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RESEARCH ARTICLE

Intraspecific variation in wing shape of *Poecilobothrus regalis* (Meigen, 1824) (Diptera, Dolichopodidae)

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Abstract: A study of 186 specimens of *Poecilobothrus regalis* was conducted in order to examine intraspecific variability of wing shape. The wing shape variation was analyzed using geometric morphometrics analyses. Significant differences in the structure of wing were found both between sexes and between populations. Differences between sexes were observed in the structure of the medium portion of wing. The first extracted canonical variate of geographic variation showed a moderately linear association with latitude and average temperature of February and March. The second canonical variate was correlated with longitude and values of average wind flow velocity. Allometric relationships were weak both between populations and sexes.

Key words: Diptera, Dolichopodidae, morphology, *Poecilobothrus*, sexual dimorphism, wing.

Introduction

Recent studies in population ecology have demonstrated that intraspecific variability should be used in environmental studies as a major source of variability and sustainability of a given community (Violle *et al.* 2011). The range of intraspecific variation should be used as a measure of dispersion of particular characters. Characters with high rate of dispersion can be used for taxonomic constructions or as bioindication (Vasil'ev 2005).

Most studies of interpopulational variation of Diptera have found significant intraspecific differences in wing structure associated with geographical location and climatic characteristics of the habitat. Clinal variation in the wing shape of *Drosophila serrata* Malloch, 1927 was analyzed by Hoffmann & Shirriffs (2002), which concluded that the significant changes were associated with the latitude. These changes were described by the distance ratio between terminal departments of R_{2+3} and M_1 vein to the wing length.

Differences in wing length associated with distinct geographical sites were found between populations of *D. melanogaster* Meigen, 1830 from Eastern Europe and Central Asia (Imasheva *et al.* 1994). Later the variations in the posterior part of the wing compartment were found for the population from these regions (Imasheva *et al.* 1995). Laboratory-based experiments demonstrated that this component of intraspecific variability might be an effect of the temperature. Griffiths *et al.* (2005) has found significant differences among Australian populations of *Drosophila birchii* Dobzhansky & Mather, 1961, but in this case the regression analysis of shape variables on latitude did not support significant differences.

Shape differences were found between colonies of *Phlebotomus sergenti* Parrot, 1917 (Dvorak *et al.* 2006) but not between sexes within each colony. Authors compared two colonies from Turkey and Israel by using three methods: Procrustes method, random amplifies polymorphic DNA (RAPD) technique and cross-mating study. The results of the geometric morphometric analysis of wing shape were confirmed by RAPD analysis. The Turkish *P. sergenti* population has a thinner wing than the Israeli one.

Another important type of intraspecific variability in Diptera is sexual dimorphism. In this case, most studies focused on qualitative characters, such as color variation and the enlargement of arista and legs (Sivinski 1997). However, wing shape dimorphism has received less attention. Gidaszewski *et al.* (2009) considered that the range of dimorphism varies among species based on the study of sexual dimorphism in nine species of the *D. melanogaster* subgroup. The most common difference in this species was on the shape of the distal portion of female wings.

In this study we focused on intraspecific variability of wing shape in *Poecilobothrus regalis* (Meigen, 1824). *P. regalis* differs from other species of the family Dolichopodidae by the dark apical spot on the wing, the presence of 2-4 strong setae on the first segment of hind tarsi and triangular cercus (Stackelberg 1933). *P. regalis* is a fairly common species in southern Europe. It is a particularly attractive species for study because it is relatively large (4-7 mm length) and has visible sexual dimorphism in the coloration of wing. Males of *P. regalis* have dark apical spot on wings, while female’s wings are hyaline. Sexual and geographic variations in wing shape of these species were evaluated using geometric morphometrics analyses.

Morphometric characters of wing are widely used in the taxonomy of the family Dolichopodidae. It indicated that wings play an important role in the vital activity of the imago. Yet little is known about factors that have an impact on structure of wing. Therefore, a detailed study of their intraspecific variability allows for a deeper analysis of evolutionary trends.

Material and methods

One hundred eighty-six specimens of *P. regalis* (136 males and 50 females), from 6 different Russian populations were collected from the natural environments for analysis of intraspecific variability (Table 1).

