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Intraspecific variation and asymmetry in wing shape of dolichopodid flies (Diptera, Dolichopodidae)

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Abstract

Intraspecific variation in wing shape of six species of the Dolichopodidae family was analyzed by the method of Procrustes superimposition. Specifically, we examined the patterns of sexual dimorphism, interpopulation variation, variation among individuals, directional asymmetry and fluctuating asymmetry (within-individual variability). The results suggest that sexual dimorphism is the most significant sources of intraspecific variation. Highly significant differences in wing shape were observed between populations of *Poecilobothrus regalis*, it reflects their adaptations to different environmental conditions. The analysis revealed significant directional asymmetry for wing shape of *Poecilobothrus regalis* from one of the studied populations; directional asymmetry had a significant allometric component. Four types of pattern of individual variation were discovered. It was shown that individual variation and fluctuating asymmetry have similar patterns. Based on previous studies of wing development, we made conclusions about the developmental processes, which cause different types of intraspecific variation.

Keywords: diptera, dolichopodidae, interpopulation variation, sexual dimorphism, fluctuating asymmetry, directional asymmetry

1. Introduction

Characters of the wing morphology have significant importance in the systematic and taxonomy of the Dolichopodidae family [1, 2]. Together with the structure of male genitalia, wing morphology gives more information on the taxonomic position of dolichopodid flies than the other parts of the body. The large interspecific variety of wing morphologies within the family suggests that flight plays an important role in the dolichopodid's survival by providing varying levels of maneuverability and velocity. Differences in the model of flight can be the force influencing the evolution of other morphological structures.

The study of the variability of wings venation in all taxonomic levels can provide data on the evolutionary path of a tax on [3, 4, 5]. It is important to examine the range and degree of intraspecific variations in order to study an adaptive optimum of a morphological trait [6]. In the first instance, patterns of sexual dimorphism, interpopulation variability, and various types of asymmetries must be taken into consideration.

Sexual dimorphism of wing shape is a feature of many species of Diptera [7, 8, 9]. It is obvious that differences exist because of the various roles of flight in males and females, so sexual shape dimorphism can be related to selective pressures of the habitat and sexual selection. Maneuverability traits may be subject to rapid evolution. There is an alternative explanation for sexual shape dimorphism, related with the ability of males to produce acoustical signals, but currently these facts have not verified statistically.

Allometry is a subject of many studies as factor playing an important role in sexual shape dimorphism [10]. Allometric relationships are central to the evolution of traits that are used in courtships or in aggressive interactions with sexual rivals. For example, allometric relationships have been discussed as an important component of wing sexual dimorphism in the Drosophila melanogaster subgroup [11].

Fluctuating asymmetry (FA) has been defined as a random, non-directional deviation from the perfect symmetry of the bilateral traits [12]. In recent studies it has been shown that forms of fluctuating asymmetry are not inherited [13, 14]. It is also suggested that FA is a result of small genetic perturbation and environmental stressors during the ontogeny of an organism [15, 16]. The results of recent studies show FA is stress, trait, and population specific [17, 18, 19]: in addition, FA showed low repeatability.

Many studies have devoted more attention to one or another type of morphological variation among individuals (for instance, sexual dimorphism or FA): however, there are a few studies concerning the comparison of different types of variation with each other. In our study we focused on intraspecific variability of wing shape in species of the Dolichopodidae family.

Wings of dolichopodid flies usually oval, about as long as body, hyaline, but sometimes with darkening spots. The following veins are present (Fig. 1): costa (C), subcosta (Sc), a radial sector with three branches (R1, R_{2+3}, R_{4+5}), a medial vein (M_{1+2}), which sometimes forked near middle of last part and formed arcuate M_1 (in the Sciapodinae subfamily), a single cubital vein (CuA_1). Anal vein is short, sometimes reduced or absent.

Most variation of wing structure is concentrated in a several morphological traits: posterior region, including medial and cubital veins and the posterior crossvein (dm-cu), and the anterior margin – costal vein and the point of its intersections with R_1 and R_{2+3}. The characters can vary within subfamilies, genera and species.

Costal vein forms the anterior margin of the wing and it experiencing the greatest air resistance during the flight. The costa reveals a trend toward thickening in the region of its meeting of vein R_1. The second apparent form of variation is the variation of R_{2+3} length. Then the ratio of costal section between the base of wing and R_1 to the wing length is used as generic character in the subfamilies Achalcinae and Dipahorinae. CuA_1 and dm-cu are present stably, although significant variations in its length and position of its meeting point are present. An interesting area for investigation is M_2 vein, which is present at Sciapodinae species and present as rudimentary stub at some Dolichopodinae species.

In the family Dolichopodidae, there are two most probable factors influencing the wing shape. First of all, dolichopodid flies are predators and widespread in various habitats, so flight performance is an important feature, which is responsible for survivability. Type of habitat (open area of a shore or dense growth of damp area in a forest) acts on both flight performance and wing shape. Secondly, for several species of the Dolichopodidae family the courtship ceremonies are described. A male of *Poecilobothrus nobilitatus* L. stand in front or to on side of a female and its wings vibrate three or more times. The milk-white spots of the apex of wings made specific visual image [20, 21, 22, 23].

Since the functional consequences of wing form determine the locomotors performance of flight then different performance requirements lead to different combinations of these traits. A clear understanding of the directions of changes is required in order to construct a robust phylogenetic hypothesis. To implement this goal in this study we focused on following questions:

1. Different types of intraspecific variability in wing shape of the Dolichopodidae species were examined and the presence of a taxonomic significant of these traits was tested.
2. We considered the questions of whether there are special patterns in different types of intraspecific variability or if the same processes control their development.
3. The role of allometry in each case was studied.

