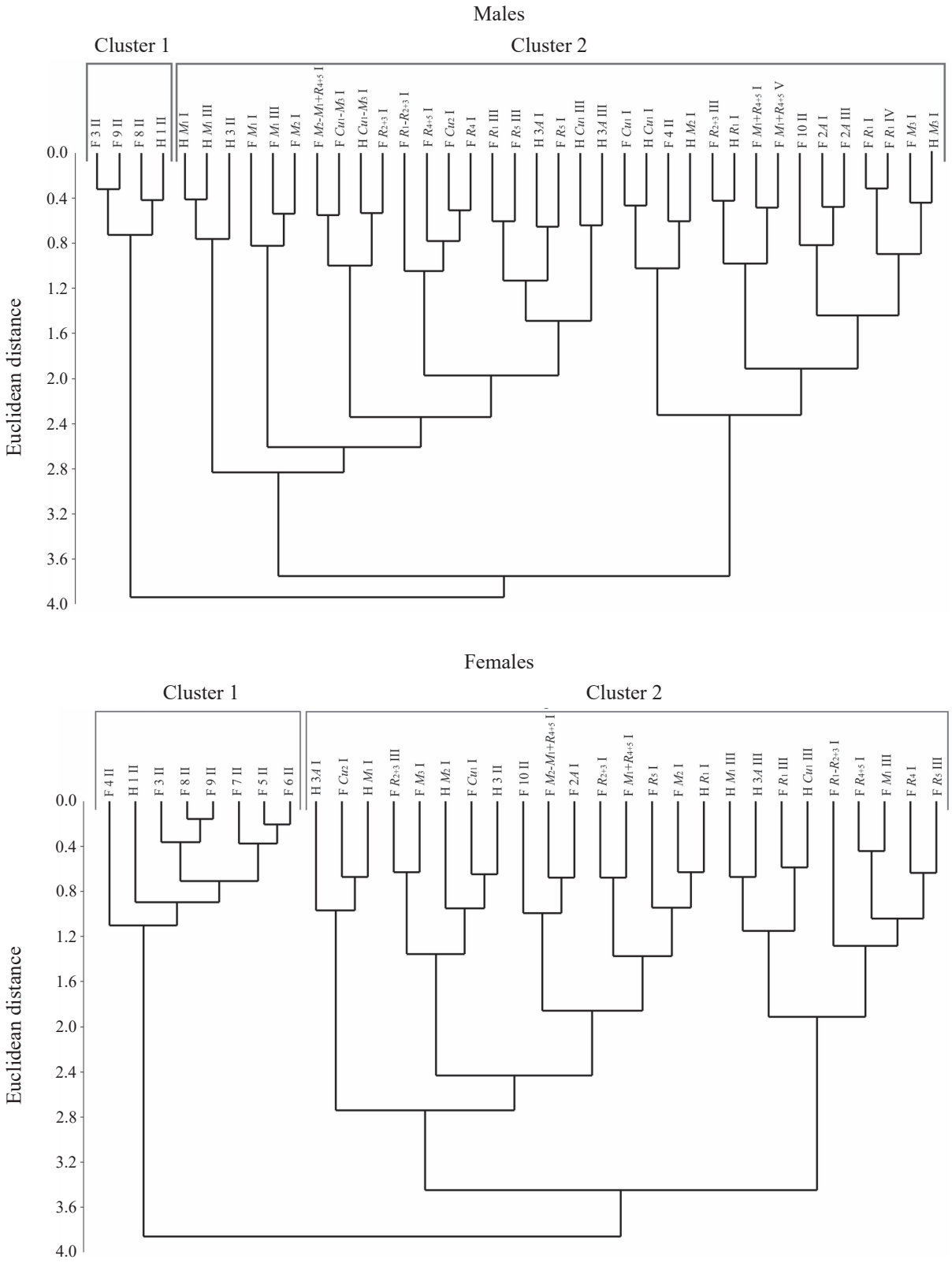

Fig. 6. Results of cluster analysis of wing venation abnormalities in *Aporia crataegi* L., obtained by metric multidimensional scaling of the correlation matrix: F, forewing; H, hindwing; the wing cells are numbered with Arabic numerals, the types of abnormalities, with Roman numerals.

