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# Wing shape is influenced by environmental variability in *Polietina orbitalis* (Stein) (Diptera: Muscidae)

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## ABSTRACT

We measured variation and covariation in wing morphology in six populations of the fly *Polietina orbitalis* (Stein) (Diptera: Muscidae) to test for geographic morphological structure. Additionally, we examined the role of environmental variables in determining geographic variation in wing shape. We sampled five populations in the state of Paraná, southern Brazil (Colombo, Fênix, Guarapuava, Jundiaí do Sul and Ponta Grossa), and one in Paraguay (Mbaracayú). We choose 15 landmarks to describe the wing shape and size and 19 environmental variables to describe the local environmental conditions. Our results showed that *P. orbitalis* wing shape, but not size, varies geographically. A canonical variate analysis showed the existence of two clusters of populations based on wing shape. These groups compare populations in which the wing is slender with groups in which the wings are broad. These shape differences were correlated with variation in elevation, precipitation and temperature but were not allometric. Taken together, these results suggest that wing shape differences in *P. orbitalis* populations are due to a plastic response to local environmental conditions.

## Introduction

Phenotypic plasticity is the ability of an organism to express different phenotypes depending on the environmental conditions faced (Agrawal, 2001). Such plasticity is therefore the consequence of the interaction between environmental variability and the developmental program (genotypic × environmental interaction; Scheiner, 1993) and is thus, one solution to the problem of adaptation to heterogeneous environments (Via et al., 1995). Plasticity arises because environmental variability induces developmental changes, which alter the expression and connection between traits (Relyea, 2004).

As a developmental response, plastic traits allow species to cope with environmental variability (temporal or spatial), enabling a fitness optimization (such as acclimatization) to these conditions (Ghalambor et al., 2007). Although phenotypic plasticity has an effect on fitness, this effect could be adaptive, maladaptive or neutral (Scheiner, 1993; Pigliucci, 2005; Ghalambor et al., 2007). Because any environmentally induced plasticity places phenotypes into different selective regimes, the fitness consequence, in both the short and long run, will determine whether the response is adaptive. For example, environmental variability that is persistent, causing persistent selection pressure, may lead to local population adaptation, with fitness peaks that are different for each population (Kawecki and Ebert, 2004).

Although morphological traits have been one of the characters most widely used to study phenotypic plasticity, any measurable trait, such as life history features, physiology and behavior could also be used (Whitman and Ananthakrishnan, 2009). Insect body size and shape strongly respond to changes in temperature, with responses ranging from population differences (e.g., Hoffmann and Shirriffs, 2002) to thermal clines in body size (Griffiths et al., 2005; van Heerwaarden and Sgrò, 2011). As size and shape impact performance and fitness (van Heerwaarden and Sgrò, 2011), these traits are of interest for the study of phenotypic plasticity.

Environmental variables are spatially structured, and this structuring could also lead to hierarchically structured morphological variation that could be either continuous (such as a cline) or discontinuous. Therefore, the recognition of such morphological discontinuities can lead to an understanding of the shaping of not only species boundaries but also intraspecific patterns of variation and covariation (Mateus et al., 2013).

The study of morphological variation has advanced at the same pace as the development of the corresponding analytical methods, thus allowing for a more accurate treatment of a variety of hypotheses (Adams et al., 2013). Some phenotypic alteration can be very subtle and difficult to detect, and morphometry can be a useful tool for detecting such change, mainly that involving the size and form of organisms (Strauss and Bookstein, 1982). The main strength of geometric morphometry over traditional morphometric methods

