# Ecotope effect in *Triatoma brasiliensis* (Hemiptera: Reduviidae) suggests phenotypic plasticity rather than adaptation

V. S. P. B A T I S T A[1], F. A. F E R N A N D E S[1], P. C O R D E I R O - E S T R E L A[2], O. S A R Q U I S[1] and M. M. L I M A[1]

[1]Laboratório de Eco-Epidemiologia da Doença de Chagas, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil and [2]Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

**Abstract.** *Triatoma brasiliensis* (Hemiptera: Reduviidae) is an important vector of Chagas' disease in both sylvatic and peridomestic ecotopes. Discriminating between these populations of Triatominae has been proposed as a means of investigating re-infestation rates of human dwellings. Geometric morphometrics have been widely applied in the study of Triatominae polymorphisms at species and population levels. This study characterizes morphometric differences between sylvatic and peridomestic populations, as well as between sexes in *T. brasiliensis* specimens from Jaguaruana, Ceará, in northeastern Brazil. No differences in either the shape or size of the cephalic capsule were apparent between sexes or ecotopes. However, the wings showed differentiation in shape and size. Sexual dimorphism was detected, with females presenting significantly higher values and conformations. Size differentiation was also evident, with sylvatic specimens being generally larger than peridomestic examples. These results indicate that differences in the wings of *T. brasiliensis* may be related to the existence of phenotypic plasticity, and variations in size and shape may be associated with different ecotopes, possibly as a result of conditions in each micro-habitat, such as temperature, relative humidity, food supply and density.

**Key words.** Triatominae, Chagas' disease, geometric morphometrics, re-infestation, Ceará.

## Introduction

Since the success of *Triatoma infestans* control in Brazil, *Triatoma brasiliensis brasiliensis* (Neiva, 1911) has been considered one of the most important native triatomine species in the country because it is frequently found colonizing houses in rural areas in the northeastern regions, where it is widespread in the drier areas of the Caatinga biome. In the Jaguaruana municipality, located in the Jaguaribe valley, Ceará State, several studies have demonstrated colonies of *T. brasiliensis* in domestic, peridomestic and sylvatic ecotopes; these colonies are often infected with *Trypanosoma cruzi* (Chagas, 1909) and therefore play an epidemiological role in the maintenance of Chagas' disease in this region (Sarquis *et al.*, 2004, 2006; Lima *et al.*, 2012). This species occupies both natural and artificial ecotopes, thereby maintaining the cycle of *T. cruzi*, carrying the parasite from wild to domestic environments and providing sources for the infestation and re-infestation of human dwellings (Silveira *et al.*, 1984; Dias *et al.*, 2000; Costa *et al.*, 2002; Sarquis *et al.*, 2006). Abundant sylvatic ecotopes are the most important sources of the re-establishment of domestic populations after control interventions (Dias & Diotaiuti, 1998; Ceballos *et al.*, 2011).

The existence of a *T. brasiliensis* species complex was recently proposed based on geography, morphology, ecology and molecular data (Costa *et al.*, 2009). Except for *Triatoma melanica*, all members of the *T. brasiliensis* species complex (*T. b. brasiliensis*, *Triatoma brasiliensis macromelasoma*, *Triatoma juazeirensis*, *Triatoma sherlocki*) are presently able to invade and colonize human dwellings, and maintain rocky
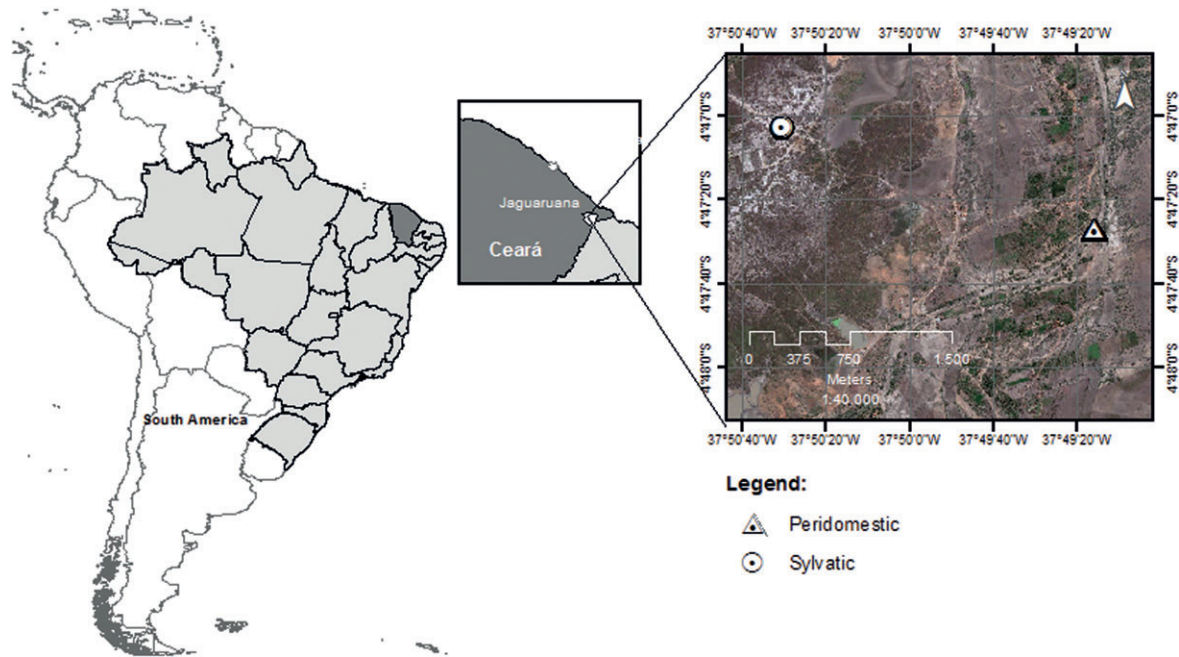
**Fig. 1.** Study area (Figueiredo do Epifânio) indicating the locations of capture (sylvatic and peridomestic ecotopes) of triatomines.