Table 1. Patterns of antimeric occurrence of wing venation abnormalities in *Aporia crataegi* L.

	Vein section or cell of the wing	Type of abnormality	*N*	Number of specimens with abnormality		Occurrence in body sides, %		p_1	*D*, %	p_2
				asymmetric	symmetric	right	left			
				Males						
Forewing	M_2–$M_1 + R_{4+5}$	I	3728	85	3	1.26	1.18	1	2.8	0.248
	R_1–R_{2+3}	I	3728	211	59	4.46	4.33	1	19.6	0.000
	R_1	I	3718	12	0	0.16	0.16	1	−0.1	1
		III	3718	72	9	1.23	1.18	1	10.5	0.003
		IV	3718	15	6	0.35	0.37	1	28.4	0.013
	R_{2+3}	I	3706	43	0	0.56	0.59	1	−0.3	1
		III	3706	16	0	0.29	0.13	0.818	−0.1	1
	$M_1 + R_{4+5}$	I	3726	143	52	3.31	3.64	1	24.9	0.000
		V	3726	15	8	0.37	0.45	1	34.6	0.002
	R_{4+5}	I	3717	18	0	0.32	0.16	0.811	−0.1	1
	R_4	I	3698	27	0	0.21	0.51	0.505	−0.2	1
	R_5	I	3674	50	8	1.21	0.56	0.113	13.4	0.006
		III	3674	14	0	0.22	0.16	1	−0.1	1
	M_1	I	3676	22	0	0.32	0.27	1	−0.1	1
		III	3676	17	1	0.22	0.30	1	5.4	1
	M_2	I	3689	22	0	0.30	0.30	1	−0.1	1
	M_3	I	3688	25	2	0.38	0.43	1	7.2	0.419
	Cu_1–M_3	I	3727	16	0	0.29	0.13	0.818	−0.1	1
	Cu_1	I	3687	136	37	2.91	2.82	1	19.9	0.000
	Cu_2	I	3668	16	0	0.13	0.32	0.797	−0.1	1
	2*A*	I	3644	77	23	1.35	2.00	0.479	22.2	0.000
		III	3644	26	1	0.46	0.30	1	3.5	1
	cell 3	II	3687	107	40	2.50	2.55	1	25.9	0.000
	cell 4	II	3670	26	1	0.22	0.57	0.465	3.5	0.832
	cell 8	II	3665	10	4	0.27	0.22	1	28.5	0.069
	cell 9	II	3651	28	9	0.68	0.57	1	24.0	0.002
	cell 10	II	3622	217	55	4.42	4.70	1	17.9	0.000
Hindwing	R_1	I	3693	12	1	0.19	0.19	1	7.6	1
	M_1	I	3661	66	5	1.00	1.05	1	6.5	0.060
		III	3661	42	8	0.73	0.86	1	15.6	0.005
	M_2	I	3656	299	54	5.41	5.70	1	12.4	0.000
	M_3	I	3661	9	1	0.19	0.11	1	9.9	1
	Cu_1–M_3	I	3701	25	2	0.51	0.27	0.797	7.2	0.419
	Cu_1	I	3666	13	0	0.16	0.19	1	−0.1	1
		III	3666	23	1	0.43	0.27	1	4.0	1
	3*A*	I	3660	190	21	3.19	3.18	1	8.3	0.000
		III	3660	40	5	0.84	0.51	0.708	10.8	0.054
	cell 1	II	3673	26	2	0.51	0.32	0.993	6.9	0.419
	cell 3	II	3633	128	15	2.20	2.14	1	9.4	0.001

Table 1. (Contd.)

