

# Morphometric Variation of the *Aedes albifasciatus* (Diptera: Culicidae) Wings in Three Populations From Different Ecoregions of Argentina

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## Abstract

Shape variability among individuals is important to understand some ecological relationships, since it provides the nexus between the genotype and the environment. Geometric morphometrics based on generalized procrustes analysis was applied on 17 landmarks of the wings of *Aedes albifasciatus* (Macquart 1838) (Diptera: Culicidae) females collected from three ecoregions of Argentina (Delta and islands of the Paraná River, Pampa, and Patagonian steppe). This methodology was used to discriminate the shapes of individuals belonging to different regions. The population of the Patagonian steppe, which was the most geographically distant, showed the most dissimilar shape. Different local variations in wing shape could have been selected according to the environmental characteristics and maintained by geographic isolation. The individuals of the two ecoregions closest to each other (Delta and islands of the Paraná River and Pampa) showed differences in shape that can be explained by a lower gene flow due to the effect of geographic isolation (by the Paraná River) and the limited dispersive capacity of *Ae. albifasciatus*. The results allow concluding that both environmental diversity and geographic barriers could contribute to local variations in wing shape.

**Key words:** environment, mosquitoes, population, shape, variation

Intra-specific variability is common in many species of insects, particularly in mosquitoes (Vinogradova 2003). Shape variability among individuals is important to understand some ecological relationships, since it is one of the most conspicuous aspects of the phenotype that provides the nexus between the genotype and the environment (Kuclu et al. 2011). Therefore, population variations in the wing shape may be related to adaptive responses to the environment (Gilchrist et al. 2000).

The floodwater mosquito *Aedes* (*Ochlerotatus*) *albifasciatus*, vector of western equine encephalitis (Avilés et al. 1992), has a wide distribution within the southern cone of South America, including different ecoregions with diverse climates and microenvironments (Garzón et al. 2013). Molecular studies have shown that variations in genetic distances correlate positively with geographic distances, as observed for the central region of Argentina, where some authors have suggested a restriction in gene flow between the breeding areas of local populations (De Sousa et al. 1999).

Geometric morphometric is a complement to molecular methods since genetic variability often results in morphological variations that allow distinguishing specimens from different regions (Bradshaw

et al. 2000, Stacey and Fellowes 2002). Comparative studies in geometric morphometrics that use as reference the configurations of the wing veins and their intersections have allowed detecting population structure as well as differentiating evolutionary units at intraspecific level and divergence genetics in local populations (De la Riva et al. 2001). Geometric morphometric techniques have been successfully complemented with molecular studies and have demonstrated comparable results (Ancca et al. 2008), suggesting that they are a robust, fast, and economic phylogeographic analysis tool.

In this work, we evaluated three populations of *Aedes albifasciatus* (Macquart 1838) (Diptera: Culicidae) by means of geometric morphometrics, under the hypothesis that morphology (wing shape) to vary according to the different environmental conditions.

## Materials and Methods

### Study Sites

Adult female mosquitoes were collected from three sites, each representing one ecoregion of Argentina (Burkart et al. 1999): 1) Campana

insular (34°14'S, 58°53'W), from the *Delta and islands of the Paraná River ecoregion*; 2) Buenos Aires (34°36'S, 58°22'W), from the *Pampa ecoregion*, and 3) Sarmiento (45°36'S, 69°5'W), from the *Patagonian steppe ecoregion* (Fig. 1). In each ecoregion, the number of collect sites was grouped to achieve an adequate sample size considering the environmental representativeness. In Delta and islands of the Paraná River ecoregion, female mosquitoes were captured in different points of one island, covering an area of approximately 200 ha. In Pampa ecoregion, the catch was carried out in three sites (distantly from each other about 25 to 300 km) that included rural areas (70 ha), peri-urban areas (60 ha), and urban parks (40 ha), covering an area of approximately 170 ha. In Patagonian steppe ecoregion, females were captured at different points (farms) covering an area of approximately 400 ha in the rural area of Sarmiento Valley.

The Pampa ecoregion covers 44.25 million ha, 62.9% of which is an anthropically modified landscape. The climate is humid to sub-humid, with warm summers. The annual rainfall ranges from 600 mm in the southwest to 1,100 mm in the northeast. Average annual temperatures range from 15°C in the south to 18°C in the north. The humid climate (79 to 63% RH) has favored the development of soils with high contents of organic matter and nutrients, with excellent agricultural aptitude. The temperate grassland is the original vegetation of this region (Burkart et al. 1999).