Wings were removed from the flies’ bodies, placed on a glass slide and covered with a cover glass. All slides were photographed by using a Levenhuk C NG microscopic camera and digitized. Wing shape was described by 11 landmarks located at vein intersections with each other and wing margin (Fig. 1). The x, y coordinates of the following landmarks for

Table 1. Material examined.

Population localities	Number of studied specimens
Saratov region, Balashov district, vill. Malaya Semyonovka	14 ♂♂, 10 ♀♀
Voronezh region, Kantemirovska district, vill. Mitrofanovka	34 ♂♂, 10 ♀♀
Voronezh region, Ertil district, vill. Rostoshi	28 ♂♂, 10 ♀♀
Stavropol Territory, Spakovsk district, vill. Mikhailovskoe	12 ♂♂, 7 ♀♀
Saratov region, Samoylov district, vill. Trudovoe	22 ♂♂, 10 ♀♀
Lipetsk region, Elets distinct, vill. Chernyshovka	26 ♂♂, 3 ♀♀

each wing were obtained in order to study wings shape: 1 – base of Sc, 2 – apex of R_1, 3 – apex of R_{2+3}, 4 – apex of R_{4+5}, 5 – apex of M_1, 6 – apex of M_4, 7 – point of confluence of dm-m and M_4, 8 – point of maximum curvature of dm-m, 9 – point of confluence of dm-m and M_1, 10 – point of maximum curvature of M_1, 11 – base of R_{2+3} and R_{4+5}. Two-dimentional Casterian coordinates of these landmarks were digitized from the photos using tpsDig-2.32 software (Rohlf 2008).

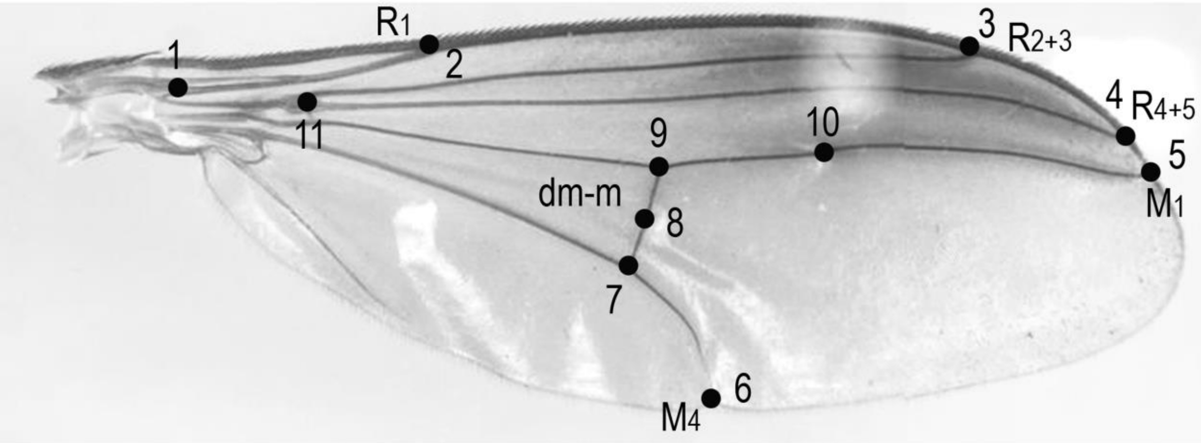

Figure 1. *P. regalis* wing and location of 11 landmarks used in the study.

The data was then analyzed by the methods of geometric morphometrics (Pavlinov & Mikeshina 2002, Klingenberg & McIntyre 1988, Hoffmann & Shirriffs 2002). Morphometric analysis was performed, using MorpholJ software (Klingenberg 2011). The statistical significance of the analysis was established with permutation test with 10000 random permutations. The centroid size of each wing (the square root of the sum of squared interlandmark distances) was calculated to characterize an overall measure of wing. These values were used in regression analysis with values of latitude and longitude of the location of population and climatic characteristics of these areas. Also centroid size was used for decomposing general variation into allometric and non-allomertic components.