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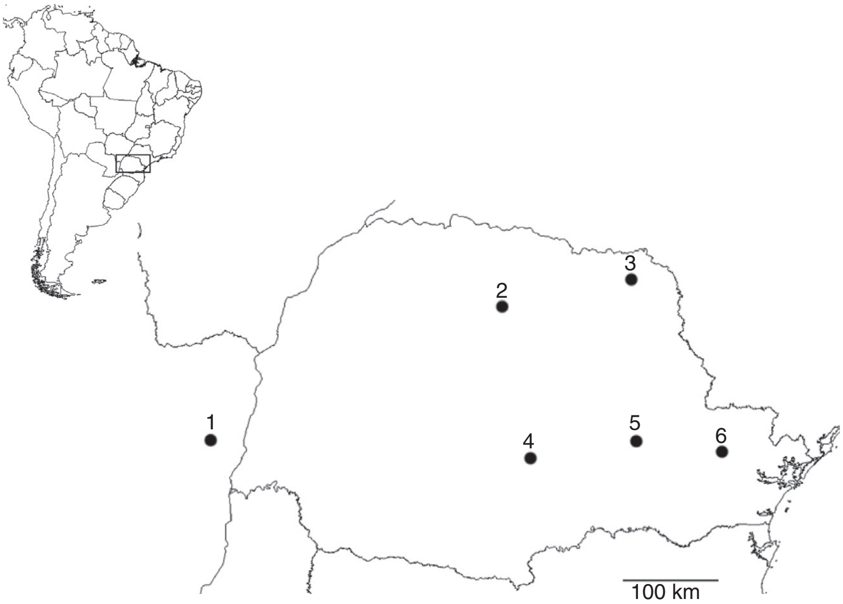

**Fig. 1.** Map showing sites where *P. orbitalis* populations were collected: 1. Mbaracayú (25°17′ S, 54°49′ W) in Paraguay and 2. Fênix (23°54′ S, 51°58′ W), 3. Jundiaí do Sul (23°26′ S, 50°14′ W), 4. Guarapuava (25°23′ S, 51°27′ W), 5. Ponta Grossa (25°05′ S, 50°09′ W) and 6. Colombo (25°17′ S, 49°13′ W) in Brazil.

is its ability to detect even subtle morphological variation. Additionally, geometric morphometry can separate the size and shape components of form (Adams et al., 2013) by permitting analysis in a high-dimensional morphological space, which enables a graphical interpretation of shape changes.

Diptera provide a standard model system to study environmentally induced phenotypic changes. Several species exhibit variations in fitness-related and morphological traits (wing size and shape) that correlate with local selective pressures, mainly thermal selection (Reeve et al., 2000; Hoffmann and Shirriffs, 2002; Soto et al., 2007; Aytekin et al., 2007; Marsteller et al., 2009; Devicari et al., 2011; Demirci et al., 2012; Kjaersgaard et al., 2013; Hidalgo et al., 2015). However, these results are biased toward groups of medical and veterinary importance as well as model species, such as those belonging to the genus *Drosophila*. Although we expect that the life history traits of all ectotherms respond to temperature (Angilletta et al., 2004), those ectothermal species that are broadly distributed are expected to face steeper climate changes along their geographic range. *Polietina orbitalis* (Stein, 1904) (Diptera: Muscidae) are broadly distributed in forested areas of South America, including Brazil, Argentina, Bolivia, Peru and Paraguay (Löwenberg-neto and de Carvalho, 2013). Thus, the geographic range of *P. orbitalis* encompasses several biome types, each one defined by a set of specific environmental characteristics. Additionally, their range spans tropical and subtropical/temperate climates. Therefore, this species seems a model candidate to determine the influence of environmental conditions on morphology because its populations along a latitudinal/longitudinal gradient are under different thermal regimes. Consequently, we used geometric morphometry to characterize geographic patterns in *P. orbitalis* morphology (wing shape), expecting them to vary according to the environmental conditions under which each population evolved. Specifically, we tested for the following patterns and associations: (a) morphological interpopulational variation, (b) an association of morphological changes with latitude or longitude and (c) the climatic variables most strongly correlated with morphological changes.