outcrops as their natural habitat (Almeida *et al.*, 2011). Diotaiuti *et al.* (2000) reported a rapid re-infestation of peridomestic ecotopes by *T. brasiliensis*, assumed to be a consequence of the abundance of this triatomine in nearby sylvatic rockpile habitats. Borges *et al.* (2005), using molecular and morphometric techniques, investigated the source of *T. brasiliensis* population infestation in three different ecotopes in Ceará, Brazil and detected the existence of a flow between sylvatic and domestic environments, an interchange for which the peridomestic population was mainly responsible. The establishment of phenotypic differences between Triatominae populations from natural and artificial ecotopes has been suggested as an important way of investigating the basis of human dwelling re-infestation and is useful for the detection and prevention of peridomestic environment re-infestation in endemic regions.

A particularly efficient method of analysing these populations is geometric morphometrics. Wing venation provides a well-defined set of morphological landmarks and is excellent material for morphometric analysis (Gumiel *et al.*, 2003), to study variability at species (Márquez *et al.*, 2011) and population (Ceballos *et al.*, 2011) levels, and for taxonomic research (Villegas *et al.*, 2002; Costa *et al.*, 2009; de la Fuente *et al.*, 2011) and the evaluation of sexual dimorphism (Dujardin *et al.*, 1999). These veins can suggest gene flow (Dujardin *et al.*, 1997a, 1997b) and provide quantitative information that contributes to understanding of the spatial structures of populations in different habitats (Jaramillo *et al.*, 2002; Schachter-Broide *et al.*, 2004; Feliciangeli *et al.*, 2007). Cephalic capsules can also contribute to investigations of changes in infestation (Hernández *et al.*, 2011) and growth in different ecotopes (Riaño *et al.*, 2009).

In order to explore the existence of phenotypic differentiations linked to the domiciliation of the species, this study set out to analyse the wings and cephalic capsules of *T. brasiliensis*. The aim was to characterize the morphological differences between sylvatic and peridomestic populations, accounting for sexual dimorphism, using geometric morphometric techniques, in an epidemiological scenario.

## Materials and methods

### Study area and insects

The triatomines were captured in 2007 in Figueiredo do Epifânio (Fig. 1), a rural area of the Jaguaruana municipality, located in a hot, dry region in the east of the State of Ceará, ~180 km from Fortaleza, the state capital. The vegetation coverage is composed of spinous caducifolius forest and dense arbustive caatinga (Sarquis *et al.*, 2006). A total of 97 *T. brasiliensis* specimens were analysed. Of these, 50 (25 male, 25 female) specimens were captured in the peridomestic environment (04°47′27″ S, 37°49′16″ W) and were collected in animal shelters (pig corrals) on one farm that had been treated with insecticide annually during 2004–2006. The other 47 (24 male, 23 female) sylvatic specimens were captured within a radius of 30 m in a single rocky outcrop (04°47′28″ S, 37°50′30″ W) about 2 km away from the peridomestic area. The specimens were identified according to Lent and Wygodzinsky (1979).

### Geometric morphometrics

Each right wing and each cephalic capsule was cleaned and photographed with a digital camera [Canon EOS Rebel Xti
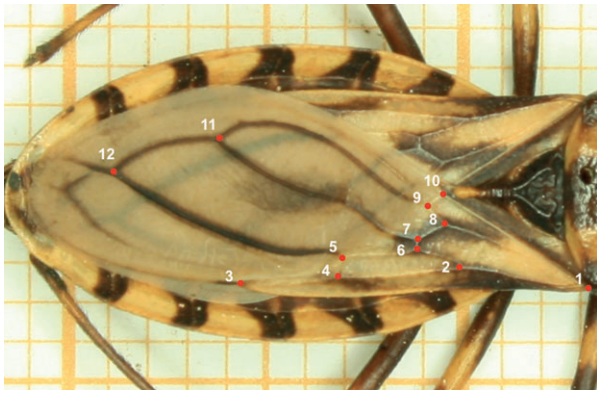
**Fig. 2.** Landmarks on the right wing of *Triatoma brasiliensis*. 1, proximal extreme point of the wing. Corium portion: 2, bifurcation of the radius (R) and media (M) veins. Corium–membrane junction: 3, in subcosta (Sc) vein at the external limit of the wing; 4, in radius vein; 5, in media vein; 6, intersection of media and cubitus (M–Cu); 7, intersection of Cu and M–Cu; 8, intersection of Cu and Cu–postocubitus (Cu–Pcu); 9, intersection of Pcu and Cu–Pcu; 10, intersection of Pcu and Pcu + first anal vein. Membrane portion: 11, intersection of Pcu and Cu; 12, intersection of M vein and extension of Cu–Pcu veins.

(10 mp); Canon, Inc., Tokyo, Japan] without zoom or flash. The camera was positioned 13.5 cm from the photographic plane. Millimetric graph paper was used as a background in each picture to indicate scale and to assist in the alignment of specimens.

We defined a total of 11 type I (juxtaposition of tissue–venation intersections) landmarks and one type II (extreme point) landmark in the wings (Fig. 2) and four type I, six type II (maximum curvature) and two type III landmarks in the cephalic capsules (Fig. 3), according to the nomenclature of Bookstein (1991). The geometric coordinates of each landmark were digitized using tpsDig Version 2.16 (Rohlf, 2010) by the same operator (VSPB).