2. Materials and methods

2.1 Flies

A study of 6 species belonging to 4 genera and 3 subfamilies of the Dolichopodidae family was conducted to examine the intraspecific variability in wing shape: *Poecilobothrus regalis* (Meigen, 1824), *Poecilobothrus varicoloris* (Becker, 1917), *Dolichopus latilimbatus* Macquart, 1827, *Dolichopus ungulatus* (Linnaeus, 1758) of the Dolichopodinae subfamily, *Campsicnemus lumbatus* Loew, 1857 of the Sympycninae subfamily and *Sciapus wiedemanni* (Fallen, 1823) of the Sciapodinae subfamily (Table 1). The specimens were collected from the natural environments in 2015-2016.

Table 1: Material examined.

Species	**Collection sites and year for the species and number of specimens**	
Campsicnemus lumbatus	Population 1	Population 2
	20 ♀, 36 ♂, 2016 55° 87´ N, 48° 67´ E	12 ♀, 32 ♂, 2016 51° 81´ N, 39° 39´ E
Dolichopus latilimbatus	58 ♀, 40 ♂, 2016 55° 87´ N, 48° 67´ E	
Dolichopus ungulatus	20 ♀, 36 ♂, 2016 55° 87´ N, 48° 67´ E	
Poecilobothrus regalis	Population 1	Population 2
	40 ♀, 84 ♂, 2015 49° 97´ N, 39° 68´ E	18 ♀, 58 ♂, 2015 51° 85´ N, 40° 78´ E
Poecilobothrus varicoloris	34 ♀, 36 ♂, 2015 44° 19´ N, 39° 96´ E	
Sciapus wiedemanni	43 ♀, 39 ♂, 2016 51° 81´ N, 39° 39´ E	

2.2 Measurements and statistical analysis

Wing were placed on a glass slide and covered with a cover glass. All slides were photographed by using a Levenhuk C NG microscopic camera and digitizes. Our analysis of intraspecific variability was based on the Procrustes technique [24]. Wing shape was described by 9 landmarks located at vein intersections with each other and wing margin (Fig. 1). Two-dimensional Casterian coordinates of the landmarks were digitized from the photo using tps Dig-2.32 software [25].

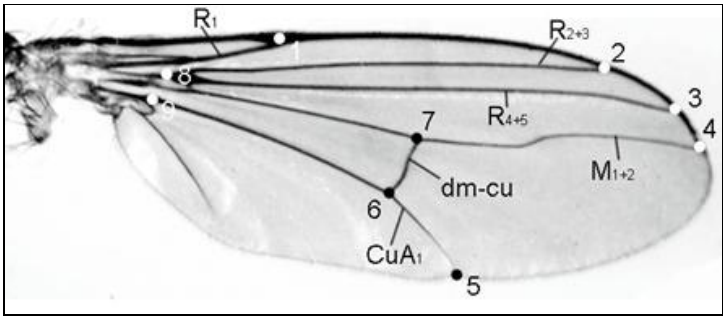

Fig 1: Landmarks used in the study (*Dolichopus ungulatus*, male).

By definition FA is a very small deviation of organism sides from the perfect symmetry, and measurements of FA are highly sensitive to measurement errors [26]. Therefore, we focus considerable attention on the estimation of measurement error. In order to take into account digitizing error, all wings were digitized two times. The time interval between the sessions was a week.

Then the Procrustes method proceed in several steps: landmarks configurations were scaled to a unit of centroid size, superimposed so that the centroid of each has coordinates (0, 0) and rotated so that the distance between landmarks of all specimens become minimal. A new set of variables (Procrustes residuals) contained the shape information. Further analyses of these variables were performed using the methods of multivariate statistics in MorpholJ software [27].

The centroid size of wing was calculated and used as characteristic of overall measure of wing. The effects of sex, population, individual and side on the wing shape were tested with ANOVA. The among-specimen effect showed individual variability (difference between specimens in a population), the effect for the side expressed directional asymmetry (difference between left and right wings), the interaction of side and specimens is used as a measure of fluctuating asymmetry, and variability among replicates of each wing was a measurement error. The effects of sexual dimorphism and interpopulation variability also were estimated. The effects of species, sex and species×sex were tested with a multivariate analysis of variance (MANOVA, type I sums of squares and cross-products).

Canonical variate analysis (CVA) allowed for the description the differences between sexes, populations, individuals and sides. The patterns of variations were displayed graphically as a plot of differences between mean shapes. To quantify the amount of differences, we used the Procrustes distance (the sum of squared of Procrustes residues of all landmarks), for example, between the mean shape of the males and females. For testing the statistical significance, we used permutation tests for pairwise distances with 10000 interactions.

To compare the patterns of shape variation, which were described for different sources, covariance matrices of the coordinates of superimposed landmarks were computed. Then we performed a comparison of the covariance matrices of different factors (sex, population, individual, fluctuating asymmetry, directional asymmetry and measurement error) and the same factor in different groups (for instance, sexual dimorphism in two populations of *Poecilobothrus regalis*). If any two factors affect the same developmental processes, the covariance matrices should be similar. Mantel test of matrix correlations with 10000 randomization rounds (permuting landmarks) was used for this purpose. The null hypothesis in this test is that two matrices are completely dissimilar.

2.3 Testing of an allometry

Allometry of variance is estimated by a regression analysis of shape (Procrustes residuals) on size (centroid size). To assess the allometric component of individual variation and FA, we used correlation analysis between shape (Procrustes residuals) and centroid size. The bootstrap test of the null hypothesis of independence between centroid size and shape was conducted.

If the difference in wing shape between group (for example, between males and females or between two populations) is entirely related with the difference in size, the samples are situated on the one regression lines. If both allometric and non-allometric changes are presented, predicted and residual components of variation correspond to these components. Therefore, if regression lines in groups are assumed to be parallel, the non-allometric component can be computed as a difference between total variation and allometric component of variation.