	Vein section or cell of the wing	Type of abnormality	*N*	Number of specimens with abnormality		Occurrence in body sides, %		p_1	*D*, %	p_2
				asymmetric	symmetric	right	left			
	Females									
Forewing	M_2–$M_1 + R_{4+5}$	I	3023	37	1	0.49	0.79	1	2.3	1
	R_1–R_{2+3}	I	3023	138	40	3.52	3.69	1	20.6	0.000
	R_1	III	3014	75	7	1.55	1.38	1	7.8	0.014
	R_{2+3}	I	3005	35	2	0.63	0.66	1	5.1	0.354
		III	3006	14	0	0.23	0.23	1	−0.1	1
	$M_1 + R_{4+5}$	I	3026	45	8	1.05	0.99	1	14.6	0.005
	R_{4+5}	I	3017	13	0	0.07	0.36	0.357	−0.1	1
	R_4	I	3010	27	1	0.53	0.43	1	3.3	1
	R_5	I	2993	62	1	0.93	1.19	1	1.1	1
		III	2993	9	2	0.23	0.20	1	18.1	0.348
	M_1	III	2993	5	4	0.20	0.26	1	44.3	0.039
	M_2	I	3003	16	3	0.40	0.33	1	15.6	0.165
	M_3	I	2995	23	1	0.50	0.33	1	4.0	1
	Cu_1	I	2995	86	14	1.95	1.85	1	13.0	0.001
	Cu_2	I	2992	22	1	0.43	0.46	1	4.1	1
	cell 3	II	3001	86	79	4.23	3.92	1	45.8	0.000
	cell 4	II	2989	30	4	0.53	0.73	1	11.5	0.095
	cell 5	II	2974	9	11	0.46	0.56	1	54.7	0.000
	cell 6	II	2980	10	6	0.36	0.36	1	37.3	0.009
	cell 7	II	2991	10	3	0.30	0.26	1	22.9	0.139
	cell 8	II	2980	17	14	0.76	0.73	1	44.8	0.000
	cell 9	II	2971	20	18	0.93	0.96	1	46.9	0.000
	cell 10	II	2954	172	57	4.72	4.90	1	22.4	0.000
Hindwing	R_1	I	2992	17	0	0.17	0.40	1	−0.1	1
	M_1	I	2975	33	0	0.43	0.66	1	−0.3	1
		III	2975	7	3	0.23	0.23	1	29.9	0.128
	M_2	I	2966	193	38	5.20	3.89	0.357	14.2	0.000
	Cu_1	III	2972	11	0	0.13	0.23	1	−0.1	1
	3*A*	I	2993	145	17	3.08	2.91	1	9.0	0.001
		III	2993	21	4	0.43	0.53	1	15.8	0.092
	cell 1	II	2980	20	11	0.60	0.83	1	35.1	0.000
	cell 3	II	2945	55	2	1.00	1.00	1	3.0	0.354

D is difference between the observed and expected frequencies of asymmetric occurrence of venation abnormalities; *N* is the number of specimens examined; p_1 is significance of differences between the frequencies of abnormalities in the right and the left body sides; p_2 is significance of differences between the observed and expected frequencies of specimens with symmetric and asymmetric occurrence of venation abnormalities.

same morphogenetic pathway, its probability depending on ecological or genetic factors. Therefore, such abnormalities cannot be regarded as independent characters, and their frequencies cannot be used to assess the level of developmental instability in the given population.

All the other studied venation abnormalities (those included in the second group) appeared independently of each other and usually asymmetrically. However, deviation (*D*) from the expected frequency of asymmetric occurrence of these abnormalities was also highly variable. In the sample of males, the value of *D* in abnormalities of the second group depended on their incidence: the more frequent abnormalities had a more symmetric occurrence ($R_S = 0.69$; $p < 0.001$). Thus, rare venation abnormalities usually appeared in males as the result of random morphogenetic errors, while the development of relatively common abnormalities was to a greater extent determined by genetic or ecological factors. This trend was non-significant in females ($R_S = 0.30$; $p = 0.15$).