*Statistical analysis*

Principal components analysis (PCA) was performed based on variance–covariance matrices of the shape variables. Individuals of each ecotope and sex were projected onto the first two components of the PCA (PC1 and PC2) to detect structuring among specimens. To compare overall wing and cephalic capsule sizes between sexes and ecotopes, we used the isometric estimator known as centroid size derived from coordinate data. Centroid size is defined as the square root of the sum of the squared distances between the centre of the landmark configuration and each individual landmark (Bookstein, 1990). Size variations in wings and cephalic capsules among sexes and ecotopes were tested using a two-factor analysis of variance (ANOVA) considering the interaction between factors and significance at 5%. Shape variables (Procrustean superimposition residuals) were obtained with the generalized Procrustean analysis (GPA) superimposition algorithm (Rohlf, 1996). The statistical significance of variations in shape between ecotopes and
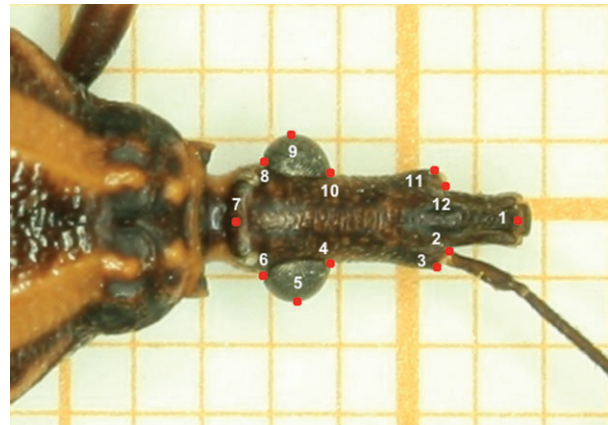


**Fig. 3.** Landmarks on the cephalic capsule of *Triatoma brasiliensis*. 1, median extreme point of the clypeus; 2, proximal point of the right anteniferous tubercle; 3, distal point of the right anteniferous tubercle; 4, intersection of the right eye and the anteocular region; 5, maximum curvature of the right eye; 6, meeting point of the right eye and the postocular region; 7, median point between the ocelli; 8, meeting point of the left eye and the postocular region; 9, maximum curvature of the left eye; 10, meeting point of the left eye and the anteocular region; 11, distal point of the left anteniferous tubercle; 12, proximal point of the left anteniferous tubercle.

sexes was tested using a Procrustean ANOVA with 1000 permutations and multivariate ANOVA (MANOVA) of superimposition residuals, both considering the interaction between factors. To examine the allometric content of shape variables, multivariate regression analysis was performed with shape as the dependent variable and centroid size as the independent variable. All tests were carried out using MorphoJ software (Klingenberg, 2011).

Discriminant function analysis was used to reclassify each individual according to the similarity of the wing and cephalic capsule shapes. A cross-validation reclassification was also performed, in which each classified individual was removed from the total sample when computing the discriminant model.

Finally, to examine whether the cephalic capsule and wing covaried together, we conducted a partial least squares analysis.

**Results**

Principal component analysis showed no structuring of individuals on the main axes of variation for either the cephalic capsule or the wing between sexes or ecotopes (data not shown).

There was no significant difference in the size of the cephalic capsule between sexes ($F = 3.31$, $P = 0.3198$) or between ecotopes ($F = 0.67$, $P = 0.5634$). The wings of the females were significantly larger than those of males and this difference was also significant between ecotopes (Table 1). The wings of sylvatic specimens were larger than those of peridomestic examples. There were statistically significant differences in wing shape between males and females (Table 1), whereby each sex displayed a different conformation of the wings. In terms of global shape differences, male wings were found to be squarer in shape than female wings, which were more elongated (Fig. 4). This difference originates mainly from the

**Table 1.** Procrustean analysis of variance for wing size and shape between sexes and ecotopes and their interaction.

| | Size | | | Shape | | |
|---|---|---|---|---|---|---|
| | MS | $F$-value | $P$-value | MS | $F$-value | $P$-value |
| Ecotope | 3280.840310 | 197.01 | **0.0453** | 0.0002121442 | 5.97 | **<0.0001** |
| Sex | 19076.647068 | 1145.54 | **0.0188** | 0.0000955620 | 2.69 | **0.0161** |
| Ecotope × Sex | 16.652915 | 0.02 | 0.8795 | 0.0000355596 | 0.88 | 0.6182 |
| Residual | 720.742279 | – | – | 0.0000405704 | – | – |

MS, mean squares.
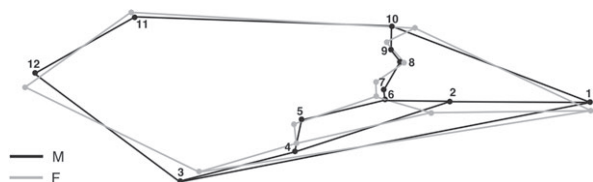Values in bold are significant at $P < 0.05$.



**Fig. 4.** Differences in wing shape between male (M) and female (F) *Triatoma brasiliensis* captured in Jaguaruana, Ceará. Male wings are more square in shape than female wings, which are more elongated.

relative displacement of the corium–membrane junction in the subcosta (Sc) vein at the external limit of the wing (landmark 3) and of the intersection of the media (M) and cubitus (Cu) veins (landmark 12). Localized shape differences showed the intersection of the postocubitus (Pcu) and Pcu + 1A (first analis) (landmark 10) to be positioned very differently in males and to be located posteriorly, which radically changes the venation shape from the triangular form found in females (Fig. 4).

The difference in wing shape between ecotopes was also statistically significant in the Procrustean ANOVA, with no interaction between sex and ecotope (Table 1). Shape differences show a clear shortening only for the membranous portion and widening of the entire wing in peridomestic compared with sylvatic specimens, and a large reduction in the Cu–Pcu (between landmarks 8 and 9) and media (between 2 and 6) veins in peridomestic specimens (Fig. 5).