The Patagonian steppe ecoregion extends over 56.5 million ha and is characterized by a cold and dry climate with an annual rainfall average lower than 250 mm (semi-desert). The area is characterized by strong winds from the west, rains or snowfall during the winter, dry summers, and frost most of the year. Average annual temperatures are in the range of 10–14°C (Burkart et al. 1999). The Patagonian valley of Sarmiento represents a humid zone within a desert matrix and it is characterized by patchy heterogeneity within an agricultural landscape. The landscape generates a mosaic of wetland areas (with natural hydrophilic herbaceous vegetation) and steppe areas (where xerophilous vegetation dominates over the shrub and sub-shrub species).

The ecoregion of the Delta and islands of the Paraná River comprises a surface area of approximately 5.7 million ha, represented

by a landscape of low and floodable islands. The climate is temperate with a mean temperature of 16.7°C and annual rainfall of 1,073 mm. Lunar tides and wind patterns affect the hydrologic regime (Kandus et al. 1999). The presence of either quiet or moving permanent bodies of water generates high local humidity and the tempering of the daily thermal extremes. There are forests and shrubs on the edges, and pastures and grasslands in the interior of the islands (Burkart et al. 1999).

### Mosquito Samples and Data Collection

In the field, female mosquito individuals that landed on the clothing of the authors of this work were captured using a mechanical vacuum cleaner during the favorable season (summer of 2011–2012). The captured individuals were killed under a temperature of –12°C (freezer) for 30 min. A total of 112 individuals were analyzed: 51 from Buenos Aires, 18 from Campana insular, and 43 from Sarmiento.

The left wing of each individual was extracted and placed between microscope slides and photographed with a camera (Leica DFC 295, Solms, Germany) coupled to a stereoscopic microscope (Leica S8 APO, Solms, Germany). Once the wing digital image was obtained, 17 reference points or landmarks (LMs) (Fig. 1) were selected and two-dimensional Cartesian coordinates were generated with the tps-DIG 2.16 software. The LM configurations obtained were translated, rotated, and scaled according to the generalized Procrustes algorithm, using the MorphoJ software 1.05 (Klingenberg 2011). In this way, the Procrustes coordinates were generated and used as shape variables.

The centroid size (CS) was used as an isometric size estimator. It is defined as the square root of the sum of the square of the distances between the center of the configuration of the LM (or centroid) and each LM (Bookstein 1991)

### Statistical Analysis

For the analysis, we focused on the wing geometry shape, because from an evolution point of view is more informative and less influenced by the environment than size (Dujardin 2008). Furthermore,

