# Relationship between Antennal Sensilla Pattern and Habitat in Six Species of Triatominae

**AL Carbajal de la Fuente, S Catalá**+

CRILAR, Mendoza y Entre Ríos, Anillaco, 5301, La Rioja, Argentina

*In order to determine if habitat similarity is correlated with a similarity of sensilla pattern, we analyzed six species of Triatominae present in two biogeographic regions of Brazil: the "caatinga" and the "cerrado". In broad terms* Triatoma infestans *(cerrado) and* T. brasiliensis *(caatinga) are found in human domiciles,* T. sordida *(cerrado) and* T. pseudomaculata *(caatinga) colonize peridomestic habitats, and* Rhodnius neglectus *(cerrado) and* R. nasutus *(caatinga) inhabit palm tree crowns. The number and distribution of four sensilla types (bristles, thin and thick walled trichoidea, and basiconica) were compared in these species. Sexual dimorphism of sensilla patterns was noted in* T. sordida, T. brasiliensis *and* T. pseudomaculata. *A principal component analysis showed three main groups: (i) species that live in the palms, (ii) domiciliated species and (iii) those living in the peridomestic habitat.* T. infestans *almost exclusively domestic, was placed at the centre of the canonical map and some individuals of other species overlapped there. These results support the idea that the patterns of antennal sensilla are sensitive indicators of adaptive process in Triatominae. We propose that those species that inhabit less stable habitats possess more types of sensilla on the pedicel, and higher number of antennal sensilla.*

Key words: Chagas disease - sensilla patterns - *Triatoma infestans* - *Triatoma brasiliensis* - *Triatoma sordida* - *Triatoma pseudomaculata* - *Rhodnius neglectus* - *Rhodnius nasutus*

As vectors of *Trypanosoma cruzi*, causal agent of Chagas disease, insects of the subfamily Triatominae (Hemiptera: Reduviidae), have special relevance in Latin America. They are obligatory haematophagous insects at all stages of their life, adapted to diverse habitats associated with mammals, birds, and in some cases reptiles and amphibians (Schofield 1988, Dujardin et al. 2000). This subfamily is believed to have evolved from other predatory Reduviidae, showing a series of morphological, physiological, behavioural, and demographical changes. These changes are mainly due to the use of vertebrate blood as feeding source, adaptation to the environment of the host, and to a progressive dependency on the vertebrate host for passive dispersal. In this way, some species have reached a close association with human dwellings, with man and his domestic animals (Schofield 1988, 1994, Schofield et al. 1999).

Schofield (1988) proposed that adaptation of Triatominae to stable habitats, such as human dwellings, would include a series of morphological and behavioural simplifications which could indicate the probability of each species to colonize the human domicile. Sensorial simplification could be linked to such morphological and behavioural simplification, and it has already been demonstrated that species adapted to a narrow range of habitats, such as *Triatoma infestans*, have a lower number of chemoreceptors on the pedicel of the antenna than species adapted to live in more diverse habitats, such as *T. sordida* (Catalá 1996, 1997, Gracco & Catalá 2000).

One hypothesis proposed by Schofield postulated that even in biogeographical different regions the species of Triatominae which live in similar habitats would have evolved similarly, and that their sensorial system would show common characteristics. To test this idea, we compared matched pairs of species which live in similar habitats of two different biogeographical regions of Brazil: the dry "caatinga" of the Northeast and the semi-arid "cerrado" of Central and Southern regions. In very broad terms, *Rhodnius neglectus* (cerrado) and *R. nasutus* (caatinga) are found at the top of palm trees, while *Triatoma infestans* (cerrado) and *T. brasiliensis* (caatinga) are well adapted to human houses. *T. sordida* (cerrado) and *T. pseudomaculata* (caatinga) exploit the peridomicile, as well as various wild habitats. It is possible that antennal sensilla patterns would show similarities in species which share the same habitat. The Brazilian caatinga, as well as the cerrado, are endemic areas for Chagas disease, and the two species mentioned as domestic are important vectors of *T. cruzi* to humans.

*T. infestans* is the most important vector of Chagas disease in the Southern cone of Latin America. Its habitat is almost exclusively domestic (Lent & Wygodzinsky 1979, Zeledón 1983). *T. infestans* was also found in wild habitats in the Cochabamba region (Bolivia) in small colonies associated with wild guinea-pigs, and in the Bolivian Chaco (Noireau 1998).