No significant differences in the shape of the cephalic capsule emerged between sexes ($F = 0.55$, $P = 0.9069$) or between sylvatic and peridomestic specimens ($F = 0.58$, $P = 0.8833$). Multivariate regression of the Procrustean coordinates that represent shape against centroid size was significant ($P = 0.005$), but showed that only 3.1% of individual wing shape distribution can be predicted by size.
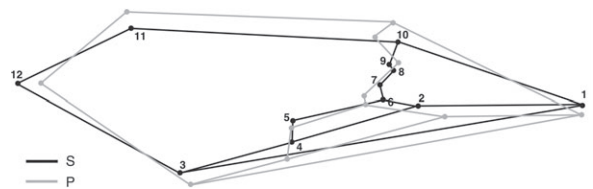


**Fig. 5.** Differences in wing shape between sylvatic (S) and peridomestic (P) individuals of *Triatoma brasiliensis* captured in Jaguaruana, Ceará. Wings of peridomestic insects are shorter and wider than those of sylvatic specimens and show reductions in the cubitus–postocubitus intersection and media veins.

Discriminant function analysis of the wing showed that correct classification percentages between sexes were 81.5% without cross-validation and 66.0% with cross-validation. Correct classification percentages for wings between peridomestic and sylvatic triatomines were 84.5% without cross-validation and 69.0% with cross-validation (Table 2).

Partial least squares analysis between the wing and cephalic capsule was not significant and indicated that these structures do not have a covariance pattern. This indicates that evolutionary forces or environmental effects affect structures independently.

## Discussion

Differentiation between sylvatic and peridomestic Triatominae populations may be epidemiologically important in facilitating the detection of sources of re-infestation after insecticide treatment. Morphometric techniques have been widely used in different morphological structures of several triatomine species (Dujardin *et al.*, 1997a, 1997b; Schachter-Broide *et al.*, 2004; Borges *et al.*, 2005; Dumonteil *et al.*, 2007; Feliciangeli *et al.*, 2007; Hernández *et al.*, 2011). Some studies have sought to identify differentiation among species (Gurgel-Gonçalves *et al.*, 2008, 2011; Campos *et al.*, 2011; de la Fuente *et al.*, 2011), but the current study examined differences at a microevolutionary scale. The significance of morphometric differences in Triatominae and their epidemiological consequences for vector surveillance have been widely debated. The use of morphometric characters in vector surveillance depends on the evolutionary forces that shape their evolution. From a microevolutionary perspective, the amount of gene flow must be quantified using neutral genetic markers. Gene flow and population size allow for the estimation of the time required to observe phenotypic differences if the character is selectively neutral. Under strong natural selection, characters can evolve much more rapidly, typically within one generation. Given this evolutionary framework and literature-based knowledge of evolutionary processes acting on phenotypic characters of triatomines, this study aimed specifically to determine morphometric variation and its possible origin.

### Shape and size variations in natural populations of triatomines

The present study shows that wings and the cephalic capsule exhibit different patterns of sexual dimorphism and ecotope

**Table 2.** Percentages of correct classification from discriminant analysis and cross-validation for wings of female, male, peridomestic and sylvatic individuals of *Triatoma brasiliensis* from Jaguaruana, Ceará, Brazil.

| | Ecotope | | | Sex | | |
|---|---|---|---|---|---|---|
| | Peridomestic | Sylvatic | Total | Female | Male | Total |
| Discriminant function | 82.0% | 80.9% | 81.5% | 79.2% | 89.8% | 84.5% |
| Cross-validation | 68.0% | 63.8% | 66.0% | 66.7% | 71.4% | 69.0% |

variation. Cephalic capsules evidence no variation in size or shape between sexes or ecotopes (sylvatic/peridomiciliar). Conversely, wing shape and size show sexual dimorphism and ecotope differences (wings are larger in females and sylvatic specimens). No study has effectively tested for sexual dimorphism and ecotope differences in both structures using geometric morphometrics. Thus, a comparison of results can only be partial. For the cephalic capsule, our results contradict those of Borges *et al*. (2000, 2005), which indicate the presence of sexual dimorphism in size and difference between ecotopes. Both works analysed *T. brasiliensis* populations. Studies in other species indicate contradicting results for *T. infestans* (Dujardin *et al*., 1997a, 1997b), claiming ecotope differences in the cephalic capsule, and congruent results for *Panstrongylus geniculatus* (Hemiptera: Reduviidae) (Aldana *et al*., 2011) because of the absence of sexual dimorphism in this structure.

The picture emerging for wing patterns is consistent because other studies have found sexual dimorphism in wing size (Schachter-Broide *et al*., 2004; Aldana *et al*., 2011) and ecotope-associated differences in wing size and shape (Dujardin *et al*., 1997a; Schachter-Broide *et al*., 2004, 2009; Dumonteil *et al*., 2007). However, *T. brasiliensis* has never been adopted as a model species and never tested for sexual dimorphism of shape. From this summary, wing patterns seem to be much more consistent than cephalic capsules and we propose that further studies should analyse both structures and consistently test sexual dimorphism in both size and shape, as well as the effect of ecotope.