Then differences in the wing shape (Procrustes coordinates) were examined using the generalized Procrustes superimposition method. For this purpose, landmarks configurations were scaled to a unit of centroid size, superimposed on an origin and rotated so that the distance between landmarks of all specimens become minimal.

Firstly, a Principal Components Analysis (PCA) was performed in order to assess individual variability. We also estimated the effects of sex, size and populations on wing shape by performing ANOVA. Following Klingenberg and McIntyre (1998) and Hoffmann and Shirriffs (2002) the contribution of each factor to size and shape variation was calculated by undertaking ANOVAs on summed means squares of each factor before calculation variance components due to sex, size and population. These calculations were made in Statistica 10 software for Windows.

Then, we used Canonical variate analysis (CVA) to find the differences between groups (sexes and populations). Canonical variates of the variance between populations were extracted and examined with correlation and regression analyses for latitudinal and longitudinal patterns and climatic characteristics. ANOVA were carried out on Procrustes coordinates to testing which landmark have the largest effect of the canonical variates. Then we used regression analysis to test a contribution of the studied factors in total variation of wing shape.

Allometry was tested using correlation analysis between canonical variates accounting for more than 10% of total variance and logarithm of centroid size of wing as independent variable. To assess the shape changes associated with allometry, decomposition of total variance into allometric and nonallometric components was made. For this purpose multivariate regression analysis of Procrustes coordinates on logarithm of centroid size was used. The sum of squares of predicted values was estimation of allometric changes in wing shape.

The 6 measurement of veins in the medium wing portion were performed in the Adobe Illustrator software, and 3 ratios were selected and tested using an analysis of variance (ANOVA). One of these characteristics was selected as an additional variable in order to characterize variation in wing derived from sexual dimorphism. The ratio of the total wing area by the area of m_1 portion can be used to estimate sexual dimorphism. An analysis of variance also was performed in Statistica 10 software for Windows to estimate the significance of differences between groups on the additional variables.

Climatic data such as monthly average temperature and monthly average wind flow velocity, relative humidity and atmosphere pressure, were obtained from the archives of meteorological stations closest to the place, where the material were collected, and climatic data used in this study were based on 30-years averages of weather station data.

Morphological terminology and abbreviations follows Grichanov & Brooks (accepted).

Results

The two principal components (PC1 and PC2) accounted for more than 60% of the general shape variation (Fig. 2). PC1, which alone accounted for 42.84% of the variation, includes x, y coordinates of landmarks 6 and 3. This component can be interpreted as a characteristic of position of the vein in terminal parts of the wing. On the other hand, PC2 accounted 24.06% of the general shape variation and included displacement of 8, 9 and 10 landmarks coordinates. PC2 illustrates changes in the point of maximum curvature of M_1 vein (landmark 10) moving towards the same spatial position of landmarks 8 and 9.

The ANOVA results indicate that the differences in the centroid size between sexes were more significant than the differences between populations (Table 2). The differences in wing form both between sexes and populations are significant. It seems important to note the