*T. brasiliensis* is the predominant domestic vector of Chagas disease in the arid caatinga of the Northeastern region of Brazil (Lent & Wygodzinsky 1979, Zeledón & Rabinovich 1981, Schofield 1994). It has similar population characteristics to *T. infestans*, occuping silvatic, peridomestic and domestic habitats (Zeledón 1983, Carcavallo & Martinez 1985). In silvatic habitats, *T.*

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+Corresponding author. Fax: +54-3827-494251. E-mail: scatala@crilar-conicet.com.ar

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*brasiliensis* is generally associated with caviid rodents (Schofield 1994).

*T. sordida* is mainly distributed in Central Brazil and parts of the chaco of Paraguay, Bolivia and Argentina (Lent & Wygodzinsky 1979, Schofield 1994). It inhabits a great variety of silvatic ecotopes, such as hollow trees, rock pile and rodent nests, but is frequently found in domestic and peridomestic habitats (Carcavallo 1975, Zeledón & Rabinovich 1981, Zeledón 1983). Similarly *T. pseudomaculata* has been collected in human houses and in peridomiciles sometimes with *T. sordida* (Lent & Wygodzinsky 1979, Barrett 1991). Its distribution is restricted to Brazil.

*R. neglectus* is widely distributed in Central Brazil, typically found in the crowns of palm trees such as *Mauritia flexuosa* and *Acrocomia aculeata*, and in rare occasions it can be seen in bird nests, mainly of the Family Furnariidae. Its presence has been mentioned in tree holes, associated with marsupials, rodents and Chiroptera (Carcavallo & Martínez 1985). This species occasionally invades human houses and peridomiciles (Lent & Wygodzinsky 1979, Barrett 1991). Zeledón (1983) and Dujardin et al. (2000) classified *R. neglectus* as insects in the process of adaptation to domestic habitats. Similarly, *R. nasutus* is primarily associated with palm tree crowns (*Copernicia prunifera*) in the caatinga region of Northeastern Brazil but occasionally invades domestic and peridomestic habitats (Lent & Wygodzinsky 1979, Barrett 1991, Dujardin et al. 2000).

The objective of this study was to compare the characteristics of the sensilla patterns of each of those pairs of species *R. neglectus, R. nasutus, T. infestans, T. brasiliensis, T. sordida* and *T. pseudomaculata,* in relation to their association with similar habitats in different geographical regions.

## MATERIALS AND METHODS

All specimens except those of *T. infestans* were provided by the Fundação Oswaldo Cruz-René Rachou Research Centre (Brazil). *T. infestans* was obtained from field material collected in the province of Córdoba (Argentina) by Catalá (1996). Ten insects of each species (five males and five females), were analyzed. One antenna per individual was removed and diafanized in Sodium hydroxide. After neutralization with acetic acid, the antenna was slide mounted in glycerine. Observations (sensilla identification and counting) were made on the ventral side of the three distal segments of the antennae using optical microscopy and a drawing chamber (Catalá & Schofield 1994, Catalá 1996). Quantitative analysis included the following receptors: bristles (BR), thin walled trichoids (TH), thick walled trichoids (TK) and basiconica (BA).

Means and standard deviations of the number of sensilla of each type on each segment were obtained. Levene's test was used to check the homogeneity of variances. Those variables which showed heteroscedasticity were analyzed using Kruskal-Wallis non-parametric test. The rest of the variables were analyzed using ANOVA, and mean values for each species were contrasted using least significant difference test.

Data for each species were log-transformed for multivariate analysis (principal components analysis and discriminant analysis; Sokal & Sneath 1963, Morrison 1976) to obtain the functions which allowed a better identification of the studied groups. Mahalanobis distances were calculated as the distance between group centroids generated by the discriminant functions.

## RESULTS

*Analysis of the antennal sensilla pattern by species* - All four types of receptor were found in both segments of the flagellum (F1 and F2) of the six species: *T. infestans, T. brasiliensis, T. pseudomaculata, T. sordida, R. nasutus* and *R. neglectus*. However, the pedicel showed an important variation in relation to the studied chemoreceptors (Table I). The two species of the genus *Rhodnius* did not have the three types of chemoreceptors in this segment. *T. sordida, T. brasiliensis,* and *T. pseudomaculata* had the three types of chemoreceptors, but *T. infestans,* in most specimens, had only one type (TH) (Table II).