To obtain allometric components of variation, we used pooled within-sex and within-population regression of shape on centroid size. Then the average observations by groups were computed. The predicted component of variation was estimated from the slope of the regression line and the deviation of the data point from the mean in the direction of size.

3. Results

The Procrustes ANOVA showed that all effects except the directional asymmetry and measurement error were statistically significant (Table 2). It is noticeable that the effect of sex was the most statistically significant for all species, except *C. lumbatus*. The highly significant fly ^{x} side interaction suggests that FA considerable affected the wing shape.

The same factors significantly affect the wing shape of males and females: FA, individual variation and interpopulation variation. The effect of directional asymmetry on shape was significant for *P. regalis* from population 2 and for *Sc. wiedemanni*, and less significant for *P. varicoloris*.

Table 2: The effect of sex, population, individual variability, direction asymmetry and fluctuating asymmetry on wing shape (results of ANOVA).

Species	Effect	D f	MS	F	P
Poecilobothrus regalis	Sex	14	0.027992	349.63	< 0.0001
	Population	14	0.000521	6.51	< 0.0001
	Fly	2730	0.000080	4.37	< 0.0001
	Side	14	0.000041	2.23	0.0053
	Fly × side	2758	0.000018	4.66	< 0.0001
	Measurement	5544	0.000004	0.76	1.0000
	Residual		0.000005		
Poecilobothrus varicoloris	Sex	14	0.015324	187.79	< 0.0001
	Fly	938	0.000082	5.60	< 0.0001
	Side	14	0.000023	1.56	0.0853
	Fly × side	952	0.000015	3.30	< 0.0001

	Measurement	1862	0.000004	0.74	0.8911
	Residual	28	0.000006		
Dolichopus latilimbatus	Sex	14	0.002188	32.74	< 0.0001
	Fly	1596	0.000067	0.19	1.0000
	Side	14	0.000063	0.18	0.9996
	Fly × side	1582	0.000344	24.51	< 0.0001
	Measurement	2016	0.000014	0.71	0.9998
	Residual	3122	0.000016		
Dolichopus ungulatus	Sex	14	0.004545	17.96	< 0.0001
	Fly	700	0.000253	7.05	< 0.0001
	Side	14	0.000035	0.97	0.4857
	Fly × side	714	0.000036	10.08	< 0.0001
	Measurement	756	0.000003	0.87	0.9789
	Residual	1456	0.000004		
Campsicnemus lumbatus	Sex	14	0.000811	4.36	0.0226
	Population	14	0.000915	4.92	0.0018
	Fly	1344	0.000186	5.66	< 0.0001
	Side	14	0.000027	0.83	0.6160
	Fly × side	1372	0.000033	5.40	< 0.0001
	Measurement	2772	0.000006	0.81	1.0000
	Residual	5544	0.000007		
Sciapus wiedemanni	Sex	14	0.006218	19.21	< 0.0001
	Fly	1148	0.000324	8.21	< 0.0001
	Side	14	0.000046	1.17	0.2892
	Fly × side	1162	0.000039	10.54	< 0.0001
	Measurement	2254	0.000004	0.97	0.6503
	Residual	14	0.000004		

MS – mean squares. D f – degrees of freedom. F – F–criterion. P – Significance.

The MANOVA results (Table 3) show the species differ in wing shape. The significance of the species×sex interaction suggests that sex dimorphism has diverged considerably among species.

Table 3: The effects of species, sex and their interaction of wing shape (MANOVA).

Effect	Wilk's lambda	F	D f num	D f den	P
Species	0.00000	25921	80	11803.27	< 0.0001
Sex	0.059029	477	32	4902.00	< 0.0001
Species × sex	0.06846	148	64	9597.51	< 0.0001

Df num – degrees of freedom for the numerator; Df den – degrees of freedom for the denominator.

3.1 Sexual dimorphism

Canonical variate analysis (CVA) was used in order to allocate the main differences between males and females. Our results showed significant differences in sexual shape dimorphism between species (Fig. 2). The variation on wing shape related to sexual dimorphism can be described as follows.

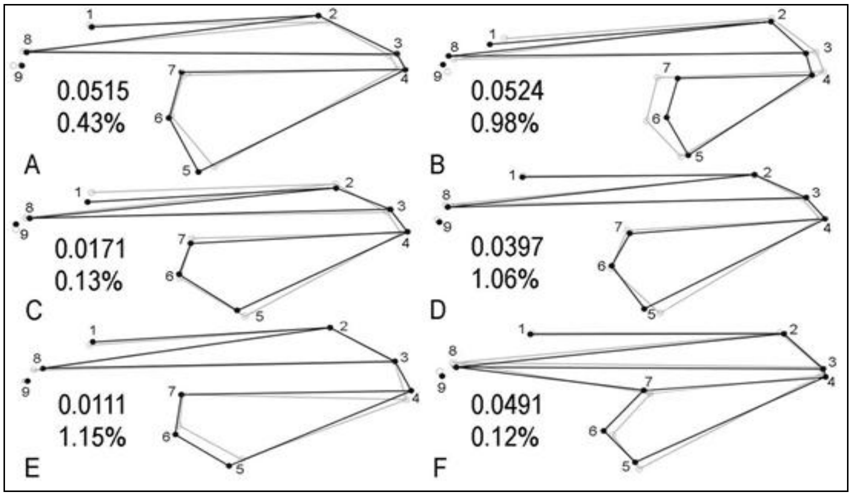

Fig 2: Shape changes associated with sexual dimorphism. Differences between the average shape of male (black outline) and female (grey outline) are presented. Differences are exaggerated tenfold for better visual perception. The values of shape variation are presented in units of Procrustes distance ($P < 0.0001$), allometric components are indicated in percentages. A, *Poecilobothrus regalis*; B, *Poecilobothrus varicoloris*; C, *Dolichopus latilimbatus*; D, *Dolichopus ungulatus*; E, *Campsicnemus lumbatus*; F, *Sciapus wiedemanni*.