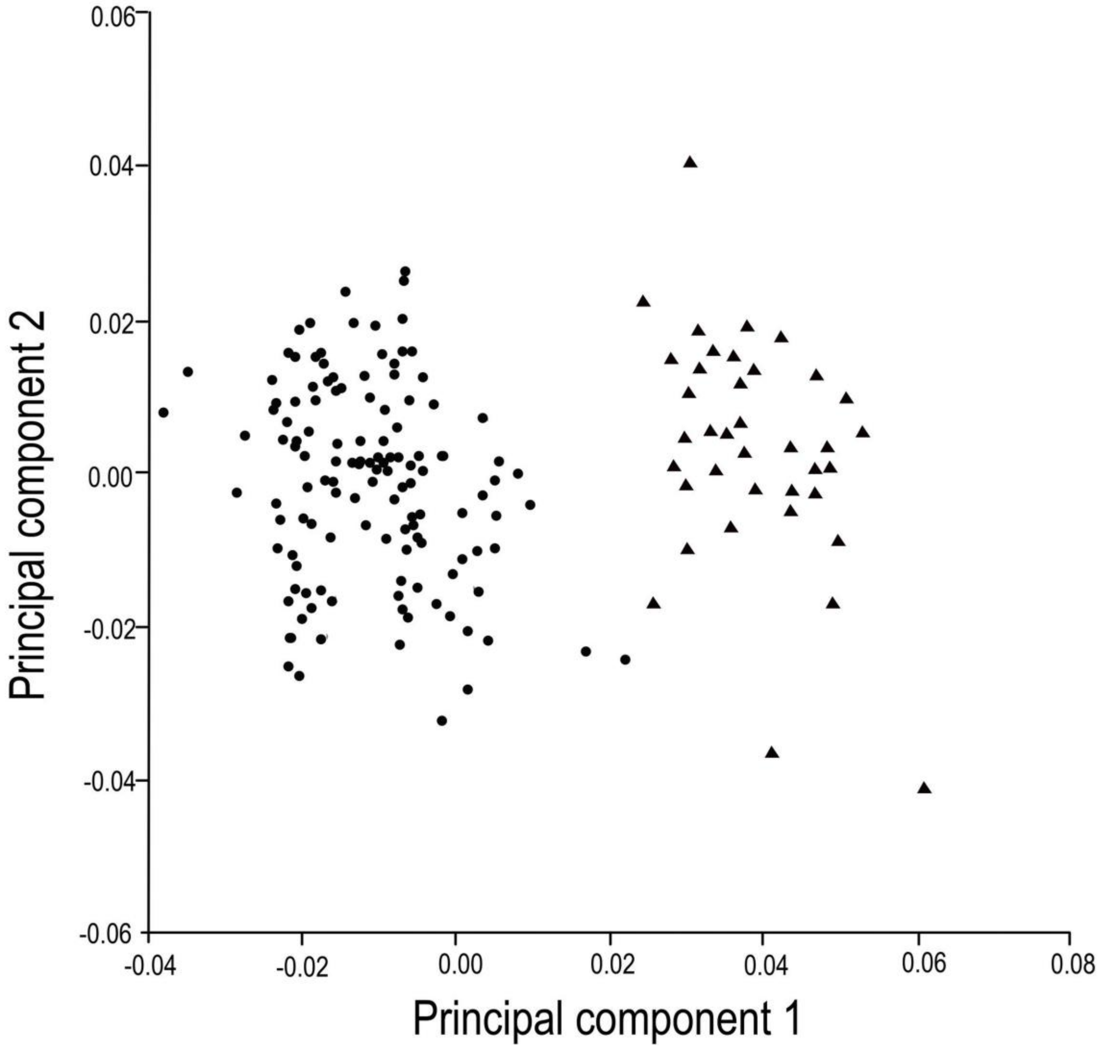

Figure 2. Morphospace of the two PCs with most of the variation PC scores are displayed (circles – male; triangles – female). Sex used as a criterion for grouping observations.

Table 2. The effect of sex, population and their interaction on centroid size, wing shape and additional variable (results of ANOVA).

Character	Effect	df	MS	F	P	% variance
Centroid size	Sex	1	5472.91	4.46	0.0036	52.63
	Population	5	2909.23	2.37	0.0420	27.98
	Sex and population	11	788.97	0.64	0.7896	7.59
	Error	148	1227.12			11.80
Shape	Sex	18	0.00397	101.64	<0.0001	94.30
	Population	90	0.00011	3.00	<0.0001	2.61
	Centroid size	252	0.00006	1.53	<0.0001	1.42
	Sex and population	198	0.00003	0.87	0.8743	0.71
	Error	2412	0.00004			0.95
Additional variable 1 (wing area/*m_1* area)	Sex	1	4.8260	337.28	<0.0001	99.31
	Population	5	0.0189	1.32	0.2629	0.39
	Error	163	0.0143			0.29

MS – mean squares, df – degrees of freedom, F – F-criterion, P – significance.

low statistical significance of the interaction "sex and population" indicates that the sexual dimorphism in wing shape is similar in populations studied. There were no significant differences between males and females from different populations, which have not been caused by the sexual dimorphism and variation between populations.