## Material and methods

We restricted our analysis to female specimens of *P. orbitalis* from southern Brazil and nearby Paraguay. The geographical extent of sampling was defined by the availability of an adequate sample size, and only females were sampled to avoid the effects of sexual dimorphism and a male sample size that was too small. The sample comprised the following specimens: 28 from Paraguay (Mbaracayú) and Jundiaí do Sul, 24 from Colombo and 30 from Fênix, Guarapuava, and Ponta Grossa (Fig. 1).

All the specimens were previously collected using Malaise traps. The Paraguayan population was collected at the Reserva Natural del Bosque Mbaracayú, and the Brazilian populations were sampled within the project "Levantamento da Fauna Entomológica no Estado do Paraná" (PROFAUPAR), from August 1986 to July 1988 (Marinoni and Dutra, 1993). All the specimens belong to the Padre Jesus Santiago Moure entomological collection housed in the Zoology Department of the Universidade Federal do Paraná (DZUP).

To test our hypotheses, we used right wings as a morphological proxy. The wings were carefully removed and placed in absolute alcohol for 20 min, xylene for 10 min and then mounted between 14 mm coverslips with Entellan®. The dorsal side of the wing was photographed using a Dino-Lite Pro® microscope at 15× magnification. Voucher specimens, together with wing mounts, were deposited in the DZUP.

We defined the wing shape through the placement of 15 anatomical landmarks at wing vein intersections (Table 1, Fig. 2). These 15 landmarks were chosen because they could capture morphological variations along the entire wing area (the base, center and tip of the wing). All the landmarks were digitized using tpsDig version 2.16 (Rohlf, 2010) and tpsUtil version 1.53 (Rohlf, 2012), free software available at http://life.bio.sunysb.edu/morph/.

The landmark configurations were superimposed using generalized Procrustes analysis (GPA, Rohlf and Slice, 1990). The first step of GPA is to center each landmark configuration at the origin that is aligning all the landmark configurations to their centroids, eliminating the effect of position. Then, the landmark configuration is scaled to unit centroid size, thus eliminating the effect of size. Next, the landmark configurations are rotated around the origin to minimize the summed square distance between homologous landmarks, which removes the effect of orientation. After this procedure, the distances (Procrustes distance) between the superimposed configurations correspond to the extents to which the configurations differ in shape, and the superimposed coordinates contain information regarding the shape. To quantify the

**Table 1**
Number and description of the 15 anatomical landmarks used to characterize wing shape in *P. orbitalis*.

| Anatomical landmark | Description (intersection between veins) |
|---|---|
| 1 | C with h |
| 2 | C with Sc |
| 3 | C with $R_1$ |
| 4 | C with $R_{2+3}$ |
| 5 | C with $R_{4+5}$ |
| 6 | C with M |
| 7 | bm-cu with M |
| 8 | bm-cu with $Rear_1$ |
| 9 | $R_{4+5}$ with r-m |
| 10 | M with r-m |
| 11 | $R_{2+3}$ with $R_{4+5}$ |
| 12 | M with dm-cu |
| 13 | $CuA_1$ with dm-cu |
| 14 | $CuA_1$ with $CuA_2$ |
| 15 | $A_1$ with $CuA_2$ |

*Abbreviations*: $A_1$, anal 1; C, costa; CuA, anterior cubital; $CuA_1$, anterior cubital 1; $CuA_2$, anterior cubital 2; M, medial; R, radial; $R_1$, radial anterior 1; $R_{2+3}$, radial 2 + 3; $R_{4+5}$, radial 4 + 5; Sc, subcosta; bm-cu, basal median cubital; dm-cu, distal median cubital; h, humeral; r-m, radial medial.