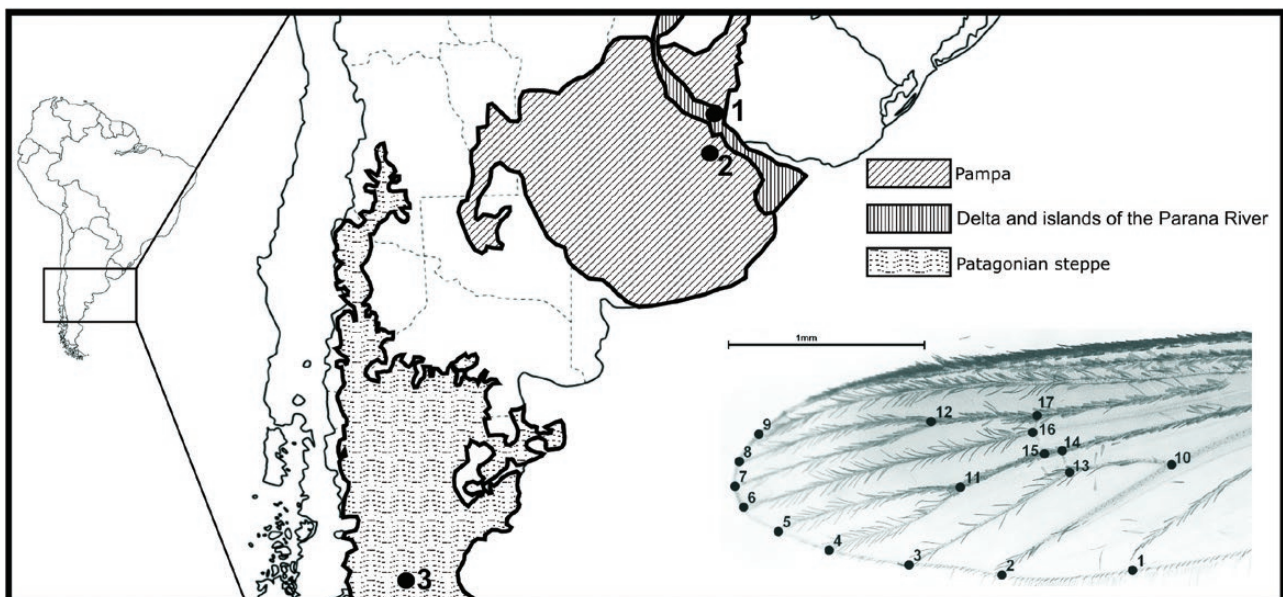


Fig. 1. Sites where the specimens of *Aedes albifasciatus* were collected: (1) Campana insular (Paraná River Corridor); (2) Buenos Aires; and (3) Sarmiento. The different ecoregions are indicated with different patterns. In the lower right corner is shown the left wing with the 17 landmarks on the vein intersection.

we have included size analysis to observe the influence of the environment on the size and its relation with the shape. For this, the logarithm of the CS [(Log CS)] was compared between populations with a nonparametric test.

To eliminate allometry (relationship between the size and wing shape), we performed a multivariate regression analysis on pooled samples using CS (geometric size estimator) as independent variable and shape (Procrustes coordinates) as the dependent variable (Zelditch et al. 2004).

After discarding the allometric effect, the shape variables (regression residuals) were summarized in new variables by applying a principal component analysis (PCA). For analysis with statistical inference, a multivariate analysis of variance (MANOVA) was performed to compare between the wing shapes from each ecoregion. To differentiate the wing shape between ecoregions, canonical variate analysis (CVA) and discriminant analysis (DA) by pairs were carried out. DA was used for comparisons of specific groups, while CVA was used for general analysis of group structure in the dataset. In addition, the DA by pairs, in MorphoJ software, automatically included a parametric T-square test for the difference between group means (Klingenberg 2011).

The Procrustes distance was a measure of the distance between shapes. In all cases, we used a significance level of  $\alpha = 0.05$  and the statistical software Infostat.

To visualize the shape changes in the wings between ecoregions, the thin-plate spline (TPS) and strain vectors (Zelditch et al. 2004) were plotted using the software MorphoJ 1.05 (Klingenberg 2011).

## Results

The wing size [Log (CS)] between populations were significantly different (Kruskal Wallis Test:  $df = 2$ ;  $H = 41.71$ ;  $P < 0.001$ ). The largest

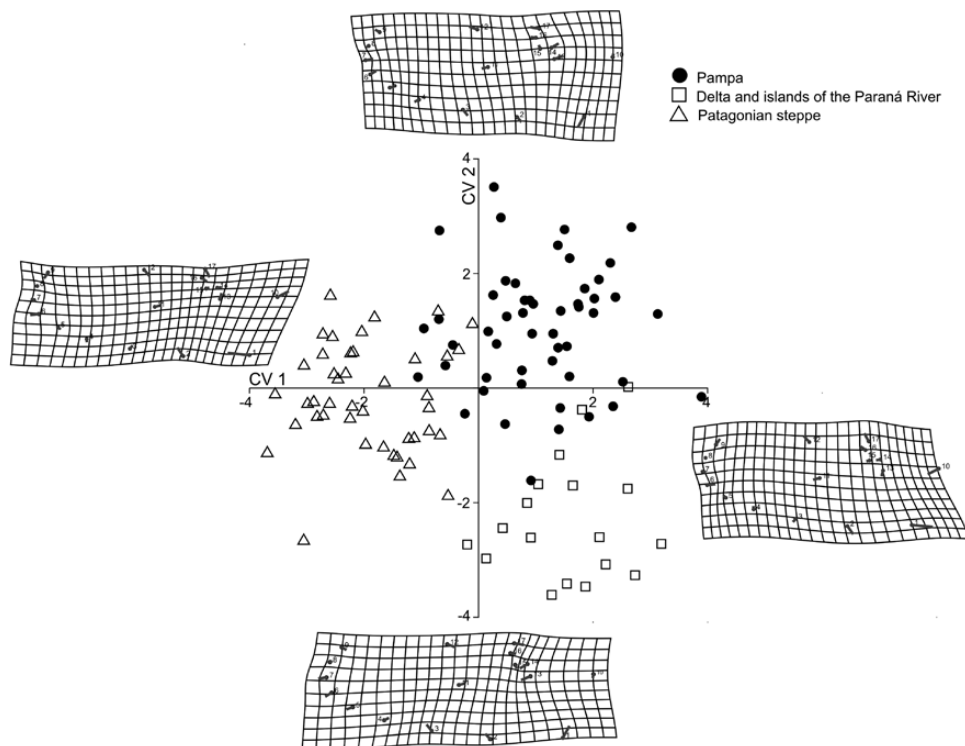
individuals were those of the Campana insular ( $7.27 \pm 0.17$  mm), while the smallest were those of Sarmiento ( $6.95 \pm 0.09$  mm). The mean size for the Buenos Aires individuals was  $7.05 \pm 0.22$  mm.