Only those venation abnormalities which show a non-significant difference (*D*) between the observed and expected frequencies of asymmetric occurrence can serve as measures of the level of developmental instability. This criterion is met by a considerable part of the studied traits: 24 out of 39 analyzed characters in males and 18 out of 32 characters in females. However, such abnormalities comprise only 21% of the total number of recorded abnormalities in males and 23%, in females. Therefore, most of the venation abnormalities analyzed herein cannot be used to characterize developmental instability. Thus, it would be impossible to assess the level of developmental instability in *A. crataegi* populations based on the total frequency of all the venation abnormalities.

Our data indicate that wing venation abnormalities in *A. crataegi* may be regarded as stable states of threshold nonmetric traits, i.e., as phenes. This result agrees with the previously described tendency of particular types of abnormalities to occur in the same sections of veins (Solonkin et al., 2017). Along with other threshold nonmetric traits described in the literature, wing venation abnormalities in *A. crataegi* may probably be interpreted as the results of certain relatively stable aberrant morphogenetic pathways (Vasil'ev, 1988, 2005).

As shown above, different venation abnormalities follow different trends. Some abnormalities (many extra veins in the wing cells) co-occur in the same individual with relatively high frequencies, while others occur independently and do not form stable phenotypic combinations. Some abnormalities (for instance, additional forks of M_1 in the hindwing, reduction of veins 2*A* in the forewing and 3*A* in the hindwing) are mostly manifested asymmetrically, randomly and independently in different body sides, whereas others (in particular, most of the extra veins and additional forks of the radial stem along the R_1–R_{2+3} section) tend to occur symmetrically. Only those wing venation abnormalities which appear independently from each other, randomly and independently in different body sides can be considered random developmental errors and indicators of developmental instability. They are usually quite rare and comprise a smaller proportion of the total number of recorded abnormalities.

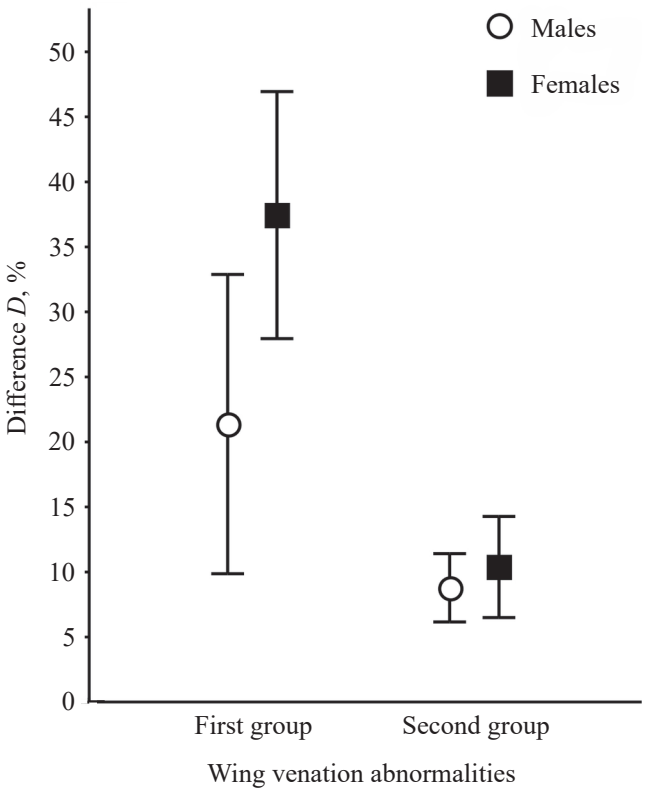

Fig. 7. Difference (*D*) between the observed and expected frequencies of asymmetric occurrence of wing venation abnormalities in males and females of *Aporia crataegi* L.: mean values and confidence intervals.