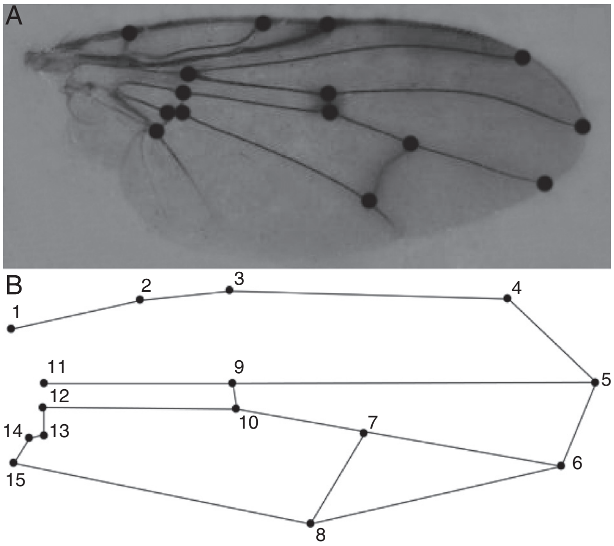

**Fig. 2.** Dorsal side of the right wing of *P. orbitalis* showing (A) the 15 (numbered points) anatomical landmarks and (B) the general shape of the wing based on their positions.

measurement error, each wing was digitized three times on separate days. We then applied a multivariate analysis of variance (MANOVA) to test for departure from random measurement errors among the replicates.

As a general measure of wing size, we used the centroid size of each specimen, which is the square root of the sum of the squared distances of all the landmarks from their centroid, the center of each configuration (Bookstein, 1991). To test the populations for differences in average size, we compared wing size among the populations using an analysis of variance (ANOVA). To assess the degree of correlation between size and shape, we fit a linear regression between the Procrustes coordinates and the centroid size.

The populations were ordered in a reduced space using canonical variable analysis (CVA). CVA is a technique that maximizes the separation of populations because each axis is constrained to represent the maximum between-group variance. To test whether the populations have diverged in shape, we used a MANOVA with the Procrustes coordinates as the response variable. The difference between groups was characterized by discriminant function analysis (DFA), and the reliability of group memberships was determined by cross-validation.

We tested for an effect of climate on phenotypic changes using a two-block partial least squares (PLS) analysis. This PLS analysis tested for covariation between blocks of variables (wing shape within populations), using the Procrustes coordinates and environmental variables (Rohlf and Corti, 2000). Climate was summarized by 19 variables representing variations in temperature and rainfall: (1) annual mean temperature; (2) mean diurnal range; (3) isothermality; (4) temperature seasonality; (5) maximum temperature of warmest month; (6) minimum temperature of coldest month; (7) annual temperature range; (8) average temperature of the wettest and (9) driest three-month periods; (10) average temperature of the hottest quarter; (11) average temperature of the coldest quarter; (12) annual precipitation; (13) precipitation in the wettest and (14) driest months; (15) seasonal variation in precipitation; and (16) precipitation in the wettest, (17) driest, (18) hottest and (19) coldest quarters. Elevation, latitude and longitude were also included in the environmental matrix. These climatic variables represent a 50-year mean trend in historical data (1950–2000) and were obtained from the WorldClim database (http://www.worldclim.org/) using DIVA-GIS version 7.5 (Hijmans et al., 2001). All the variables were standardized before analysis. All analysis was performed using MorphoJ® version 1.05d (Klingenberg, 2011) and PAST® version 2.17b (Hammer et al., 2001).

## Results

The error due to landmark digitization was random for both shape (ANOVA; $p = 0.971$, $SS = 0.004$, $F = 0.36$, $df = 32$) and size (ANOVA; $p = 0.988$, $SS = 1905$, $F = 0.010$, $df = 2$); thus, any differences between populations were unrelated to image or digitization errors. There was no size (wing centroid size) variation between among the *P. orbitalis* populations (ANOVA; $F = 0.004$, $p = 0.998$, $df = 2$), with the size ranging from 5 to 6 mm. In contrast with wing size, wing shape differed among all the populations (MANOVA; Wilks' $\Lambda = 0.030$, $p < 0.001$, $df = 150$, $F = 5.38$). There was no allometric component because the shape and size variables were not correlated ($r = 0.02$, $p = 0.1873$). Therefore, the wing shape difference among the populations was independent of the size of the specimens.