Sexual Dimorphism

Canonical variate analysis (CVA) showed that the main differences between males and females include x coordinates of landmarks 6 (the apex of M_4) and 9 (the middle of the dm-m) and x coordinate of landmark 3 (position of the terminal part of R_{2+3}) (Fig. 3, 4). ANOVA allows for the estimation the effects of changes providing of each landmark on sexual shape dimorphism. These effect were largest for landmarks 3 (40.93% of variance), 9 (14.40% of variance) and 6 (11.67% of variance).

The ANOVA analysis results (Table 2) show that there are significant differences between males and females for the additional variable (ratio “total wing area/m_1 area”) ($p<0.0001$).

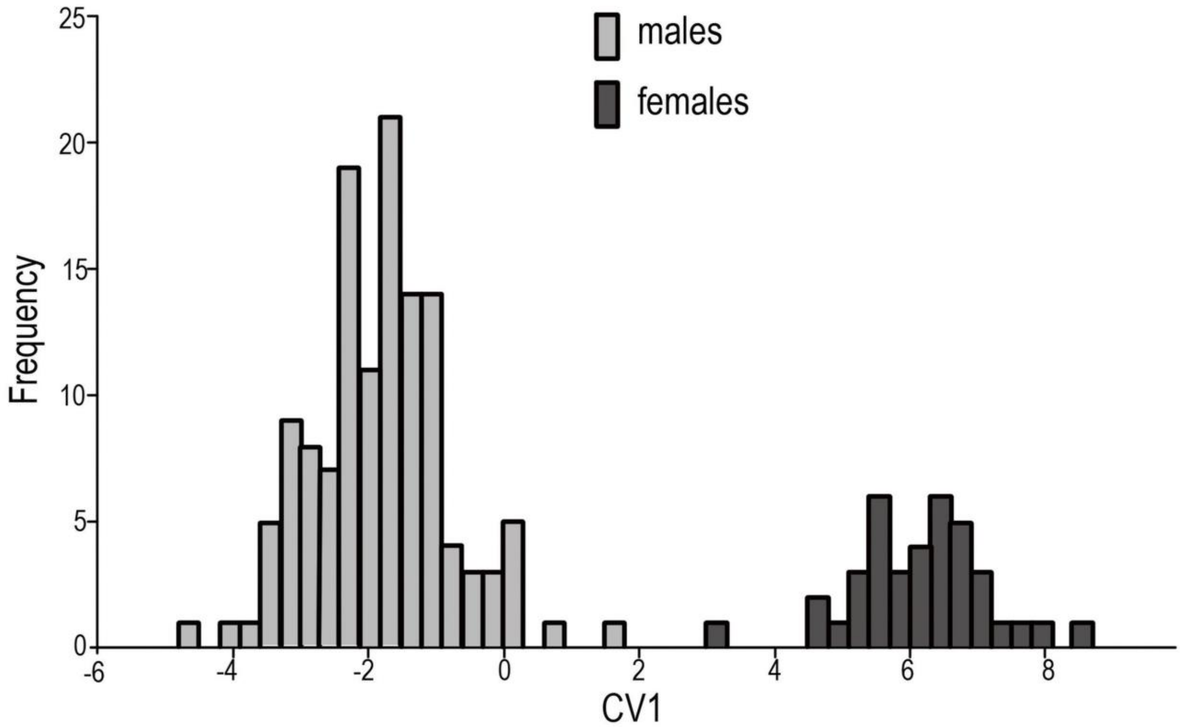

Figure 3. Scatter plot of the CV scores for sexual shape dimorphism.

Geographic Variation

Four canonical variates each with more than 10% of the total variation of wing shape were obtained and examined using correlation and regression tests with geographical latitude, longitude and climatic characteristics.

The first variate accounted for 41.20% of the total shape variation is strongly associated with the transformations of y coordinates of landmarks 1, 2, 7 and 11, and x coordinates of landmarks 1 and 7. Landmarks 1 and 7 introduce the largest contribution on first canonical variate. The percents of variance are 55.63% for landmark 1 and 30.02% for