Thus, frequencies of wing venation abnormalities may probably be used to describe the epigenetic landscape

of insect populations (Vasil'ev, 1988, 2005), but they should be utilized with caution when assessing the level of developmental instability. The approach used in this work can be applied to analysis of wing venation variability in other insect taxa characterized by active flight and relatively stable, species-specific venation patterns.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

REFERENCES

Akahira, Y. and Sakagami, S.F., Observations on the variability of wing venation in the honeybees, *J. Fac. Sci. Hokkaido Univ. Ser. 6 Zool.*, 1959, vol. 14, no. 2, p. 175.

Astaurov, B.L., *Nasledstvennost' i razvitie* (Heredity and Development), Moscow: Nauka, 1974.

Berry, R.J. and Searle, A.G., Epigenetic polymorphism of the rodent skeleton, *Proc. Zool. Soc. London*, 1963, vol. 140, p. 557.

Clarke, G.M., Patterns of developmental stability of *Chrysopa perla* L. (Neuroptera: Chrysopidae) in response to environmental pollution, *Environ. Entomol.*, 1993, vol. 22, no. 6, p. 1362.
https://doi.org/10.1093/ee/22.6.1362

Debat, V. and David, P., Mapping phenotypes: canalization, plasticity and developmental stability, *Trends Ecol. Evol.*, 2001, vol. 16, no. 10, p. 555.
https://doi.org/10.1016/S0169-5347(01)02266-2

Dongen, S.V., Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future, *J. Evol. Biol.*, 2006, vol. 19, no. 6, p. 1727.
https://doi.org/10.1111/j.1420-9101.2006.01175.x

Eligül, H., Özkan Koca, A., and Kandemir, İ., Forewing deformations in Turkish honey bee populations, *Uludag Bee J.*, 2017, vol. 17, no. 2, p. 72.

Gülmez, Y., Teratology in the solitary wasp family Sphecidae (Insecta: Hymenoptera), *Biologia*, 2019, vol. 74, p. 1349.
https://doi.org/10.2478/s11756-019-00254-7

Hallgrímsson, B., Donnabhárin, B.Ó., Blom, D.E., Lozada, M., and Willmore, K.T., Why are rare traits unilaterally expressed?: trait frequency and unilateral expression for cranial nonmetric traits in humans, *Am. J. Phys. Anthropol.*, 2005, vol. 128, no. 1, p. 14.
https://doi.org/10.1002/ajpa.20187

Hammer, Ø., Harper, D.A.T., and Ryan, P.D., PAST version 2.17. Paleontological Statistics software package for education and data analysis, *Palaeontol. Electron*., 2001, vol. 4, no. 1, p. 1.

Klingenberg, C.P., Phenotypic plasticity, developmental instability, and robustness: the concepts and how they are connected, Front. Ecol. Evol., 2019, vol. 7, art. 56.
https://doi.org/10.3389/fevo.2019.00056

Łopuch, S. and Tofilski, A., The relationship between asymmetry, size and unusual venation in honey bees (*Apis mellifera*), *Bull. Entomol. Res.*, 2016, vol. 106, no. 3, p. 304.
https://doi.org/10.1017/S0007485315000784

Martynova, O.M., Individual variation in the wings of Mecoptera, *Izv. Akad. Nauk SSSR Ser. Biol.*, 1948, no. 2, p. 193.

Orlov, L.M., Wing venation in the lacewing *Chrysopa adspersa* Wesm. (Chrysopidae, Neuroptera) as a model for microevolution research, *Zh. Obshch. Biol.*, 1975, vol. 35, no. 6, p. 902.

Padró, J., Carreira, V., Corio, C., Hasson, E., and Soto, I.M., Host alkaloids differentially affect developmental stability and wing vein canalization in cactophilic *Drosophila buzzatii*, *J. Evol. Biol.*, 2014, vol. 27, no. 12, p. 2781.
https://doi.org/10.1111/jeb.12537

Palmer, A.R., Developmental origins of normal and anomalous random right–left asymmetry: lateral inhibition versus developmental error in a threshold trait, *Contrib. Zool.*, 2012, vol. 81, no. 2, p. 111.
https://doi.org/10.1163/18759866-08102006

Porporato, M., Laurino, D., Balzola, L., and Manino, A., Wing venation teratology in *Apis mellifera* L., *Redia*, 2014, vol. 97, p. 157.