Females *P. regalis* have narrower wings (particularly in the region between landmarks 2 and 5) and shorter (between landmarks 3 and 8) that males. Landmarks 2 and 5 moved distally, that reflected the shift of the intersections of costa and R_{2+3} and apex of CuA_1 along the proximal-distal axis of wing. The other pattern of sexual dimorphism was found in *P. varicoloris*. Females have longer and wider wings, but moving of landmarks 2 and 5 is insignificantly. Landmarks 6 and 7 moved proximally together, so the posterior crossvein of female was closer to the wing base that in male's wings, thus affecting in the area of the portion of wing between M_{1+2}, CuA_1 and dm-cu (m_1 portion).

Wings of females *D. latilimbatus* were wider then males and the most significant widening were observed in the region between landmarks 5 and 7 along the anterior-posterior axis of wing. The similar pattern was revealed for *D. ungulatus*, but in this case wing widening is associated with the moving of landmark 5 to the distal end of wing. Wings of females *C. lumbatus* were narrower and shorter that males' wings. The narrowing occurred as shifts of landmarks 5 and 6 along the anterior-posterior axis of wing. The wing lengths of males and females *Sc. wiedemanni* were not significantly different, but females had wider wings with posterior crossvein shifted distally.

The shape changes associated with sexual dimorphism were statistically significant in all cases, distinct among species and ranged from 0.0111 (*C. lumbatus*) to 0.0524 (*P. varicoloris*). The differences between regression vectors for males and females were also statistically significant for all species. Sum of squared ranged from 0.29 ($P < 0.0001$) of *C. lumbatus* to 0.10 ($P = 0.0022$) of *P. varicoloris*. This fact reflected that separation of allometric and non-allometric components of variation was reliable. Hence there was no evidence for allometric relationships relating with sexual dimorphism.

Comparisons of covariation matrices for the effect of sexual dimorphism in the different populations were conducted. Test showed that sexual shape dimorphism in the different populations of the same species were similar: the matrix correlation were high and statistically significant (MC = 0.90, $P < 0.0001$ in *P. regalis*; MC = 0.97, $P < 0.0001$ in *C. lumbatus*).

3.2 Interpopulation variation

The canonical variate (CV1) of interpopulation variation of males *P. regalis* described a relative increase of the wing area coupled with extension of the posterior cross vein and shortening of R_1 (Fig. 3). Males from population 1 have significantly smaller area of the wing than males from population 2 ($P = 0.04$).

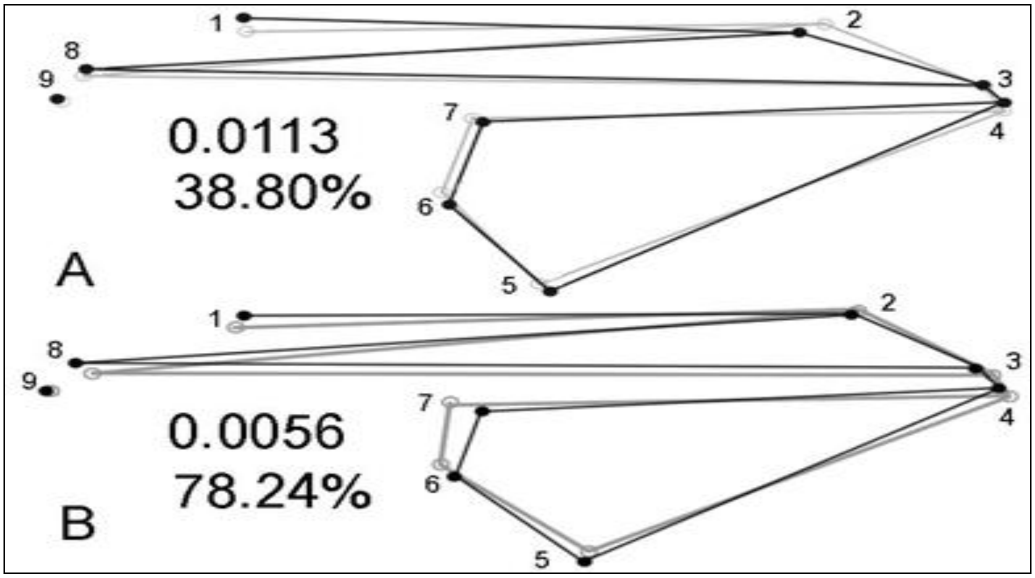

Fig 3: Shape changes associated with interpopulation variation. Differences between the average shape of population 1 (black outline) and population 2 (grey outline) of *Poecilobothrus regalis* are presented. Differences are exaggerated tenfold for better visual perception. The values of shape variation are presented in units of Procrustes distance ($P < 0.0001$), allometric components are indicated in percentages. A, male; B, female.

A similar pattern of interpopulation variability has been found in females, and difference in wing area was more significant ($P < 0.0001$). The increase of wing area was followed by extension of the posterior cross vein and its displacement along longitudinal axis of wing toward wing apex. So the total form differences between females of two populations were more statistically significant, than between males. The allometric component of interpopulation variation was also greater in female.

The increase of wing area of males *C. lumbatus* from population 2 occurred together with widening of the distal part of the wing (particularly in the region between landmarks 2 and 5) and shortening the apical part of CuA_1 (Fig. 4). Landmarks from 2 to 5 formed a pointed or a blunter apex of the wing. The changes in females' wing were related with decrease of the area of m_1 portion and shortening of the posterior crossvein. Allometric components were also relatively large. The matrix correlation between males and females covariance matrices reflected their similarity (MC = 0.95, $P = 0.0002$).