TABLE I

Presence (*) of basiconic (BA) and trichoidea thin (TH) and thick (TK) walled on the pedicel of the six species

| Species | TH | TK | BA |
|---|---|---|---|
| *Rhodnius neglectus* | | | |
| *R. nasutus* | | | |
| *Triatoma infestans* | * | | |
| *T. brasiliensis* | * | * | * |
| *T. pseudomaculata* | * | * | * |
| *T. sordida* | * | * | * |

Lengths of antennal segments were positively correlated (pedicel/flagellum 1: $r = 0.73$; pedicel/flagellum 2: $r = 0.74$; flagellum 1/flagellum 2: $r = 0.94$; $p < 0.05$). However, there was no correlation between the length of antennal segments and the number of each type of sensillum arranged over them.

Discriminant analysis (DA) was highly significant for the six species studied ($F = 14.81$, $p < 0.0000$). Four significant functions were generated at $p < 0.01$ and 100% of individuals were correctly classified. Table III shows Mahalanobis distances to illustrate differences between the sensilla patterns of the six species.

*Sexual differences in the sensilla pattern* - Sexual differences were compared using ANOVA or Kruskal-Wallis test. All levels of significance were adjusted according to Bonferroni's test (Sokal & Rohlf 1995). Neither the two species of *Rhodnius* nor *T. infestans* showed significant differences between males and females. In *T. sordida* and T. *pseudomaculata*, there was a significant increase of TH in the first segment of the male flagellum. In *T. brasiliensis*, a significantly higher number of TK in flagellum 1 of females was observed (Table IV).

*Analysis of the antennal sensilla pattern in function of the habitat* - Principal component analysis (PCA) was carried out to compare the similarity of individuals. PC 1 separated the two species which live in palm trees (*R.*

TABLE II

Length and sensilla number on each segment of the antennae of *Triatoma infestans*, *T. brasiliensis*, *T. sordida*, *T. pseudomaculata*, *Rhodnius nasutus* and *R. neglectus*; n = 60. Mean (standard deviation) P: pedicel; F1: flagellum, first segment; F2: flagellum, second segment; BR: bristle; TH: thin walled trichodea; TK: thick walled trichodea; BA: basiconic

| | Length | | | Sensilla on Pedicel | | | | Sensilla on Flagel 1 | | | | Sensilla on Flagel 2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | P | F1 | F2 | BR | TH | TK | BA | BR | TH | TK | BA | BR | TH | TK | BA |
| *T. infestans* | 19.09<br>(2.22) | 16.82<br>(1.96) | 12.89<br>(0.55) | 167.8<br>(20.17) | 158.1<br>(25.74) | 0<br>0 | 0<br>0 | 20.9<br>(3.72) | 91.2<br>(21.18) | 403.5<br>(65.88) | 27.2<br>(12.66) | 12.9<br>(2.13) | 40.8<br>(6.66) | 270.2<br>(35.88) | 23.4<br>(6.31) |
| *T. brasiliensis* | 17.76<br>(1.51) | 14.09<br>(1.29) | 11.26<br>(0.98) | 151.5<br>(19.72) | 65.5<br>(28.15) | 0.8<br>2.53 | 0.3<br>0.48 | 22.5<br>(2.95) | 67.6<br>(23.07) | 279.2<br>(67.70) | 60.4<br>(32.14) | 11.9<br>(2.81) | 40.7<br>(8.02) | 190.7<br>(40.64) | 61.7<br>(23.29) |
| *T. sordida* | 12.99<br>(0.99) | 9.55<br>(0.47) | 7.17<br>(0.23) | 79.8<br>(11.57) | 175.3<br>(47.08) | 185.3<br>(64.71) | 8.5<br>(4.20) | 16<br>(3.40) | 72.7<br>(17.20) | 240.2<br>(50.47) | 35.5<br>(21.72) | 9<br>(1.94) | 31.1<br>(10.74) | 119.8<br>(14.77) | 17.1<br>(5.99) |
| *T. pseudomaculata* | 13.86<br>(1.95) | 10.18<br>(0.49) | 7.43<br>(0.4) | 97.78<br>(37.05) | 179<br>(91.56) | 4.33<br>(7.24) | 3<br>(3.04) | 16.11<br>(4.48) | 62.89<br>(27.24) | 155.4<br>(34.64) | 26.56<br>(11.52) | 8.11<br>(2.47) | 29.33<br>(6.32) | 111.9<br>(29.35) | 23.78<br>(7.66) |
| *R. nasutus* | 13.66<br>(2.12) | 10.99<br>(0.73) | 7.66<br>(0.56) | 117.3<br>(15.09) | 0<br>0 | 0<br>0 | 0<br>0 | 37.6<br>(6.59) | 86.5<br>(26.3) | 114.5<br>(22.98) | 27.3<br>(7.41) | 17<br>(2.45) | 65.1<br>(11.04) | 131.6<br>(14.53) | 24.6<br>(9.24) |
| *R. neglectus* | 16.75<br>(1.28) | 9.7<br>(0.65) | 7.62<br>(0.42) | 198.7<br>(30.2) | 0<br>0 | 0<br>0 | 0<br>0 | 51.6<br>11.38) | 59.8<br>(15.34) | 162.2<br>(47.7) | 15<br>(8.15) | 19.3<br>(4.71) | 53.6<br>(15.36) | 229.9<br>(42.36) | 19.2<br>(7.72) |

