Wing geometry in *Triatoma infestans* (Klug) and *T. melanosoma* Martinez, Olmedo & Carcavallo (Hemiptera: Reduviidae)

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**Abstract.** Geometric morphometrics is a novel approach to biological shape analysis, and its application to medical entomology is just beginning. Here, we use it to examine geographical and interspecific variation in the *Triatoma infestans* complex, vectors of Chagas disease in southern Latin America. Using six landmarks defining the membranous part of the hemelytra, we analysed the geographical patterns of variation in several populations of *T. infestans*, including the recently described ‘dark morph’ of *T. infestans* from hollow trees in the Bolivian Chaco. As a potential outgroup, *T. melanosoma* was added to the sample. A consistent geographical differentiation was observed, but no evidence was found in either sex to consider either the dark morph or *T. melanosoma* as a species distinct from *T. infestans*. *Triatoma melanosoma* is relegated to synonymy with *T. infestans* (syn.n.).

**Introduction**

*Triatoma infestans* (Klug) (Hemiptera: Reduviidae: Triatominae) is the main vector of Chagas disease in the Southern Cone countries of Latin America. It has a wide and probably recent geographical distribution (Schofield, 1988; Dujardin et al., 1998a,b), over which it does not show marked morphological changes, except for general size (Dujardin et al., 1998b). There are, however, two chromatic variants. An entirely melanic form from Misiones in northwestern Argentina was originally described as a subspecies, *T. infestans melanosoma* Martinez, Olmedo & Carcavallo, 1987, and subsequently proposed as a species, *T. melanosoma*, mainly on the argument that the black forms are ‘true breeding’ (Lent et al., 1994). Recently, the examination of sensilla pattern, a good indicator of geographical structuring in Triatominae (Catalá & Dujardin, 2001), could not distinguish *T. melanosoma* from *T. infestans* (Catalá & Torres, 2001). A second dark form, recently discovered in the Bolivian Chaco, was described simply as a ‘dark morph’ of *T. infestans* (Noireau et al., 1997, 2000).

To date, molecular studies of different *T. infestans* populations have not revealed consistent genetic heterogeneity (Monteiro et al., 1999; Noireau et al., 2000), supporting the idea that the melanic forms of *T. infestans*, either ‘dark morphs’ or ‘*T. melanosoma*’, are different ecotypes rather than different species (Dujardin et al., 1999b).

This study explored the usefulness of geometric morphometrics in separating specific from geographical differences in wing shape. We expected to see more variation in shape between putative species (*T. infestans* and *T. melanosoma*), and possibly between the ‘dark morph’ and other samples, than among domestic populations of *T. infestans*.

Instead of measurements of distances between landmarks, the geometric method uses Cartesian coordinates of the landmarks. This technique takes into account all information about the spatial relationships between the

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landmarks, which gives the method greater statistical power (Rohlf & Marcus, 1993). By constructing distinct variables for (isometric) size and shape, it allows separate examination of these two metric features, as well as an indirect assessment of residual allometry (Baylac & Daufresne, 1996). We examined six landmarks on the membranous part of the hemelytra in samples of *T. melanosoma* and *T. infestans* coming from different geographical regions. Thus, the size and shape of the wing could be compared separately within and between two taxa, allowing analysis of geographical variation as well as taxonomic divergence.

**Materials and methods**

**Insects**

Four hundred and forty-eight wings were examined: forty-one of *T. melanosoma*, sixty of the dark morph of *T. infestans* and 347 of domestic *T. infestans* (Table 1). The insects originated from four countries of the Southern Cone of Latin America: Argentina, Bolivia, Chile and Paraguay (Fig.1). All *T. melanosoma* specimens, offspring from specimens originally collected in Argentina (Province of Misiones), came from an established insectary colony (Lent et al., 1994). The ‘dark morphs’ of *T. infestans* came from sylvatic ecotopes in Santa Cruz (Bolivia), although twenty-eight were the F1 of a recently established laboratory colony. Most of the 347 remaining wings came from specimens collected directly from domestic habitats in Argentina (La Rioja, seventy-five specimens), Bolivia (Chaco, sixty-four and Cochabamba, fifty-eight), Chile (Region II, Atacama, eighty-three) and Paraguay (Departments of Presidente Hayes and Paraguarí, sixty-seven).