Many studies have tried to explain the origin of morphometric disparities among triatomines collected in different ecotopes. Some have argued that these disparities may indicate adaptation to a particular ecotope (Dujardin *et al*., 1997a, 1997b) or, more generally, that these differences are genetically based (Schachter-Broide *et al*., 2009). Experimental evidence has demonstrated that colony individuals are smaller than parental sylvatics (Jaramillo *et al*., 2002; Gurgel-Gonçalves *et al*., 2011) and that size differences can arise as a result of density and feeding frequency (Riaño *et al*., 2009). Thus, in both cases, morphometric differences cannot arise from genetic differences because such differences between parental sylvatics and descendants in laboratory colonies are probably minimal (Jaramillo *et al*., 2002; Gurgel-Gonçalves *et al*., 2011) or occur between inbred lines (Riaño *et al*., 2009). Furthermore, in order for genetically based morphometric differences to arise within populations, specific demographic or selective conditions would have to be met. Either gene flow between subpopulations (domestic/peridomestic and sylvatic) must diminish or intense selection must act on these characters. In *T. brasiliensis*, empirical observations (Diotaiuti *et al*.,

2000) and neutral genetic markers (Borges *et al*., 2005) have quantified substantial gene flow between sylvatic and peridomestic/domestic habitats.

Gene flow between ecotopes is also pervasive in other species, including *Triatoma dimidiata* (Dumonteil *et al*., 2007), *Triatoma vitticeps* (Souza *et al*., 2008), *T. infestans* (Marcet *et al*., 2008) and *Triatoma pseudomaculata* (Carvalho-Costa *et al*., 2010). The selective hypothesis implies that certain shapes increase fitness enough to be associated with specific ecotopes within one or two generations, but few studies have evaluated this conjecture. Only Payet *et al*. (2009) have shown that domestic *T. dimidiata* has a higher fecundity than its sylvatic counterpart. If selection is strong, colonies should retain sylvatic phenotypes, which is not the case. Based on these observations, it is most likely that the repeated findings of morphometric differences between sylvatic and domestic/peridomestic specimens represent instances of phenotypic plasticity.

Phenotypic plasticity can be defined generally as the property of a genotype to produce different phenotypes depending upon environmental variation [see Debat & David (2001) for further detail]. At the population level, we assume that individuals that develop in domestic/peridomestic or sylvatic habitats will produce different phenotypes. For example, phenotypic plasticity of wing shape has been described in heliconius butterflies (Jorge *et al*., 2011). No study has yet tested these hypotheses for shape variables in triatomines. Whether this is an adaptive property of triatomines (see Almeida *et al*., 2011) or whether a gene × environment interaction occurs (see Debat *et al*., 2009) should be further investigated using an appropriate experimental design.

In the present study, as well as in Dujardin *et al*. (1999), Schachter-Broide *et al*. (2004) and Rodríguez *et al*. (2007), females were found to have larger wings than males. Wing sexual dimorphism is likely to be a characteristic associated with dispersal. That female *T. infestans* are more likely to disperse than males (Gurevitz *et al*., 2006; Minoli *et al*., 2006) and that the flight muscle is more frequently absent in males (Gurevitz *et al*., 2007) seem to support the idea that flight is more important to females and thus females may be more prone to selection. Finally, sexual dimorphism had no interaction with ecotope variation, which implies that sexes can be pooled (thus avoiding multiple testing).

### *Functional and epidemiologic significance of form differences*

The wings of triatomine specimens captured in peridomestic habitats in Jaguaruana were smaller than those of sylvatic

examples. The same pattern has been observed in other triatomines (Dujardin *et al.*, 1999; Jaramillo *et al.*, 2002), which suggests that the ecotope may be responsible for these differences, and the availability of food may be related to ontogenesis in the domestic and sylvatic environments. Whereas larger specimens apparently fare better in sylvatic conditions as a result of their greater capacity to survive temporary food shortages, smaller individuals survive better under laboratory or domestic conditions, in which food availability is less restricted (Dujardin *et al.*, 1997a, 1997b; Jaramillo *et al.*, 2002; Schachter-Broide *et al.*, 2004; Rodríguez *et al.*, 2007).

Large bloodmeals in the fifth-instar stage can lead to distension of the abdomen, causing the insect to secrete ecdysteroids, which are responsible for the cessation of growth and result in premature metamorphosis and smaller adults (Nijhout, 2003; Schachter-Broide *et al.*, 2009). Therefore, the greater availability of food may influence the emergence of smaller individuals, as witnessed in the peridomestic population.

Geographical variation in phenotypes was detected for *Triatoma melanosoma*, *T. infestans* and *Rhodnius ecuadoriensis* (Hemiptera: Reduviidae) (Gumiel *et al.*, 2003; Schachter-Broide *et al.*, 2004; Villacís *et al.*, 2010). In another example, Almeida *et al.* (2011) tested the flight potential of two species and laboratory hybrids and confirmed that longer wings may enable more efficient dispersion. This interspecific differentiation, which was also observed at the intraspecific level in the wing shape of *T. brasiliensis* populations in Jaguaruana, may have an analogous effect on flight ability. The shortening and widening of wings in peridomestic specimens may affect dispersion, resulting in a lower frequency of flight, as we expected in peridomestic populations.

Schofield *et al.* (1992) reported that *T. infestans* is able to fly further than 500 m and Schweigmann *et al.* (1988) reported that in the field, this species can achieve flights of 1500 m. This range was verified between the ecotopes analysed in the present study in Jaguaruana, which found that small distances could be covered by generations. Despite this argument, we cannot conclude that the re-infestation of this ecotope occurred as a result of the recent immigration of sylvatic insects.

In the Jaguaruana municipality, some areas have always been infested by *T. brasiliensis*, which has almost always carried high rates of *T. cruzi* infection (Sarquis *et al.*, 2006). Despite a control strategy that includes the periodic insecticide spraying of all domiciliary units in which the presence of this insect has been confirmed, these vectors are still found frequently, mainly in peridomestic areas (Sarquis *et al.*, 2004, 2006; Gonçalves *et al.*, 2009; Lima *et al.*, 2012). Control trials with insecticides against domestic and peridomestic populations suggest that *T. brasiliensis* specimens from neighbouring sylvatic populations have a great capacity to repopulate treated habitats, making their elimination more complex (Diotaiuti *et al.*, 2000).