*nasutus* - *R. neglectus*), while PC 2 separated specimens belonging to domiciliated species (*T. infestans* - *T. brasiliensis*) from those corresponding to peridomiciliated species (*T. sordida* - *T. pseudomaculata*). There was some overlap between individuals of the more domiciliated species (*T. infestans*) and some individuals of *R. nasutus*, *T. brasiliensis*, *T. pseudomaculata*, and *T. sordida* (Figure).

## DISCUSSION

During their evolution, Triatominae have conquered different habitats (Schofield 1988). In epidemiological terms and regarding the control of Chagas disease, the tendency of these insects to develop in human dwellings is of great importance. In this study, we postulated that sensilla patterns would be indicators of the adaptation of Triatominae to habitats of different complexity and stability. McIver (1987) stated that sensilla patterns in haematophagous insects reflected ancestral patterns modified by the adaptation to different hosts and habitats. On the other hand, Catalá and Schofield (1994), Catalá (1997), and Gracco and Catalá (2000) already demonstrated that antennal sensilla patterns allowed the separation of genera and species of Triatominae. Our results, based on the analysis of antennae of the three pairs of species which inhabit the Brazilian cerrado and caatinga, showed significant differences in those species that develop their life cycle in different habitats.

Multivariate analyses (PCA and DA) showed similarities in species which use the same habitat in the two biogeographic regions: *T. infestans* and *T. brasiliensis*: domestic, *T. sordida* and *T. pseudomaculata*: peridomestic, and *R. nasutus* and *R. neglectus*: palm trees. Considering that the domestic habitat has its main representative in *T. infestans*, and based on PCA, we believe that some specimens of *T. brasiliensis*, *T. pseudomaculata*, and *R. nasutus* share more similarity with the domiciliated *T. infestans*. This could indicate an antennal sensilla pattern better adapted to function within the domestic habitat.

The absence of chemoreceptors in the pedicel of the two species of the genus *Rhodnius* is characteristic of the Rhodniini (Catalá & Schofield 1994) – evidence supporting the idea of monophyletic origin of this group (Schofield 1988). On the other hand, the great similarity of the antennae of the two species of *Rhodnius*, their lower complexity and the absence of sexual differences, could be related to the similarity of habitat, since both species are associated with the specific and quite stable environment of palm tree crowns.

Diversity in the type and number of receptors on the pedicel was observed in the four species of the genus *Triatom*a, which could be related to the characteristics of the habitat where each species develops. *T. infestans* and *T. brasiliensis*, well adapted to the human domicile, showed a less complex pedicel than *T. sordida* and *T. pseudomaculata*, which have a greater amplitude of habitat.

Moreover, in *T. sordida* and *T. pseudomaculata* marked sexual differences were observed. We postulate that the sexual dimorphism in the antenna may be linked to differences in sensing sexual pheromones. Baldwin et al. (1971) suggested the presence of a sexual pheromone in *R.*