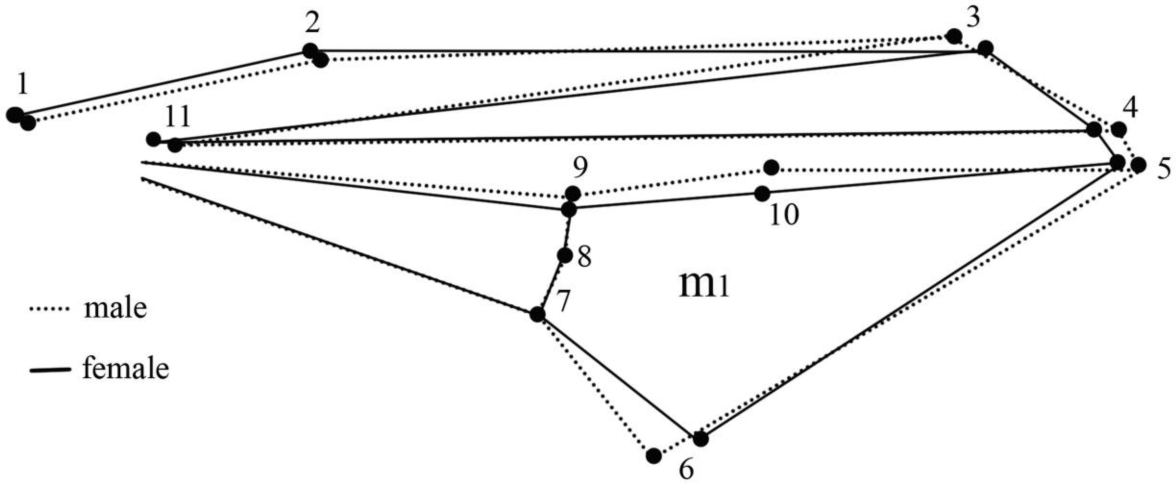

Figure 4. Shape changes associated with sexual dimorphism in *P. regalis*. Landmark positions are indicated by number. Differences are exaggerated tenfold for better visual perception.

landmark 7. The first canonical variate shows moderate correlation with longitude (r = 0.44) and average temperatures of February and March (r = –0.42 and r = –0.53) (Fig. 5). For landmark 7, populations from region with lower temperatures in February and March had Procrustes residuals with lower y-values.

The second variate accounted for 28.45% of the total dispersion includes the transformations of y coordinate of landmark 3 and x coordinate of landmark 7. The effects of second variate for landmarks 3 and 7 are 35.76% and 41.25%. The second canonical variate was correlated with latitude (r = 0.45) and average summer wind flow velocity (r = –0.54) (Fig. 6). So the average shape of wing of the flies from the region with higher wind flow velocity is narrower, and this change is the cause of position of landmark 3.

Regression analysis showed significant linear component in both cases: as for the first canonical variate and temperature and for the second canonical variate and average summer wind flow velocity. The angular coefficient (b±SE) of the linear relationship between the first canonical variate and average temperatures for March and February, is 22.16±5.63 (P<0.0001). The angular coefficient (b±SE) of the linear relationship between the second canonical variate and average wind flow velocity is 1.91±0.24 (P<0.0000001). As a result of the multiple regression analysis, we obtained no strong relationship between the centroid size of wing and climatic characteristics. No significant correlations were found between centroid size of wing and latitude, longitude and climatic characteristics.

Allometry

Correlations between canonical variate and logarithm of centroid size among sexes were –0.12. Correlations between five main canonical variates and centroid size among populations were –0.07, –0.19, –0.18, –0.08 and 0.08 respectively. Allocation of regression residuals showed that allometric relationships among localities are 3.24% and among sexes – 0.14%. So, there were no significant allometric relationships among sexes and among populations.