Vector control has substantially reduced the incidence of Chagas' disease, particularly since the eradication of *T. infestans* in Brazil (Ferreira *et al.*, 2006). However, transmission by triatomines that re-infest domestic spaces persists, principally by *T. brasiliensis*, which is currently considered the most important vector in semi-arid areas of northeastern Brazil (Dias *et al.*, 2000). An important epidemiological challenge in controlling Triatominae concerns the identification of the origin of insects that re-infest treated areas, especially when re-infestation occurs during the first 2 years following insecticide application and in the absence of insecticide resistance (Dujardin *et al.*, 2007). Thus, entomological surveillance is critical to achieve longterm interruption of transmission. Insecticides have been sprayed in the peridomestic areas referred to in the present study annually since 2004. Because we detected phenotypic differentiation among individuals in both environments analysed, we suggest that peridomestic re-infestation may reflect the inefficient application of insecticide in this ecotope.

Although Diotaiuti *et al.* (2000) refuted this conjecture, they found peridomestic triatomines of all development stages just 4 months after spraying in the municipality of Independência, also in Ceará, and discovered that 9.7% of houses contained triatomines, which suggested that the insects had migrated from neighbouring localities. This exemplifies the suggestion that re-colonization probably results from a few insects that remain hidden or come from adjacent areas, and that if these insects have access to shelter and food sources, their survival, even after insecticide spraying, can be assumed.

Without effective surveillance and elimination of vectors in domestic and peridomestic environments, not only will insect migration represent a considerable risk factor, but the maintenance of infestations by residual individuals after insecticide spraying will remain of great epidemiological importance.

## Acknowledgements

## References

Aldana, E., Heredia-Coronado, E., Avendaño-Rangel, F. *et al.* (2011) Análisis morfométrico de *Panstrongylus geniculatus* de la ciudad de Caracas, Venezuela. *Biomédica*, **31**, 108–117.

Almeida, C.E., Oliveira, H.L., Correia, N. *et al.* (2011) Dispersion capacity of *Triatoma sherlocki*, *Triatoma juazeirensis* and laboratory-bred hybrids. *Acta Tropica*, **122**, 71–79.

Bookstein, F.L. (1990) Introduction to methods for landmark data. *Proceedings, Michigan Morphometrics Workshop* (ed. by F.J. Rohlf & F.L. Bookstein), Special Publication No. 2, pp. 216–225. University of Michigan Museum of Zoology, Ann Arbor, MI.

Bookstein, F.L. (1991) *Morphometric Tools for Landmarks Data: Geometry and Biology*. Cambridge University Press, New York, NY.

Borges, E.C., Dujardin, J.P., Schofield, C.J., Romanha, A.J. & Diotaiuti, L. (2000) Genetic variability of *Triatoma brasiliensis* (Hemiptera: Reduviidae) populations. *Journal of Medical Entomology*, **37**, 872–877.

Borges, E.C., Dujardin, J.P., Schofield, C.J., Romanha, A.J. & Diotaiuti, L. (2005) Dynamics between sylvatic, peridomestic and domestic populations of *Triatoma brasiliensis* (Hemiptera: Reduviidae) in Ceará State, northeastern Brazil. *Acta Tropica*, **93**, 119–126.

Campos, R., Botto-Mahan, C., Coronado, X., Jaramillo, N., Panzera, F. & Solari, A. (2011) Wing shape differentiation of *Mepraia* species (Hemiptera: Reduviidae). *Infection, Genetics and Evolution*, **11**, 329–333.

Carvalho-Costa, F.A., dos Santos, S.M., Pires, M.Q., Lopes, C.M., Noireau, F. & Pacheco, R.S. (2010) Sylvatic and peridomestic populations of *Triatoma pseudomaculata* are not significantly structured by habitat, as revealed by two genetic markers. *Journal of Vector Ecology*, **35**, 295–300.

Ceballos, L.A., Piccinali, R.V., Marcet, P.L. *et al.* (2011) Hidden sylvatic foci of the main vector of Chagas' disease *Triatoma infestans*: threats to the vector elimination campaign? *PLoS Neglected Tropical Diseases*, **5**, e1365.

Costa, J., Peterson, A.T. & Beard, C.B. (2002) Ecologic niche modelling and differentiation of populations of *Triatoma brasiliensis* Neiva 1911, the most important Chagas' disease vector in northeastern Brazil (Hemiptera, Reduviidae, Triatominae). *Journal of Tropical Medicine Hygiene*, **67**, 516–520.

Costa, J., Peterson, A.T. & Dujardin, J.P. (2009) Morphological evidence suggests homoploid hybridization as a possible mode of speciation in the Triatominae (Hemiptera: Heteroptera: Reduviidae). *Infection and Genetic Evolution*, **9**, 263–270.

Debat, V. & David, P. (2001) Mapping phenotypes: canalization, plasticity and developmental stability. *Trends in Ecology and Evolution*, **16**, 555–561.

Debat, V., Debelle, A. & Dowrkin, I. (2009) Plasticity, canalization and developmental stability of the *Drosophila* wing: joint effects of mutations and developmental temperature. *Evolution*, **63**, 2864–2876.

Dias, J.C.P. & Diotaiuti, L. (1998) Vectores secudários de la enfermedad de Chagas en el Brasil y perspectivas para su control. *Control de Tripanosomiasis Americana y Leishmaniosis: Aspectos Biológicos, Genéticos y Moleculares* (ed. by F. Guhl & C.A. Jaramillo), pp. 154–159. Universidad de los Andes, Santafé de Bogotá.