The allometric effect was 3.01% and the permutation test against the null hypothesis of independence was significant ( $P = 0.0009$ ; randomization rounds = 1,000). Therefore, the regression residuals were used as the shape variables free of the size effect.

The first 10 principal components (eigenvalues greater than 1 according to Kaiser's criterion) accounted for 77% of the total variance that summarized the shape information. The MANOVA indicated that the wing shape of the populations of each ecoregion differed significantly (Pillai's test:  $F [20, 202] = 5.6$ ,  $P < 0.0001$ ; a posteriori Hotelling's test  $P < 0.0001$ ).

In the CVA, the ecoregion populations were discriminated by the wing shapes, with minimal overlap, in the morpho-space formed by the first (60.4%) and second (39.6%) canonical variates (Fig. 2). The Procrustes distances between groups were: 0.0166 for Pampa-Delta, 0.019 for Pampa-Patagonian Steppe and 0.0224 for Delta-Patagonian Steppe. The  $P$ -values from permutation tests (10,000 permutation rounds) for the distances between groups were all significant ( $P < 0.0001$ ). The TPS indicated the relative change in wing shape in the canonical analysis. The LMs for the first axis (CV1) that had greater contribution to the shape change were LMs 1, 2, 6, and 9 on the edge of the wing and LMs 12, 17, 10, and 11 in the center of the wing, whereas those for the second axis (CV2) were LMs 1, 2, 3, 6, and 7 on the edge of the wing and LMs 17, 14, 12, and 13 in the center of the wing (Fig. 2).

The DA showed that the highest classification error was that for the comparison between the Pampa and Delta and islands of the Paraná River ecoregions (28%), if all misclassified individuals among the pairs are summed over the total analyzed,  $(13 + 6) / (51 + 18)$ . This coincided with smaller differences between the shapes



**Fig. 2.** Distribution of the females of *Aedes albifasciatus*, grouped a priori by ecoregions, along the first (CV1:60.4%) and second axis (CV2:39.6%) derived from the CVA of wing shape variables. The TPS and deformation vectors along CV1 and CV2 showed the shape changes associated with the canonical variates.

(Procrustes distances = 0.0166). The DA also showed that the lowest global classification error (16%) was in the Delta and the islands of the Paraná River and Patagonian steppe,  $(5 + 5)/(18 + 43)$ , which coincided with the largest differences between shapes (Procrustes distances = 0.0224) (Table 1).

## Discussion

Geometric morphometrics used in the *Ae. albifasciatus* wings allowed to discriminate individuals from three sites studied with different environmental conditions. This indicates that variation in the wing shape correlates with the population of origin, suggesting possible local adaptations that generate geographical variants.

Despite the wing size differences between populations, possibly due to local rearing conditions (Motoki et al. 2012), this feature did not affect the shape. The absence of allometry associated with geographical variations in wing shape suggests that other factors, regardless of size, could be influencing the wing geometry (Alves et al. 2016).

In the geographic center of Argentina, the genetic structure of *Ae. albifasciatus* and the presence of subpopulations have been studied at distances greater than 25 km (De Sousa et al. 1996). The results of the present study could complement this idea at the morphological level and contribute with a possible approach to the hypothesis of population structure. In *An. darlingi*, ecoregions has represented barriers that limited dispersal and influenced the population structuring (Motoki et al. 2012). Similar structuring results, further supported by molecular studies, have been found in sandflies (Dvorak et al. 2006).