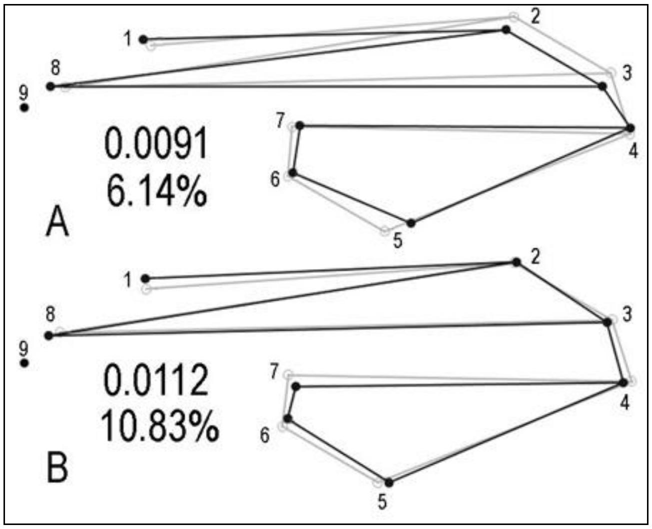

Fig 4: Shape changes associated with interpopulation variation. Differences between the average shape of population 1 (black outline) and population 2 (grey outline) of *Campsicnemus lumbatus* are presented. Differences are exaggerated tenfold for better visual perception. The values of shape variation are presented in units of Procrustes distance ($P < 0.0001$), allometric components are indicated in percentages. A, male; B, female.

We should also point out highly significant differences between centroid size both males and females of *P. regalis* from two populations ($T_{male} = -55.2$, $T_{fem} = -20.80$, $P < 0.0001$). Specimens from population 2 had larger wings, then specimen from population 1. This fact should be considered in the study of allometric relationships. Differences in centroid size between specimens of *C. lumbatus* from two populations also were significant ($T_{male} = -5.65$, $T_{fem} = -4.84$, $P < 0.0001$). The matrix correlations between covariance matrices of interpopulation variation and different types of variation showed that interpopulation variation and directional asymmetry in *P. regalis* are highly correlated (MC = 0.999, $P < 0.0001$). Probably this high correlation is due to the high contribution of allometric component, which was relatively higher than in other types of variation. Interpopulation variation in *C. lumbatus* was strongly correlated with both sexual dimorphism (MC = 0.99, $P < 0.0001$) and individual variation (MC = 0.93, $P < 0.0001$).

3.3 Individual variation

The matrix correlations between two covariance matrices of individual variability in different populations showed their high similarity both for males and females: MC = 0.88, $P < 0.0001$ (*P. regalis*) and MC = 0.87, $P < 0.0001$ (*C. lumbatus*). The matrix correlations between covariance matrices of sexual dimorphism and individual variability were high and statistically significant: MC = 0.94, $P < 0.0001$ (*P. regalis*); MC = 0.93, $P < 0.0001$ (*D. latilimbatus*); MC = 0.91, $P < 0.0001$ (*D. ungulatus*); MC = 0.93, $P < 0.0001$ (*C. lumbatus*); MC = 0.87, $P < 0.0001$ (*Sc. wiedemanni*); MC = 0.76, $P = 0.0018$ (*P. varicoloris*). These high correlations are explained to involving in individual variability the same landmarks as in sexual dimorphism (primary 5, 6 and 7), because most of the changes in both cases occurred in the cubital part of the wing.

The first three canonical variates of both the males and females individual variance were associated with displacements of landmarks 5, 6 and 7 (Figs 5, 6). More detailed analysis allows for allocation of four patterns of individual variation. The first combination of landmarks displacements is typical of males of *Campsicnemus lumbatus*, *D. ungulatus* and *D. latilimbatus*.

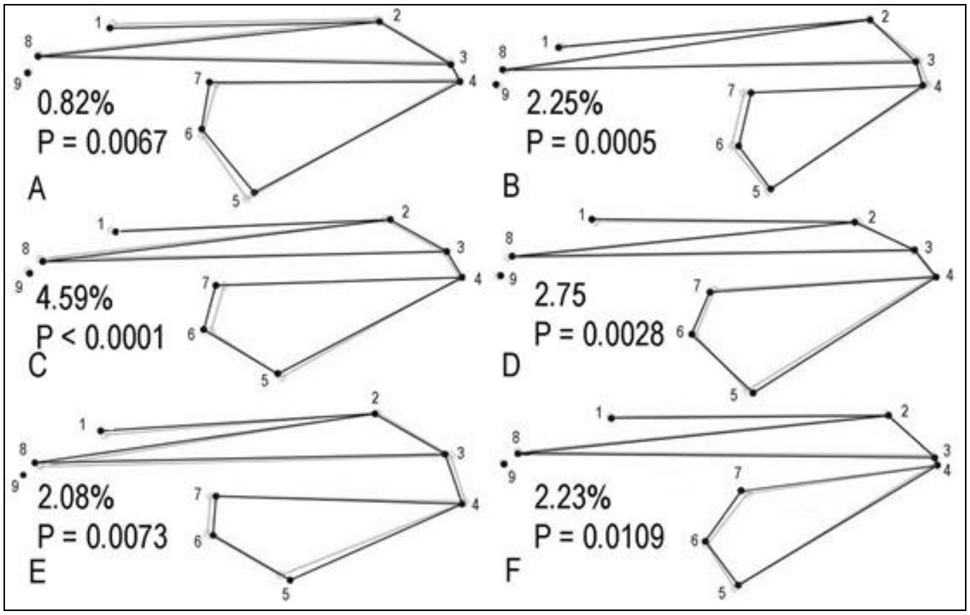

Fig 5: Shape changes associated with individual variation of males. Differences are exaggerated tenfold for better visual perception. Allometric components are indicated in percentages and the P-value for the bootstrap test of the null hypothesis of independence between centroid size and Procrustes residuals is given. A, *Poecilobothrus regalis*; B, *Poecilobothrus varicoloris*; C, *Dolichopus latilimbatus*; D, *Dolichopus ungulatus*; E, *Campsicnemus lumbatus*; F, *Sciapus wiedemanni*.