R Core Team, R: A Language and Environment for Statistical Computing, Vienna: R Foundation for Statistical Computing, 2019 (accessed 15.11.2019).
https://www.R-project.org

Ross, K.G. and Robertson, J.L., Developmental stability, heterozygosity, and fitness in two introduced fire ants (*Solenopsis invicta* and *S. richteri*) and their hybrid, *Heredity*, 1990, vol. 64, no. 1, p. 93.
https://doi.org/10.1038/hdy.1990.12

Sereno, P.C., Logical basis for morphological characters in phylogenetics, *Cladistics*, 2007, vol. 23, no. 6, p. 565.
https://doi.org/10.1111/j.1096-0031.2007.00161.x

Shishkin, M.A., Phenotypic reactions and the evolutionary process: the evolutionary significance of modifications revisited, in *Ekologiya i evolyutsionnaya teoriya* (Ecology and the Theory of Evolution), Gall, Ya.M., Ed., Leningrad: Nauka, 1984, p. 196.

Smith, D.R., Crespi, B.J., and Bookstein, F.L., Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization, *J. Evol. Biol.*, 1997, vol. 10, no. 4, p. 551.
https://doi.org/10.1046/j.1420-9101.1997.10040551.x

Solonkin, I.A., Zakharova, E.Yu., Shkurikhin, A.O., and Oslina, T.S., Classification and trends of wing venation abnormalities in whites (Lepidoptera: Pieridae), by the example of the black-veined white *Aporia crataegi* L., *Euroasian Entomol. J.*, 2017, vol. 16, no. 6, p. 579.

Sotavalta, O., Studies on the variation of the wing venation of certain tiger moths (Lepidoptera, Arctiidae, subfamilia Arctiinae), in *Annales Academiae Scientiarum Fennicae. Series A. IV. Biologica*, Helsinki: Suomalainen Tiedeakatemia, 1964, p. 1.

Takahashi, K.H., Multiple modes of canalization: Links between genetic, environmental canalizations and developmental stability, and their trait-specificity, *Semin. Cell Dev. Biol.*, 2019, vol. 88, p. 14.
https://doi.org/10.1016/j.semcdb.2018.05.018

Vasil'ev, A.G., Epigenetic variation: nonmetric threshold characters, phenes, and their compositions, in *Fenetika prirodnykh populyatsii* (Phenetics of Natural Populations), Yablokov, A.V., Ed., Moscow: Nauka, 1988, p. 158.

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

Vasil'ev, A.G. and Vasil'eva, I.A., *Gomologicheskaya izmenchivost' morfologicheskikh struktur i epigeneticheskaya divergentsiya taksonov: osnovy populyatsionnoi meronomii* (Homologous Variation of Morphological Structures and Epigenetic Divergence of Taxa: Basic Population Meronomy), Moscow: KMK Scientific Press, 2009.

Waddington, C.H., The basic ideas of biology, in *Na puti k teoreticheskoi biologii* (Towards a Theoretical Biology), *Vol. 1*, Waddington, C.H., Ed., Moscow: Mir, 1970, p. 11.

Willmore, K.E., Young, N.M., and Richtsmeier, J.T., Phenotypic variability: its components, measurement and underlying developmental processes, Evol. Biol., 2007, vol. 34, no. 3, p. 99.
https://doi.org/10.1007/s11692-007-9008-1

Zakharov, V.M., *Asimmetriya zhivotnykh (populyatsionno-fenogeneticheskii podkhod)* (Asymmetry in Animals: A Population Phenogenetic Approach), Moscow: Nauka, 1987.