The ordination of the populations by CVA showed that the first and second canonical variables jointly explained 81% of the observed variation. The first canonical axis (accounting for 51% of the variance) ordered the populations in a wider to narrower wing shape gradient. The populations with positive loadings (positive CV1 values, Fig. 3), such as Ponta Grossa, Guarapuava and Colombo, had certain landmarks of the costal margin (landmarks 1,2 and 3) and of the posterior wing margin (landmarks 8 and 15) displaced toward the center of the wing. Additionally, landmarks 4 and 7 displaced toward the wing tip. Together, these landmark displacements shorten the wing blade, resulting in a narrow wing (Fig. 4). The populations with negative loadings on CV1, such as Mbaracayú and Fênix (Figs. 3 and 4), showed a contrasting wing shape compared with those in the positive quadrant. The displacement of the costal (landmarks 1, 2 and 3) and posterior (landmarks 8 and 15) wing margin landmarks expanded the wing blade (Fig. 4). Additionally, landmarks 4 and 7 moved toward the wing base. These rearrangements resulted in an enlarged wing.

The second canonical axis (CV2, accounting for 30% of the variance) showed only local changes (Fig. 5), in contrast with CV1, for which the morphological changes were global. The landmark displacement occurred mostly in the basal region (landmarks 1, 2, and 11). The wings of populations loaded with positive scores (Fênix, Colombo and Guarapuava) had a slight expansion of the anterior

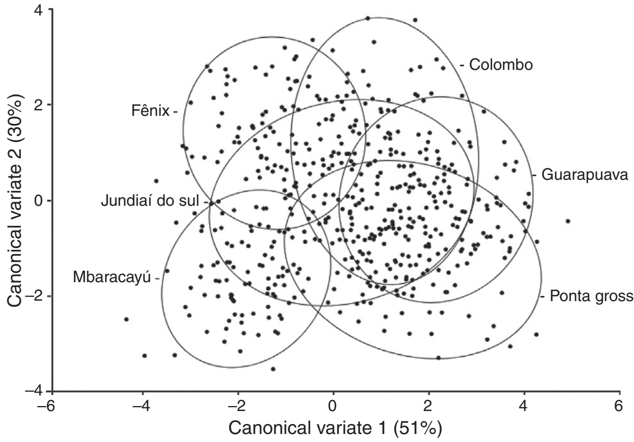

**Fig. 3.** Position of the *P. orbitalis* populations in the space determined by the first two canonical axes. The ellipses indicate 95% confidence intervals.

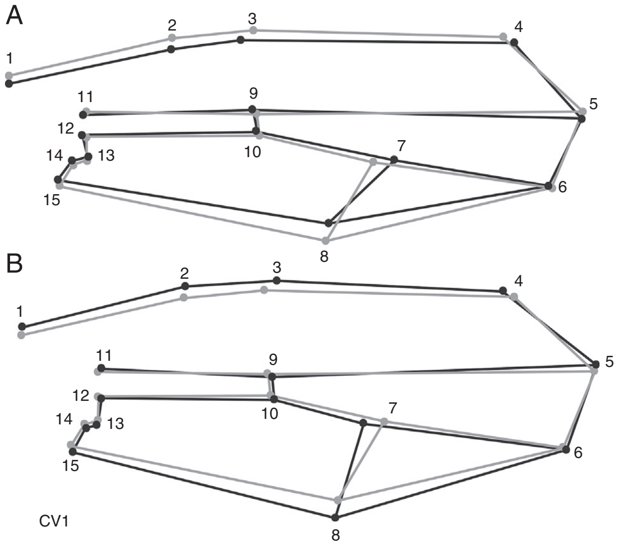

**Fig. 4.** Graphic reconstruction of the wing shape of individuals with (A) positive and (B) negative scores on the first canonical axis (CV1, increased 10 times). The lines in gray represent the average configuration of the wing, and those in black represent the canonical first variable.