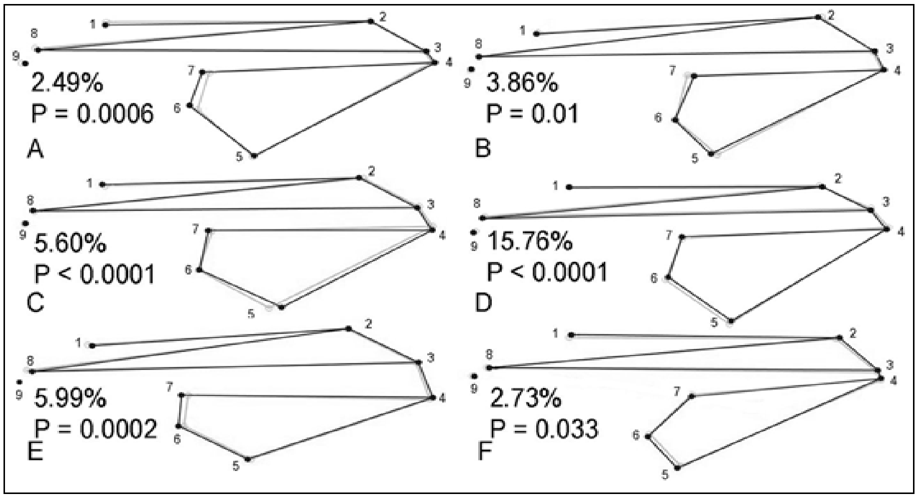

Fig 6: Shape changes associated with individual variation of females. Differences are exaggerated tenfold for better visual perception. Allometric components are indicated in percentages and the P-value for the bootstrap test of the null hypothesis of independence between centroid size and Procrustes residuals is given. A, *Poecilobothrus regalis*; B, *Poecilobothrus varicoloris*; C, *Dolichopus latilimbatus*; D, *Dolichopus ungulatus*; E, *Campsicnemus lumbatus*; F, *Sciapus wiedemanni*.

Landmarks 6 and 7 moved distally together, reflecting shift of the posterior crossvein. It leads to the widening of the distal part of the wing; however the angle between dm-cu and longitudinal axis of the wing did not change.

The other pattern is presented of *P. regalis* and *Sc. wiedemanni* males and also of *P. varicoloris* and *D. latilimbatus* females. Widening of the distal part of the wing was caused by changes of the angle between dm-cu and longitudinal axis of the wing, while the position of landmark 6 was stable.

The third pattern was observed at *P. varicoloris*, *P. regalis* and *C. lumbatus* females and males *P. varicoloris*. Landmarks 6 and 7 (dm-cu) moved toward the wing apex, but it was not associated with displacement of landmark 5 and significant widening of the wing. Thereby the individual variation of both males and females was related to the changes of the wing area of m$_1$ portion.

Finally, the fourth pattern was a widening of the cubital compartment of the wing. The landmark of the apical part of CuA$_1$ (5 and 6) moved together toward the posterior margin of the wing and caused the widening. This type was characterized of *Sc. wiedemanni* females and *D. ungulatus* females.

There was a difference in a value of allometric components between species and between males and females of each species. In all studied cases allometry played a larger role for individual variation of females, than males. Allometric components were correlated with a small elongation of the basal part of the wing, which include a shift of landmarks 1 and 8 (*Sc. wiedemanni*) or 1 and 9 (*D. ungulatus*) along Y-axis. In other cases allometric components consisted a small narrowing of the distal part of the wing, because there was a shift of landmark 5 along X-axis (*P. regalis* and *P. varicoloris*). A shift of landmark 7 along Y-axis caused the decrease of the area of m$_1$ portion.

To answer the question whether the patterns of males and females individual variation are similar, the correlation between corresponding covariance matrices was computed. Correlations between male and female individual changes were statistically significant (MC = 0.77, P = 0.0004 for *P. regalis*; MC = 0.83, P < 0.0001 for *P. varicoloris*; MC = 0.80, P < 0.0001 for *D. latilimbatus*; MC = 0.57, P = 0.0003 for *D. ungulatus*; MC = 0.90, P = 0.0003 for *C. lumbatus*; MC = 0.52, P = 0.0006 for *Sc. wiedemanni*), but lower than the correlation between different types of variation within the sex.

3.4 Directional asymmetry

Significant differences in shape between left and right wings were discovered in both males and females *P. regalis* from population 2 (results of ANOVA: F = 2.07, P = 0.012). Canonical variate of the between-sides variance was related to displacements of landmarks 1, 3 and 5 along anterior-posterior axis of the wing (Fig. 7).

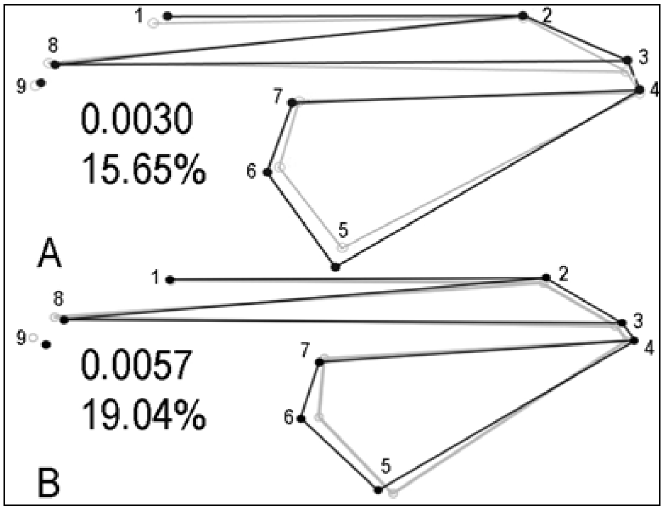

Fig 7: Shape changes associated with directional asymmetry. Differences between the average shape of right wing (black outline) and left wing (grey outline) of *Poecilobothrus regalis*. Differences are exaggerated tenfold for better visual perception. The values of shape variation are presented in units of Procrustes distance; allometric components are indicated in percentages. A, male; B, female.