The populations studied were distanced by 1,500 Km (Sarmiento) or geographically isolated by a water basin (Campana insular). Similar differences between wing shapes have been observed in other *Aedes* species, although domiciliary, between five cities within 38,000 Km<sup>2</sup> of surface (Gafur and Ajizah 2008).

In the present study, the wing shapes of specimens from Sarmiento were the most morphologically separated; it could be explained by geographic separation. Studies carried out by Morais et al. (2010) on the wing geometric morphometrics of *Culex pipiens* L. (Diptera: Culicidae) showed differences between Brazilian and Argentinean populations and, in turn, indicated morphological differences between the populations of northern (tropical zone) and southern (subtropical) Brazil, possibly due to geographic barriers. These authors suggested a population structure supported by a low gene flow between areas, which was then genetically confirmed (Morais et al. 2010). In our case, the results observed in population structure should also be complemented by molecular studies. The wing shape may be an indicator of population genetic structure as it is determined by quantitative genetic inheritance (Dujardin 2008).

The shape changes illustrated in the TPS along the canonical axes indicated that LMs 1, 2, and 6 for the edge of the wing and LMs 12 and 17 for the center of the wing (which coincided on both axes) were the most important to differentiate between the wing shapes from the different regions. Most changes would be non-uniform, indicated by local distortions, unlike the uniform changes indicated in the parallelism of the grid. Although venation is genetically and conservatively determined, differences between populations represented in certain LMs may indicate some degree of divergence or genetic variation (Gafur and Ajizah 2008).

The isolation of *Ae. albifasciatus* specimens from Campana insular (Delta and islands of the Paraná River ecoregion) resulted in shape differences, respect to those from Buenos Aires (60 km distance) despite the relative proximity of the two populations. This may be explained by a lower flow of individuals from the mainland to the islands (therefore low genetic flow), due to the effect of the isolation and the intrinsic dispersive capacity of *Ae. albifasciatus* of 500 m with respect to the breeding place (Gleiser and Gorla 1997).

The population structure in insects exists both on a small scale, as in the case of bugs, where morphometric differences between biotopes are observed within the same locality (Schachter-Broide et al. 2004), and in a large scale, as is the case of *Ae. albifasciatus* from relatively more geographically distant locations (De Sousa et al. 1999). This suggests that the morphological heterogeneity could be due to variation ranging from physical isolation to associations between biotic and abiotic factors. Therefore, both environmental diversity and geographic barriers could contribute to possible population fragmentation or structuring of *Ae. albifasciatus*. The variation in wing geometry may result from different ecological and environmental determinants (microclimate, environmental determinants, vegetation structure, and availability of larval habitat) in each ecoregion (Motoki et al. 2012).

The results of our study do not allow us to determine whether the proportion of differentiation is due to genetic differences or due to possible phenotypic plasticity of individuals growing in different environments. Phenotypic plasticity arises from the interaction between environmental variability and the genetically determined development program (Scheiner 1993). In *Ae. albifasciatus*, it could happen that larval rearing conditions influence phenotype (wing morphometry) of the adult. For example, this could be observable in wing size where field rearing conditions (temperature, density, nutrients among others) most probably affected the final size of the adult in each local environment (Clements 1992). Otherwise, wing shape differences among populations could be the result of a plastic response to local environmental conditions (Alves et al. 2016). A common garden experiment could determine whether the size and shape have a genetic component or not (de Villemereuil et al. 2016). Nevertheless, the environmental variables spatially

**Table 1.** Cross-validation resulting from the DA by pairs for the three ecoregions

Groups compared	Misclassification proportion			Procrustes distances	T-square	P-value
	Pampa	Delta and islands of Paraná River	Patagonian steppe			
Pampa-Delta and islands of Paraná River	13/51	6/18		0.0166	173.26	0.0003
Pampa-Patagonian steppe	11/51		7/43	0.0190	243.62	<0.0001
Delta and islands of Paraná River -Patagonian steppe		5/18	5/43	0.0224	341.62	<0.0001

A parametric T-square test for the difference between group means is included, with a significance level of 0.05.

structured, could also lead to structured morphological variation that could be either continuous (such as a cline) or discontinuous (Alves et al. 2016). The shape variation between these three sites could describe the limits of dispersion and patterns of intra-specific variation (Mateus et al. 2013).

The results of this geographical variation study at the morphological level between populations leave questions to be resolved for future molecular genetic studies that complement our findings.

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