**Collection of landmark data**

Each wing was mounted separately in Hoyer’s medium between microscope slides and examined for six landmarks (Fig. 2). For each individual, one wing was reflected so that both the left and right wings could be used in the subsequent analyses. Insect wings are excellent material for morphometric analyses because they are essentially two dimensional and the wing veins provide many well defined morphological landmarks (Klingenberg & McIntyre, 1998). We did not use all possible landmarks, to maintain a relationship of four times as many individuals as landmarks in the smallest group (Bookstein, 1996).

Camera lucida drawings of the wings were made using a microscope at a magnification that allowed the maintenance of a consistent plane of focus to control distortion. The use of camera lucida drawings is an inexpensive way to collect coordinates. It can be affected by small positional changes of the camera lucida relative to the optical apparatus. Such artefacts only affect size variation, not shape, and may be reduced by carefully verifying the scales of the drawings before each set of measurements.

**Statistical analyses**

Because the total sample of insects represented captures during different years, from 1996 to 1999, we were obliged to assume that the metric properties of *T. infestans* were not affected by variation over this period of time. This seemed a reasonable assumption given that the total time span represents only three to six generations (Gorla & Schofield, 1989). The present results can therefore be assumed to reflect either spatial or specific variation only.

To compare localities, left and right wings were not averaged by individual but were used separately as individual units. This procedure allowed the doubling of sample sizes. For each sex, we examined size and shape variation separately.

Size was computed as centroid size, i.e. the square root of the sum of squared distances from the landmarks to the centroid of the landmarks. In the absence of allometry, this is the only size measure that is uncorrelated with all shape variables (Bookstein, 1991). Its variation among species,

<table>
<thead>
<tr>
<th>Species/sample</th>
<th>Origin</th>
<th>Males</th>
<th>Females</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triatoma melanosoma mel</td>
<td>(Misiones, Argentina)</td>
<td>19</td>
<td>22</td>
<td>1997</td>
</tr>
<tr>
<td>Triatoma infestans</td>
<td>(183)</td>
<td>(224)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dkm</td>
<td>Santa Cruz</td>
<td>25</td>
<td>35</td>
<td>1996</td>
</tr>
<tr>
<td>Arg</td>
<td>Anillaco, La Rioja</td>
<td>41</td>
<td>34</td>
<td>1997</td>
</tr>
<tr>
<td>Cha</td>
<td>Tarija</td>
<td>24</td>
<td>40</td>
<td>1996</td>
</tr>
<tr>
<td>Cha</td>
<td>Cochabamba</td>
<td>28</td>
<td>30</td>
<td>1999</td>
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<td>19</td>
<td>25</td>
<td>1997</td>
</tr>
<tr>
<td>Par</td>
<td>Presidente Hayes</td>
<td>10</td>
<td>13</td>
<td>1997</td>
</tr>
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*Laboratory specimens.
*Hollow tree specimens, mixed with laboratory specimens.
*Domestic and peridomestic specimens, mixed with some laboratory specimens.
Fig. 1. Geographical origin of the *Triatoma infestans* samples used in the study. 1, *Triatoma melanoma*; a laboratory sample (LNIRTT, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil) originally collected from its only known locality in the Province of Misiones, Argentina; 2, Cordillera Province, Department of Santa Cruz, Bolivia; 3, Anilaco, Province of La Rioja, Argentina; 4, Province of Gran Chaco, Department of Tarija, Bolivia; 5, Cercado Province, Department of Cochabamba, Bolivia; 6, Atacama, Chile; 7, Departments of Paraguary and Presidente Hayes, Paraguay.

geographical populations and sex was illustrated by quantile box plots, and the significance of the pairwise comparisons (Student’s *t*-test after a one-way ANOVA) was verified with a Bonferroni correction (*P* = 0.001) (Sokal & Rohlf, 1995). The assumption of equal variances was verified by Bartlett’s test (*P* = 0.34 for males and *P* = 0.53 for females). Separate statistical comparisons were performed for sexual size dimorphism, also applying a Bonferroni correction (at *P* = 0.001), using the nonparametric Wilcoxon test (Wilcoxon, 1945).