Widening of the wing was caused of increase of the distance between landmarks 2 and 5. Allometric component of the directional asymmetry was relatively large, but the differences between left and right wings in units of Procrustes distance were small and not statistically significant (P-values 0.163 for males and 0.107 for females). Therefore, the allometric component of variation should not be interpreted with certainty.

3.5. Fluctuating asymmetry

The changes associated with fluctuating asymmetry were located in the same points as individual variation. Canonical variates were correlated with displacements of landmark 5, 6 and 7 (Figs 8, 9). The patterns of fluctuating asymmetry and individual variation showed significant similarity. This fact is also supported by the strong correlation between covariance matrices of individual variation and fluctuating asymmetry (MC ranged 0.92 for *Sc. wiedemanni* to 0.95 for *P. regalis*, P < 0.0001).

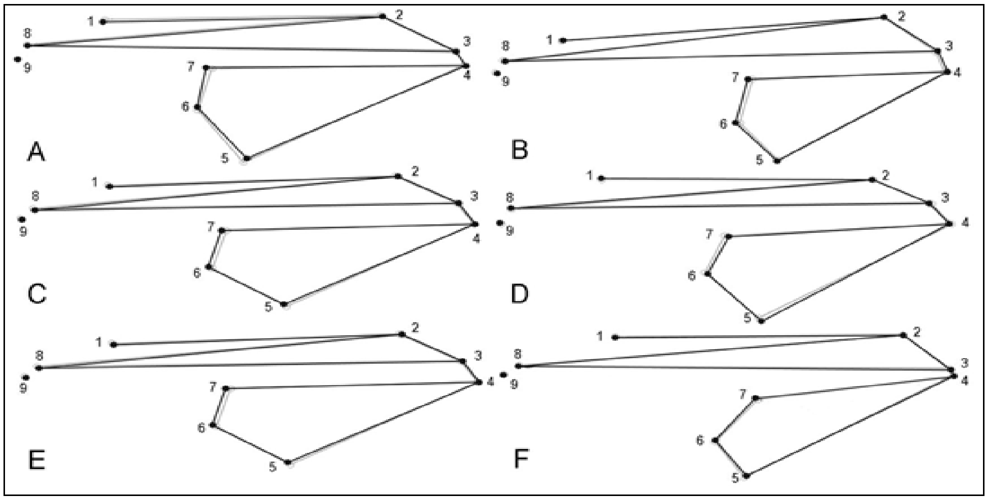

Fig 8: Shape changes associated with fluctuating asymmetry of males. Differences are exaggerated tenfold for better visual perception. A, *Poecilobothrus regalis*; B, *Poecilobothrus varicoloris*; C, *Dolichopus latilimbatus*; D, *Dolichopus ungulatus*; E, *Campsicnemus lumbatus*; F, *Sciapus wiedemanni*.

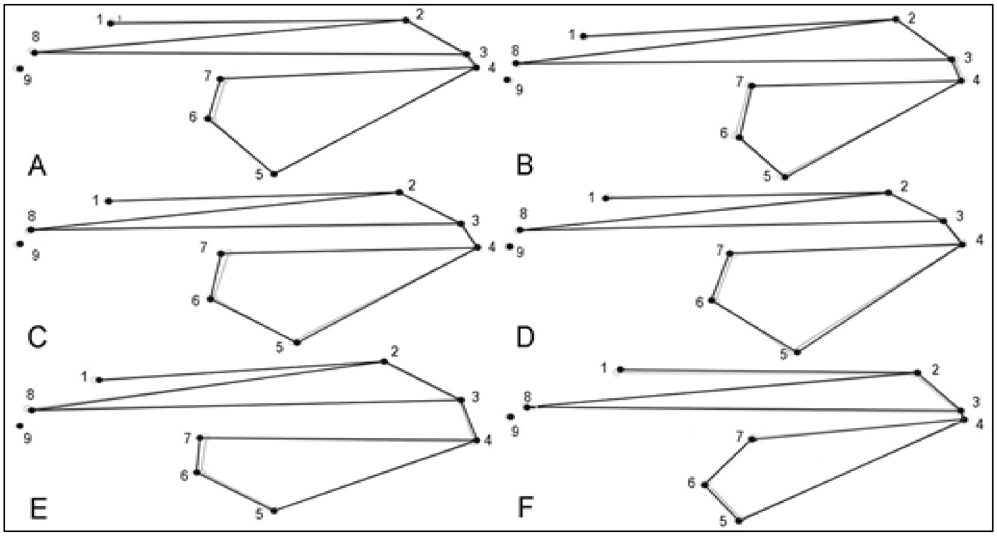

Fig 9: Shape changes associated with fluctuating asymmetry of females. Differences are exaggerated tenfold for better visual perception. A, *Poecilobothrus regalis*; B, *Poecilobothrus varicoloris*; C, *Dolichopus latilimbatus*; D, *Dolichopus ungulatus*; E, *Campsicnemus lumbatus*; F, *Sciapus wiedemanni*.

4. Discussion

Our results showed that different factors affect the wing shape of Dolichopodidae flies. Wing shape can vary according to sex, population, specimen and side; the variations tend to affect parts of the wing differently. Namely, these factors affected the cubital part of the wing, the base of the costal vein and the apex of the wing. Thus, the characteristics, widely used in supraspecific taxonomy, have considerable magnitude in the intraspecific variation.