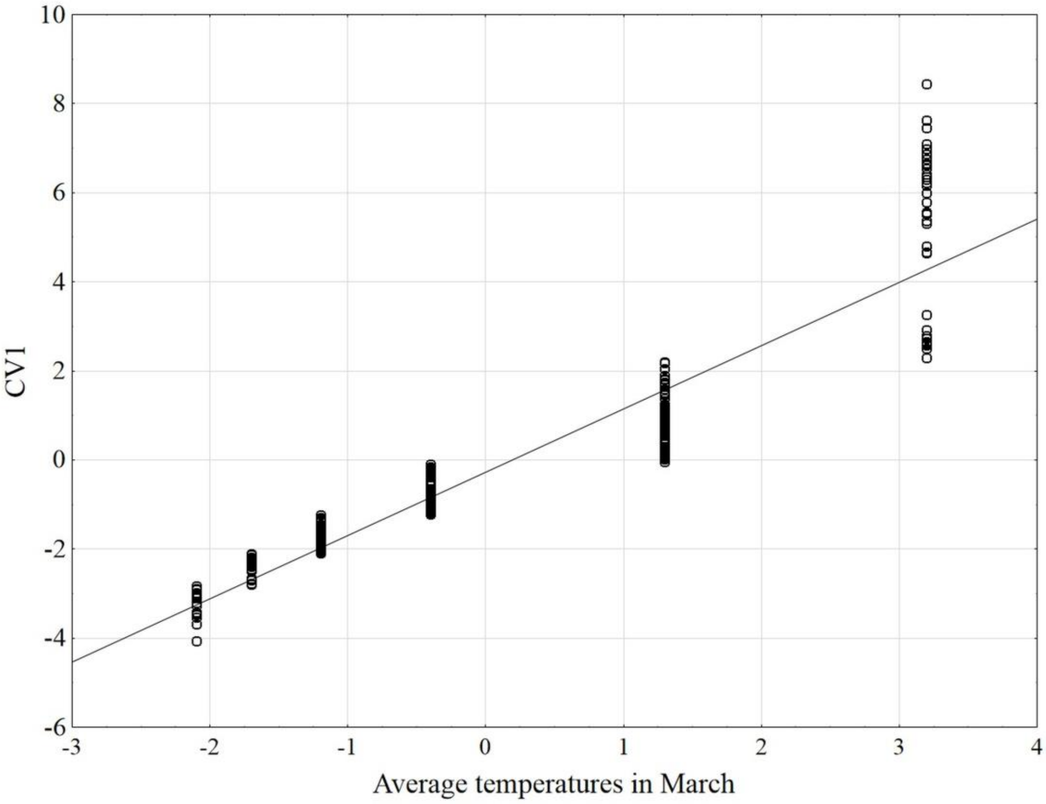

Figure 5. Quantile-quantile scatterplot of CV1 against average temperature in March.