**Table 2**
Percent correct classification of populations of *P. orbitalis* based on wing morphology and discriminant function analysis (DFA).

| Population | Allocation value (%) |
|---|---|
| Colombo | 97 |
| Fênix | 95 |
| Guarapuava | 99 |
| Jundiaí do Sul | 98 |
| Mbaracayú | 97 |
| Ponta Grossa | 96 |

region (landmarks 1 and 2) and at landmark 11 in a more apical position. The populations with negative loadings on CV2 (Mbaracayú and Ponta Grossa) showed less basal area than that shown by populations on the positive CV2 quadrant due to the basal displacement of landmark 11 and a slightly posterior displacement of landmarks 1 and 2. The cross validation tests indicated that an average of 97% (ranging between 95% and 99%) of individuals were correctly allocated to their respective populations (Table 2).

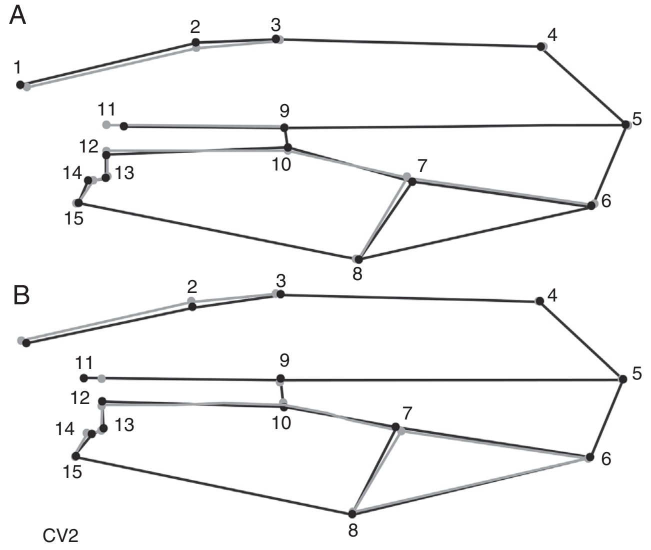

**Fig. 5.** Graphic reconstruction of the wing shape of individuals with (A) positive and (B) negative scores on the second canonical axis (CV2, increased 10 times). The lines in gray represent the average configuration, and those in black represent the second canonical variable.

All dimensions of the PLS analysis (Table 3) were statistically significant (all with $p < 0.01$), but the PLS1 axis alone explained 92% of the variation in the shape variables. Elevation and precipitation in the driest month were negatively correlated with PLS1 (−0.79 and −0.20, respectively), whereas the minimum temperature of the coldest month and average temperature of the driest quarter were positively correlated (0.23 and 0.25, respectively) with PLS1 (Fig. 6). The populations clustered in two groups along this axis: Guarapuava, Ponta Grossa and Colombo in one group and Mbaracayú, Fênix and Jundiaí do Sul in another. The variation in shape was independent of latitude and longitude. Thus, higher elevations and greater rainfall were associated with narrower wings, and lower elevations and temperature were associated with wider wings (Fig. 7).

## Discussion

Our results showed that *P. orbitalis* wing shape varies geographically, but no such variation was detected for wing size (centroid size). Additionally, all the populations differed in wing shape, which led to a high success rate (an average of 97%) in predicting the population in which each individual belonged. Although all the populations had average wing shapes that differed from one another, the CVA showed two clusters of populations based on wing shape (narrow wing and broad wing clusters), with the shape of both groups correlated with elevation, precipitation and temperature but not with wing size. Taken together, these findings suggest that shape differences in *P. orbitalis* result from a plastic response to local environmental conditions.