Procrustes ANOVA showed that sexual dimorphism has the most significant effect on the wing shape and that there were significant differences in sexual shape dimorphism between species. Shape variations associated with sexual dimorphism mostly affected the landmarks and were related with the overall shape. Our results lead to the following conclusions. Sexual shape dimorphism has diverged among the dolichopodid species. The biological interpretation of the changes is difficult and requires additional study of behavior and the aerodynamics of flight. Currently, little is known about the behavior of these species; however, the presence of sexual shape dimorphism shows that optimal shape differs for males and females.

A common trait of sexual dimorphism is the change of the overall shape of the wing blade. There was a general narrowing from male to female (*C. lumbatus*, *P. regalis*) or from female to male (*D. latilimbatus*, *Sc. wiedemanni*), and a general widening and elongation from male to female (*P. varicoloris*). Additionally, the general narrowing or elongation was accompanied by various displacements of landmarks.

Allometric relationships, which were described for the majority of sexual selected characters (ornaments) in previous studies, have not been revealed in our study. The shape variation attributable to sex is similar for different populations, although size differences between populations were significant. This indicates that sexual shape dimorphism has a functional significance and is a characteristic trait of the species. Finally, it is noticeable that sexual dimorphism is shown not only in the difference between the mean wing shapes of the sexes, but also in the difference between the trends of individual variation for males and females.

Interpopulation patterns of wing shape variation are largely dependent on size. An increase in wing size caused the forming of a wing with a more obtuse apex. Displacement and elongation of the posterior crossvein could result from the displacement of the wing's center of gravity. Studies of aerodynamics [28, 29, 30, 31] suggested that the instantaneous force on a wing section depends particularly on wing length, wing width (chord) and shape factor. High aspect-ratio wings with a more obtuse apex can produce more lift. Because the wing aspect-ratio shows a response to variation of temperature in wild populations [32, 33, 34] and in laboratory culture of fruit flies [35], the variation of the wing aspect ratio seems to be largely adaptive.

Significant directional asymmetry was found in only one population, indicating this type of asymmetry is not a characteristic trait of *P. regalis*, but can occur in certain climatic conditions. The allometric nature of the directional asymmetry explained its similarity to the interpopulation variation. We can make a preliminary assumption that long-wing flies should be more asymmetrical for allometric reasons, although this pattern is opposite of the results obtained by Møller [36].

Individual variation involved the landmarks describing of the cubital compartment of the wing. A comparison of the individual variations allowed for the allocation of four types of patterns of covariance among the landmarks. A pattern of individual variation can occur in both males and females and can be combined with other types from the opposite sex. The second type of individual variation (widening of the distal part of the wing caused by changes of the angle between the posterior crossvein and longitudinal axis of the wing) is the most common and can be combined with first and third types.

A more detailed analysis of intraspecific variation requires a discussion of the developmental processes. According to Waddington [37], transformations of the wing shape can be made at the several developmental stages. First of all, the process of wing development begins with the delineation of the wing region within the epithelium of the imaginal bud. During the next stage, the wing blade consists of two epithelia layers; some alterations to the wing shape can be caused by the directions of mitotic spindles in the dividing cells.

Anyway, in the case of sexual dimorphism and interpopulation variation, changes to the wing shape caused the changes in vein pattern, which suggests that development of sexual dimorphic traits and interpopulation variation precedes the changes related to individual variation. This is because in the first case, the changes are related to the overall shape of the wing, and in the second case, with slight shifts of the landmarks in the cubital part of the wing.

Individual variation can result from the next stage of development. After a blowup by pressures of the haemolymph, the two epithelial layers connect everywhere except for the lines that will become the veins. The prospective veins are considerably wider than the veins that will be formed [38], and at the next stage, these lines will narrow. Therefore, there is a certain magnitude of vein movement during the ontogenesis. The posterior crossvein is the last region of epithelia connection. Therefore the shape changes associated with individual variation occur in the final stages of wing development.

Our analysis of individual variation and fluctuating asymmetry has demonstrated their considerable correspondence. Besides, the patterns of individual variation and fluctuating asymmetry in wing shape of different populations were significantly similar. We can conclude that the developmental processes influencing the individual variation also affect the occurrence of fluctuating asymmetry. This result is in good agreement with the results of phenogenetic research [39]. A study of phenotypic plasticity showed that morphological fluctuating is largely determined by endogenous factors of development and less determined by exogenous factors, such as climatic conditions and trophic factors.

The summarizing of shape variation allowed for the allocation of the following trends. Most of the variability affects the posterior crossvein. It is well known that the location of this element is affected by all previous events of wing development, such as the position of longitudinal veins and the distance between them.

Our results showed that the shift of posterior crossvein along the longitudinal veins toward the base of the wing was often related to an increase in centoid size and wing area (sexual dimorphism of *P. varicoloris*, interpopulation variation of *P. regalis*). This variation may be a consequence of the variation in distance between CuA_1 and M_{1+2}.

Variation in the angle between the posterior crossvein and longitudinal veins was found for individual variability. One of the possible explanations for this fact may be that it is a consequence of the overall wing shape. Because the connection between the two-epithelium layers begins in the distal part of the wing, the apical parts of longitudinal veins are deflated first. This indicates that the displacement of the apical part of CuA_1 was often caused by changes in the length and angle of the posterior cross vein.

The significant amount of variation showed that longitudinal veins inserted the wing margin at a narrow angle [40]. Our results showed that narrowing of the wing blade was usually associated with an increase of R_{2+3} length, which caused the formation of a more obtuse apex of the wing.

It is evident that the patterns for shape variation are the result of both sexual selection and natural selection. Although at this stage it is impossible to interpret our data biologically, we can make preliminary recognition that this characteristic is particularly important for the future direction of evolutionary trends.

5. References

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