Shape variables were obtained by mathematically removing the effects of variation in size, location and orientation using generalized Procrustes analysis (Rohlf & Slice, 1990). All pairs of populations were tested for differences in wing shape using a Bonferroni-corrected non-parametric randomization test (*n* = 1000, *P* = 0.001). A multivariate discriminant analysis was also applied on the same samples after their projection on to a Euclidean tangent space (Rohlf, 1996). A factor map of the first two canonical factors was used to illustrate the main results, where each population was presented as a circle corresponding to the 95% confidence region (Mardia et al., 1979). Mahalanobis distances (Mahalanobis, 1948) were derived from the multivariate discriminant analysis, and the statistical significance of their correlation with geographical distances was tested using the non-parametric Mantel test (Sokal & Rohlf, 1995). Finally, to verify the contribution of size variation in each sex to the total shape heterogeneity, a linear multivariate regression of all shape variables was performed against the corresponding centroid size of wings across all localities. This procedure indirectly quantified the contribution of allometry to shape variation. Calculations were performed using the following packages: TPS (Thin Plate Spline) series of Rohlf (1998a,b,c); MORPHEUS et al. (Slice, 1999); JMP® (SAS Institute, 1997) and STATA (Computing Resource Center, 1992).

Results

Size

A consistent sexual size dimorphism was revealed: female wings were on average larger than male wings. This was clear for each locality (Fig. 3). However, a non-parametric comparison of size between sexes, after Bonferroni correction, indicated significant sexual differences only in populations of *T. infestans* from Argentina, Cochabamba and Chile. For each sex, the smallest wings were those of *T. melanoma* and Paraguayan *T. infestans*. The largest wings were those of Andean *T. infestans* (Cochabamba) and the Argentine population. In most of the twenty-one intergroup comparisons of size, in particular for females, differences were significant (Table 2).
Shape

Table 2 also shows the twenty-one pairwise comparisons of wing shape for each sex. Most of these comparisons revealed significant differences. In both sexes, shape was similar between *T. melanosoma* and *T. infestans* from Cochabamba (see *mel* and *Cba*, Table 2), and between *T. infestans* from Argentina and Paraguay (see *Arg* and *Par*, Table 2). The ‘dark morphs’ of *T. infestans* showed more distinctiveness (differences in size and shape) in males than in females, but the reverse was true for *T. melanosoma* (Table 2).

The first two discriminant factors (CV1 and CV2, see Fig.4) represented 78% of the total variation in males (*P* < 0.001) and 91% of the total variation in females (*P* < 0.001). In females, *T. melanosoma* formed an isolated group on CV1 (Fig.4), whereas the ‘dark morphs’ of *T. infestans* did not depart from the Paraguayan and Argentine populations. In males, *T. melanosoma*, the ‘dark morph’ specimens of *T. infestans* and the Andean *T. infestans* constituted a more-or-less isolated group along the CV1 axis (Fig.4). Within this group, the ‘dark morph’ specimens were distinguished by the CV2 axis.

Both sexes were found to be similar in shape for the following relationships (Fig.4): (1) *T. melanosoma* and *T. infestans* presented shape differences lower than those observed for some geographical populations of *T. infestans*, such as those of Cochabamba and Chaco; (2) *T. melanosoma* was more similar to the Cochabamba sample than the Cochabamba sample was to other domestic populations (see Chaco, Fig.4); (3) two geographical populations, Chile and the Bolivian Chaco, tended to form distinct groups, separated from the remaining populations by the first canonical factor; and (4) the domestic populations of Paraguay and Argentina largely overlapped.

Size and shape

The relationships of overall shape variation with size revealed a significant but low allometric residue: 15% in females (*P* < 0.001) and 24% in males (*P* < 0.001).

Metric divergence and geography

There was no significant correlation between Mahalanobis distances separating groups and the corresponding geographical distances (Mantel test; *P* = 0.30, detailed results not shown).

Discussion

We compared the shape and the size of the fore wings (hemelytra) from geographical samples of both sexes of *T. infestans* and *T. melanosoma*, including a dark form of *T. infestans* known as ‘dark morph’, recently described from Bolivia (Noireau et al., 1997). According to the present study, the ‘dark morphs’ and *T. melanosoma* are both compatible with characterization as chromatic variants of *T. infestans*. The absence of consistent differences between the two taxa is in agreement with previous molecular studies using isoenzymes and mitochondrial DNA sequence variation (Monteiro et al., 1999), randomly amplified polymorphic DNA fragments (Noireau et al., 2000) and

![Fig.3. Sexual size dimorphism of the wings according to locality. The quantile boxes graphically summarize the distribution of points for each sex and locality. Each box shows the group median as a line across the middle and the quartiles (25th and 75th percentiles) at the ends. The 10th and 90th quantiles are shown as lines above and below the box. * Significant sexual difference in size after a Bonferroni correction (see localities inside boxes). Males are the boxes in bold.](image-url)
morphological comparisons based on the analysis of antennal sensilla patterns (Catalá & Torres, 2001). Accordingly, we hereby formally recognize T. melanosoma Martinez, Olmeido & Carcavallo (syn.n.) as a junior synonym of T. infestans (Klug).