Dias, J.C.P., Machado, E.M.M., Fernandes, A.L. & Vinhaes, M. (2000) General situation and perspectives of Chagas' disease in northeastern region, Brazil. *Cadernos de Saude Publica*, **16**, 13–34.

Diotaiuti, L., Faria Filho, O.F., Carneiro, F.C.F., Dias, J.C.P., Pires, H.H.R. & Schofield, C.J. (2000) Operational aspects of *Triatoma brasiliensis* control. *Cadernos de Saude Publica*, **16**, 61–68.

Dujardin, J.P., Bermudez, H., Casini, C., Schofield, C.J. & Tibayrenc, M. (1997a) Metric differences between sylvatic and domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. *Journal of Medical Entomology*, **34**, 544–551.

Dujardin, J.P., Bermudez, H. & Schofield, C.J. (1997b) The use of morphometrics in entomological surveillance of sylvatic foci of *Triatoma infestans* in Bolivia. *Acta Tropica*, **66**, 145–153.

Dujardin, J.P., Steindel, M., Chavez, T., Machane, M. & Schofield, C.J. (1999) Changes in the sexual dimorphism of Triatominae in the transition from natural to artificial habitats. *Memórias do Instituto Oswaldo Cruz*, **94**, 565–569.

Dujardin, J.P., Beard, C.B. & Ryckman, R. (2007) The relevance of wing geometry in entomological surveillance of Triatominae, vectors of Chagas' disease. *Infection, Genetics and Evolution*, **7**, 161–167.

Dumonteil, E., Tripet, F., Ramirez-Sierra, M.J., Payet, V., Lanzara, G. & Menu, F. (2007) Assessment of *Triatoma dimidiata* dispersal in the Yucatan Peninsula of Mexico by morphometry and microsatellite markers. *Tropical Medicine and Hygiene*, **76**, 930–937.

Feliciangeli, M.D., Sanchez-Martin, M., Marrero, R., Davies, C. & Jujardin, J.P. (2007) Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). *Acta Tropica*, **101**, 169–177.

Ferreira, I.L.M. & Silva, T.P.T. (2006) Transmission elimination of Chagas' disease by *Triatoma infestans* in Brazil: an historical fact. *Revista da Sociedade Brasileira de Medicina Tropical*, **39**, 507–509.

de la Fuente, A.L.C., Jaramillo, N., Barata, J.M.S., Noireau, F. & Diotaiuti, L. (2011) Misidentification of two Brazilian triatomes, *Triatoma arthurneivai* and *Triatoma wygodzinskyi*, revealed by geometric morphometrics. *Medical and Veterinary Entomology*, **25**, 178–183.

Gonçalves, T.C.M., Freitas, A.L.C. & Freitas, S.P.C. (2009) Surveillance of Chagas' disease vectors in municipalities of the State of Ceará, Brazil. *Memórias do Instituto Oswaldo Cruz*, **104**, 1159–1164.

Gumiel, M., Catala, S., Noireau, F., Rojas de Arias, A., Garcí, A. & Dujardin, J.P. (2003) Wing geometry in *Triatoma infestans* (Klug) and *T. melanosoma* Martinez, Olmedo & Carcavallo (Hemiptera: Reduviidae). *Systematic Entomology*, **28**, 173–179.

Gurevitz, J.M., Ceballos, L.A., Kitron, U. & Gürtler, R.E. (2006) Flight initiation of *Triatoma infestans* (Hemiptera: Reduviidae) under natural climatic conditions. *Journal of Medical Entomology*, **43**, 143–150.

Gurevitz, J.M., Kitron, U. & Gürtler, R.E. (2007) Flight muscle dimorphism and heterogeneity in flight initiation of field-collected *Triatoma infestans* (Hemiptera: Reduviidae). *Journal of Medical Entomology*, **44**, 186–191.

Gurgel-Gonçalves, R., Abad-Franch, F., Ferreira, J.B.C., Santana, D.B. & Cuba, C.A. (2008) Is *Rhodnius prolixus* (Triatominae) invading houses in central Brazil? *Acta Tropica*, **107**, 90–98.

Gurgel-Gonçalves, R., Ferreira, J.B.C., Rosa, A.F., Bar, M.E. & Galvão, C. (2011) Geometric morphometrics and ecological niche modelling for delimitation of near-sibling triatomine species. *Medical and Veterinary Entomology*, **25**, 84–93.

Hernández, M.L., Abrahan, L.B., Dujardin, J.P., Gorla, D.E. & Catalá, S.S. (2011) Phenotypic variability and population structure of peridomestic *Triatoma infestans* in rural areas of the arid Chaco (western Argentina): spatial influence of macro- and microhabitats. *Vector-Borne and Zoonotic Diseases*, **11**, 503–513.

Jaramillo, N., Castillo, D. & Wolff, M. (2002) Geometric morphometric differences between *Panstrongylus geniculatus* from field and laboratory. *Memórias do Instituto Oswaldo Cruz*, **97**, 667–67.

Jorge, R.L., Cordeiro-Estrela, P., Klaczko, L.B., Moreira, G.R.P. & Freitas, A.V.L. (2011) Host-plant dependent wing phenotypic variation in the neotropical butterfly *Heliconius erato*. *Biological Journal of the Linnean Society*, **102**, 765–774.

Klingenberg, C.P. (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resource*, **11**, 353–357.

Lent, H. & Wygodzinsky, P. (1979) Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas' disease. *Bulletin of the American Museum of Natural History*, **163**, 125–520.

Lima, M.M., Sarquis, O., Oliveira, T.G. *et al.* (2012) Investigation of Chagas' disease in four periurban areas in northeastern Brazil: epidemiologic survey in man, vectors, non-human hosts and reservoirs. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **106**, 143–149.