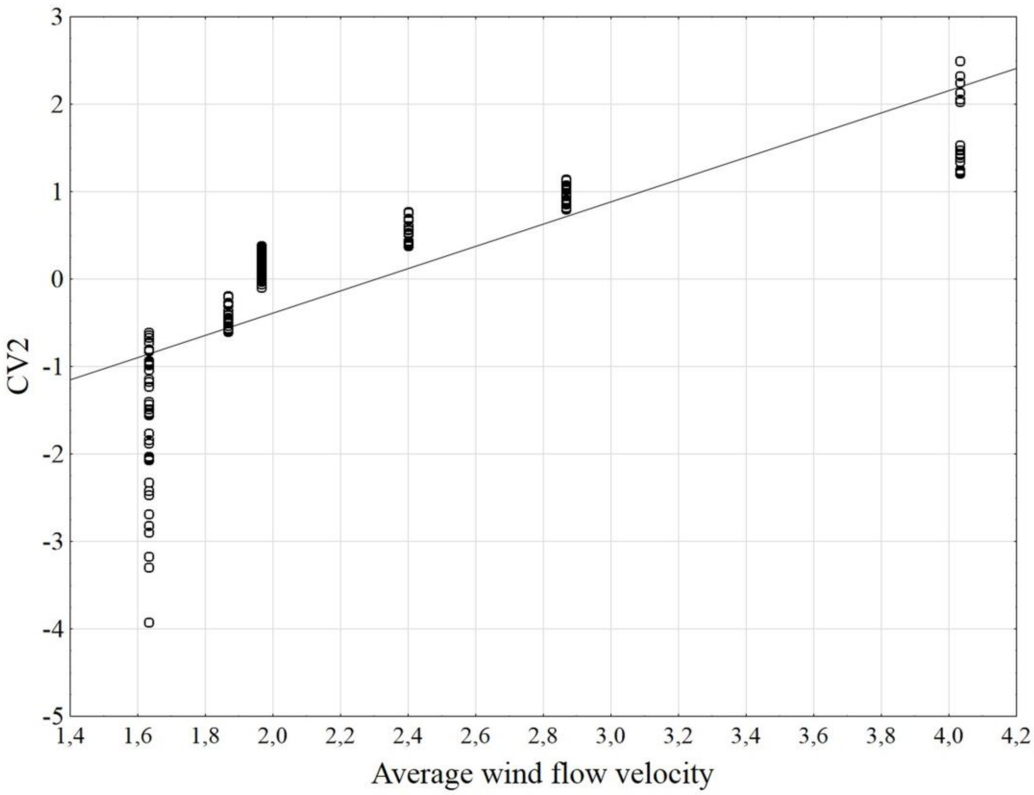

Figure 6. Quantile-quantile scatterplot of CV2 against average wind flow velocity.

Discussion

Several species of Dolichopodidae are interesting due to some features exhibited by males, which are used in courtship behavior according to some authors (Van Duzee *et al.* 1921; Land 1993a). Currently, there is no consensus about interpretation of the dark spot on male wing of some Diptera species. When analyzing the courtship behavior of Dolichopodidae species, Zimmer *et al.* (2003) concluded that sexually dimorphic badges in males are the adaptation for static courtship. Possibly, dark apical spot on wings acts as a signal from males to females. However, an experiment performed by Sivinski and Pereira (2005) did not support any evidence that amplification of wing coloration leads to an increase in the sexual success rate.

Analysis of the courtship behavior of *Poecilobothrus nobilitatus* males (Land 1993a,b; Smith & Empson 1995), that have white apical spot on the wing showed that it is related to specific flight features. For example, rotation component of the flight has been detected for males only, whereas females are characterized only by the forward flight.

In our study of the wing shape in *P. regalis*, we concluded that the differences between males and females are based on relative areas of m_1 and m_4 section. This difference was observed in all the studied populations and it has a great level of statistical significance.

The first and the second canonical variates of geographical changes in wing shape were moderately associated with latitude and longitude, temperatures and the wind flow velocity. Significant associations between latitude and longitude and main shape canonical variates, which were found in the first step of the study, indicate a relation between climatic characteristics of the geographical position of areas. As such, the dependence of the wing shape to the climatic factors should be more carefully studied in the future.

These results are in good agreement with previously studies. Laboratory experiments repeatedly demonstrated the sensitivity of the wing structure to temperature (Cavicchi *et al.* 1985, 1991). Flies of the family Dolichopodidae overwinter in the pupal stage. The fact that the variation of wing shape was affected most by the average temperatures in March and February may mean that the wing shape is undergoing significant changes at this stage. In this case, we can assume that lower temperature in February and March may result in a broader wing because of the elongation of the posterior transverse vein (landmark 7).

Dolichopodidae are active predators, so the flight plays an important role in their ability to live. Thus, morphology of wing is an important factor of performance in flight, and wing shape might be adaptive with regard to wind flow velocity. Aerodynamic studies have suggested that elongated wings generate more force per area (Spedding 1992; Combes, Daniel 2001); therefore, the wing width is one of the most important aerodynamic characteristic of wing. Our results indicate patterns in wing length that depend on the wind flow velocity: specimens of *P. regalis* with narrower distal part wings were found in regions with higher wind flow velocity. However this study can not reveal the mechanism of the wings adaptation to environmental factors. This subject requires additional investigations.

Centroid size of the wing and total size of the insects apparently does not depend on climatic, geographical and sexual factors and varies considerably within each population. Perhaps it depends on the conditions of supply and intra-competition for food resources.

In conclusion, it seems important to note that the variation in the length of dm-m and its location relative to M_1 vein are important taxonomical characters in dolichopodid species. A majority of morphometric studies (Negrobov & Chursina 2012; Chursina *et al.* 2014) have shown that the posterior part of Dolichopodidae wing varies considerably within subfamilies and family, but intraspecific differences in wing morphometry are subtle and can be analyzed only using geometric morphometrics methods. As far as sexual variation of wing shape in *P. regalis* was evident, preliminary we can recognize this character as having a particular importance for taxonomical and phylogenetic studies. However, sexual dimorphism of wing shape in other Dolichopodidae species requires additional studies.

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