Among the T. infestans populations studied, the sexual dimorphism of wing size followed the same trend observed for head and body dimensions (Dujardin et al., 1999a). In each locality, the tendency was for larger wings in females, although this difference was not always significant.

Triatoma ‘melanosoma’ was among the specimens with the smallest wings. Two considerations make this difference unlikely to be attributable to specific traits. First, small size was also apparent for the Paraguayan population of T. infestans (Fig. 3). Second, the reduced size of the T. ‘melanosoma’ wings could be a reflection of the laboratory colony having been reared for several generations, as has been observed for other populations (Dujardin et al., 1999b).

The shape variation among the samples could be attributable, at least in part, to size variation. We verified this using a multivariate regression, which indicated that the contribution of size to shape variation was significant, but low (from 15 to 24%). This means that simple growth variation could not explain very much of the observed shape heterogeneity, suggesting that other factors could be involved, such as specific differences, i.e. evolutionary background or geographical separation, or both.

Again, specific divergence did not appear as a likely cause of shape variation, as allegedly interspecific differences were less than geographical, intraspecific ones. For example, T. infestans from Cochabamba was more similar to T. ‘melanosoma’ than were populations of T. infestans from Cochabamba and Chaco or Chile (Fig. 4). Similar results were obtained based on the sensilla pattern analyses of the antennae (Catalá & Torres, 2001) or randomly amplified polymorphic DNA fragments (Noireau et al., 2000): neither the ‘dark morph’ nor the T. ‘melanosoma’ specimens showed differences with T. infestans higher than currently observed between geographical populations. This was also confirmed by multilocus enzyme electrophoresis and by mitochondrial DNA sequencing (Monteiro et al., 1999). Thus, the metric variation observed here probably does not reflect any specific separation, and is attributable to environmental, intraspecific sources. The latter could include climatic differences, ecological adaptations or the degree of physical separation between populations reflecting past or present gene exchange. Due to a lack of reliable data on temperature and humidity, we could not explore the relationships of morphological variation to environmental parameters, but two of our populations are known to exhibit different ecological adaptations, living in sylvatic ecotopes rather than the more usual domestic habitat of T. infestans. The ‘dark morphs’ are known from birds’ nests and tree holes in the Bolivian Chaco, whereas T. ‘melanosoma’ is generally found in peri-domestic arboreal habitats in the argentine province of Misiones (R. Carcavallo, personal communication). Neither of these populations, however, showed significantly more metric divergence than any of the domestic samples.

Because the population structure of T. infestans has been shown to be compatible with an ‘isolation by distance’ model (Dujardin et al., 1998b), physical separation could be proposed as a key factor in shape differentiation. Under such a hypothesis, we should have found agreement between geographical distances and shape differences. This was not the case in the overall analysis (see nonsignificant Mantel test), but two indirect arguments supporting the idea are suggested by the close resemblance between paraguayan and argentine populations, and by the similarity between populations from Chile and the Bolivian Chaco. One explanation for these similarities might be the history of T. infestans during the last century (Scholfield, 1988; Dujardin et al., 1998a,b). The migration history between Argentina and Paraguay is poorly known, but recent cytogenetic studies highlight the resemblance between T. infestans from Paraguay and northwestern Argentina, suggesting past genetic exchanges between populations in
Fig. 4. Factor maps showing the shape variation in females and males. For each map, CV1 and CV2 are the first and second canonical factors derived from a discriminant analysis of the total shape variation (uniform and non-uniform components). The circles are areas corresponding to the different groups under study, where each individual has a 95% probability of belonging to that group. The percentage contribution of each factor to the total shape variation is shown in parentheses. *mel* = *Triatoma melanosoma* (a laboratory sample); dkm = ‘dark morphs’, Santa Cruz, Bolivia; Arg = Anillaco, Argentina; Cha = Gran Chaco, Tarija, Bolivia; Cba = Cochabamba, Bolivia; Chi = Atacama, Region II, Chile; Par = Paraguay, both departments.

dthese two countries (F. Panzera *et al.*, unpublished data). The same cause, i.e. past gene flow, could account for the shape similarity between the Bolivian Chaco and Chile; between these two regions there is indeed a known history of passive transportation of *T. infestans* (Neghme, 1960).

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