Although elevation was the single variable that most influenced the PLS axis, in general, the axis could be viewed as a temperature/precipitation gradient because elevation and temperature are negatively correlated. Temperature is one of the most important drivers of phenotypic plasticity in insects (Lomônaco and Prado, 1994; Imasheva et al., 1995; Whitman and Ananthakrishnan, 2009). The temperature-size rule proposes that insects developing under high temperature have a smaller size (Atkinson, 1994). As wing size correlates with body size, we would expect smaller wings at high temperatures, a hypothesis we reject because we did not find any difference in wing size among the populations. However, temperature also affects wing load (Gilchrist and Huey, 2004), which is dependent on the shape of the wing. Thus, we would expect that a population from cooler areas would have a reduced wing load

**Table 3**
PLS result between the shape and environmental variables. Notice that the greater PLS1 has the strongest correlation (all $p < 0.01$).

| Variable | Dimension | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Latitude | 0.033 | −0.083 | 0.057 | −0.067 | 0.227 |
| Longitude | −0.051 | −0.059 | −0.054 | 0.159 | −0.091 |
| Altitude | −0.790 | 0.254 | 0.090 | 0.145 | 0.089 |
| Annual mean temperature | 0.178 | 0.007 | 0.064 | 0.123 | 0.091 |
| Mean diurnal range | 0.117 | −0.005 | 0.409 | −0.221 | 0.111 |
| Isothermality | 0.011 | −0.104 | 0.279 | −0.264 | 0.233 |
| Temperature seasonality | 0.121 | 0.304 | −0.197 | 0.129 | −0.282 |
| Maximum temperature of warmest month | 0.149 | 0.057 | 0.090 | 0.142 | −0.038 |
| Minimum temperature of coldest month | 0.235 | −0.056 | −0.038 | 0.392 | 0.167 |
| Temperature annual range | 0.111 | 0.106 | 0.146 | 0.032 | −0.128 |
| Average temperature of wettest quarter | 0.116 | −0.007 | 0.060 | 0.158 | 0.029 |
| Average temperature of driest quarter | 0.253 | −0.094 | 0.145 | 0.063 | 0.407 |
| Average temperature of warmest quarter | 0.163 | 0.064 | 0.040 | 0.134 | −0.031 |
| Average temperature of coldest quarter | 0.184 | −0.036 | 0.145 | 0.144 | 0.101 |
| Annual precipitation | −0.007 | 0.156 | −0.206 | −0.154 | 0.384 |
| Precipitation wettest month | 0.057 | 0.097 | −0.454 | −0.244 | −0.055 |
| Precipitation driest month | −0.201 | 0.420 | −0.060 | 0.542 | 0.319 |
| Precipitation seasonality | 0.141 | −0.521 | −0.298 | 0.165 | 0.214 |
| Precipitation of wettest quarter | 0.034 | −0.011 | −0.332 | −0.096 | −0.097 |
| Precipitation of driest quarter | −0.122 | 0.442 | −0.000 | −0.028 | 0.221 |
| Precipitation of warmest quarter | 0.007 | −0.105 | −0.404 | −0.130 | 0.364 |
| Precipitation of coldest quarter | −0.045 | 0.314 | 0.032 | −0.349 | 0.249 |
| Singular values | 0.00138 | 0.00040 | 0.00011 | 0.00007 | 0.00001 |
| Covariation (%) | 92 | 7 | 0.6 | 0.3 | 0.1 |
| Correlations | 0.678 | 0.401 | 0.315 | 0.362 | 0.213 |

Coefficient of association between blocks is 0.2179.

to compensate for reduced flight performance due to the smaller adult size (Dudley, 2000). A reduced wing loading could be achieved by increasing the wing area (Dudley, 2000), but populations from colder climates (positive CVA quadrant and negative correlations with PLS) had long and slender wings, which did not match the prediction of the wing load hypothesis. Thus, the wing shape changes we described are not totally related to an aerodynamic hypothesis. Our findings highlight the complex interaction between genotype and environment.