Marcet, P.L., Mora, M.S., Cutrera, A.P., Jones, L., Gürtler, R.E., Kitron, U. & Dotson, E.M. (2008) Genetic structure of *Triatoma infestans* populations in rural communities of Santiago del Estero, northern Argentina. *Infection, Genetics and Evolution*, **8**, 835–846.

Márquez, E., Jaramillo, N., Gómez-Palacio, A. & Dujardin, J.P. (2011) Morphometric and molecular differentiation of a *Rhodnius robustus* like form from *R. robustus* Larousse, 1927 and *R. prolixus* Stal, 1859 (Hemiptera, Reduviidae). *Acta Tropica*, **120**, 103–109.

Minoli, S.A. & Lazzari, C.R. (2006) Take-off activity and orientation of triatomines (Heteroptera: Reduviidae) in relation to the presence of artificial lights. *Acta Tropica*, **97**, 324–330.

Nijhout, H.F. (2003) The control of body size in insects. *Developmental Biology*, **261**, 1–9.

Payet, V., Ramirez-Sierra, M.J., Rabinovich, J., Menu, F. & Dumonteil, E. (2009) Variations in sex ratio, feeding and fecundity of *Triatoma dimidiata* (Hemiptera: Reduviidae) among habitats in the Yucatan Peninsula, Mexico. *Vector-Borne and Zoonotic Diseases*, **9**, 243–251.

Riaño, H.C., Jaramillo, N. & Dujardin, J.P. (2009) Growth changes in *Rhodnius pallescens* under simulated domestic and sylvatic conditions. *Infection, Genetics and Evolution*, **9**, 162–168.

Rodríguez, J.R., Gonzálelez, O.F., Nodarse, J.F., Fidalgo, L.M. & Dujardin, J.P. (2007) Morphometric changes of *Triatoma flavida* Neiva, 1911 (Hemiptera: Triatominae) in the transition from sylvatic to laboratory conditions. *Revista do Instituto de Medicina Tropical de São Paulo*, **49**, 127–130.

Rohlf, F.J. (1996) Morphometric spaces, shape components and the effects of linear transformations. *Advances in Morphometrics*, *NATO ASI, Series A, Life Sciences* (ed. by L.F. Marcus, M. Corti, A. Loy, G.J.P. Naylor & D. Slice), pp. 117–129. Plenum Publishing, New York, NY.

Rohlf, F.J. (2010) *TpsDig, Digitize Landmarks and Outlines, Version 2.16.* Department of Ecology and Evolution, State University of New York at Stony Brook, New York, NY.

Sarquis, O., Borges-Pereira, J., Mac Cord, J.R., Gomes, T.F., Cabello, P.H. & Lima, M.M. (2004) Epidemiology of Chagas' disease in Jaguaruana, Ceará, Brazil: I. Presence of triatomines and index of *Trypanosoma cruzi* infection in four localities of a rural area. *Memórias do Instituto Oswaldo Cruz*, **99**, 263–270.

Sarquis, O., Sposina, R., Oliveira, T.G., Mac Cord, J.R., Cabello, P.H., Borges-Pereira, J. & Lima, M.M. (2006) Aspects of peridomiciliary ecotopes in rural areas of northeast Brazil associated to triatomine (Hemiptera, Reduviidae) infestation, vectors of Chagas' disease. *Memórias do Instituto Oswaldo Cruz*, **101**, 143–147.

Schachter-Broide, J., Dujardin, J.P., Kitron, U. & Gürtler, R. (2004) Spatial structuring of *Triatoma infestans* (Hemiptera, Reduviidae) populations from northwestern Argentina using wing geometric morphometry. *Journal of Medical Entomology*, **41**, 643–649.

Schachter-Broide, J., Gürtler, R.E., Kitron, U. & Dujardin, J.P. (2009) Temporal variations of wing size and shape of *Triatoma infestans* (Hemiptera: Reduviidae) populations from northwestern Argentina using geometric morphometry. *Journal of Medical Entomology*, **46**, 994–1000.

Schofield, C.J., Lehane, M.J., McEwan, P., Catalá, S.S. & Gorla, D.E. (1992) Dispersive flight by *Triatoma infestans* under natural climatic conditions in Argentina. *Medical and Veterinary Entomology*, **6**, 51–56.

Schweigmann, N., Vallve, S., Muscio, O., Ghillini, N., Alberti, A. & Wisnivesky-Colli, C. (1988) Dispersal flight by *Triatoma infestans* in an arid area of Argentina. *Medical and Veterinary Entomology*, **2**, 401–404.

Silveira, A.C., Feitosa, V.R. & Borges, R. (1984) Distribuição de triatomíneos capturados no ambiente peridomiciliar, no período de 1975/83, Brasil. *Revista Brasileira de Malariologia e Doenças Tropicais*, **36**, 15–31.

Souza, R.C.M., Barbosa, S.E., Sonoda, I.V., Azeredo, B.V.M., Romanha, A.J. & Diotaiuti, L. (2008) Population dynamics of *Triatoma vitticeps* (Stål, 1859) in Itanhomi, Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*, **103**, 14–20.

Villacís, A.G., Grijalva, M.J. & Catalá, S.S. (2010) Phenotypic variability of *Rhodnius ecuadoriensis* populations at the Ecuadorian Central and Southern Andean Region. *Journal of Medical Entomology*, **47**, 1034–1043.

Villegas, J., Feliciangeli, M.D. & Dujardin, J.P. (2002) Wing shape divergence between *Rhodnius prolixus* from Cojedes (Venezuela) and *Rhodnius robustus* from Mérida (Venezuela). *Infection, Genetics and Evolution*, **2**, 121–128.