Variations in size between populations can be associated with alterations in shape (Dujardin, 2008); however, we did not find this trend. The absence of allometry associated with geographical variations in wing shape has also been found in *Drosophila serrata* Malloch (1927) (Hoffmann and Shirriffs, 2002). Therefore, this absence of allometric patterns means that factors other than allometry are influencing wing shape. Additionally, the PLS analysis did not show significant correlation between shape and geographical distance, as observed in other studies (Motoki et al., 2012), perhaps as a result of sampling a larger geographic area in those studies compared with that sampled by our study (Monroy et al., 2003).

An effect of elevation has been reported in other studies. For instance, Belen et al. (2004) found that *Phlebotomus papatasi* (Scopoli, 1786) populations from a higher elevation formed a distinct group. In studies with *Culex theileri* Theobald (1903), Demirci et al. (2012) also found wing shape modifications in populations between elevations of 808 and 2130 m. While evaluating the traditional and geometrical morphometry of *Drosophila mediopunctata* Dobzhansky & Pavan (1943), Bitner-Mathé et al. (1995) also observed an association of form with elevation, indicating clinal variation with elevation in this species. Additionally, we can establish a morphological pattern in *P. orbitalis* related to elevation, in that below or above an elevation of 900 m, populations showed a broad- or thin-wing phenotype, respectively. The standard thin wing found in populations at higher elevations could confer an

**Fig. 6.** Results of the PLS analysis, with Colombo (6), Guarapuava (4) and Ponta Grossa (5) showing negative values and Fênix (2), Jundiaí do Sul (3) and Mbaracayú (1) showing positive values.

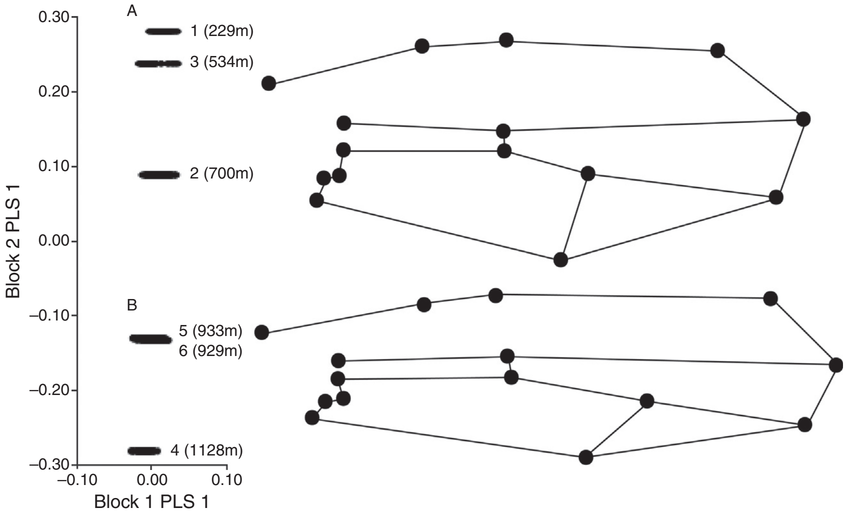

**Fig. 7.** Comparison between the PLS analysis (with elevation) and CVA (CV1) showing that individuals with a wider wing phenotype occur in populations living at a maximum elevation of 700 m (A) and those with a thinner wing phenotype occur at elevations over 900 m (B). Localities are as follows: 1. Mbaracayú, 2. Fênix, 3. Jundiaí do Sul, 4. Guarapuava, 5. Ponta Grossa and 6. Colombo.

advantage because the reduction of air density at higher elevations can interfere with the aerodynamic forces of insect wings (Dudley, 2000). For populations at lower elevations, the characteristic broad-wing phenotype can lead to an increased flight energy cost (Ayala et al., 2011). However, the organisms can slow down the wing to maintain sustentation flight, which economizes energy (Dillon et al., 2006).

In the aforementioned manner, the morphometric variations that we found in *P. orbitalis* are basically associated with organismal alterations in the face of environmental modifications. Our results showed two basic wing shapes (narrow and broad), which are correlated with elevation, precipitation and temperature and do not scale allometrically with size.

## Conflicts of interest

The authors declare no conflicts of interest.

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