TABLE III

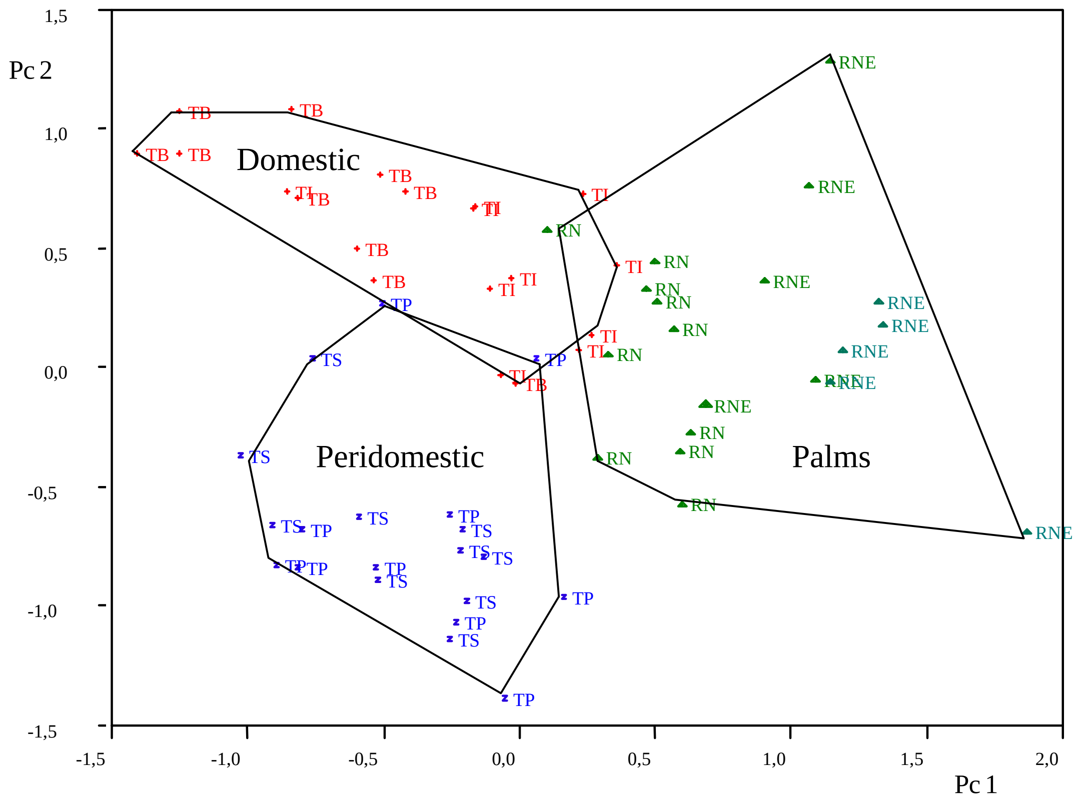
Mahalanobis distances obtained from discriminant analysis of sensilla patterns. The second column illustrates the degree of similarity to *Triatoma infestans*

| Species | Mahalanobis distance | | | | |
|---|---|---|---|---|---|
| | *T. infestans* | *T. brasiliensis* | *T. sordida* | *T. pseudomaculata* | *R. neglectus* |
| *T. infestans* | | | | | |
| *T. brasiliensis* | 14.87 | | | | |
| *T. sordida* | 35.34 | 45.5 | | | |
| *T. pseudomaculata* | 30.06 | 25.28 | 11.98 | | |
| *Rhodnius neglectus* | 92.29 | 101.4 | 145.79 | 101.4 | |
| *R. nasutus* | 81.52 | 53.19 | 85.15 | 53.19 | 28.15 |

TABLE IV

Number (mean and standard deviation) of thin walled trichodea (TH) and thick walled trichodea (TK) on the first segment of flagellum. *Triatoma infestans (T. inf)*, *Rhodnius neglectus (R. neg)* and *R. nasutus (R. nas)* do not exhibit sexual differences. *T. brasiliensis (T. bras)*, *T. pseudomaculata (T. pseud)* and *T. sordida (T. sord)* show sexual dimorphism

| | Sex | *T. bras* | *T. inf* | *T. pseud* | *T. sord* | *R. nas* | *R. neg* |
|---|---|---|---|---|---|---|---|
| TH on flagellum 1 | F | 52(14) | 93(17) | 46 (6) | 60 (8) | 94 (34) | 53(8) |
| | M | 83(20) | 90(26) | 89 (27) | 86 (13) | 79(17) | 67(18) |
| TK on flagellum 1 | F | 331 (47) | 430(74) | 146(31) | 265(56) | 115(23) | 147(20) |
| | M | 227 (38) | 377(50) | 175(39) | 215(31) | 114(26) | 118(64) |



Principal components analysis using antennal sensilla variables. Each point represents one specimen on the cannonical axis (PC 1 and PC 2). Polygons enclose specimens of each group (habitat): domestic, peridomestic and palms. TB: *Triatoma brasiliensis*; TI: *T. infestans*; TP: *T. pseudomaculata*; TS: *T. sordida*; RN: *Rhodnius nasutus*; RNE: *R. neglectus* *prolixus*, since males were attracted towards mating pairs. Lorenzo Figueiras et al. (1998) obtained evidence in *T. infestans* and *R. prolixus* of the existence of a chemical signal produced during mating which promoted the aggregation of males around pairs, while females were not attracted. It may be that some of the TH sensilla, more abundant in males, are receiving the putative sex pheromone.

Results obtained in this study support the hypothesis of Catalá (1997) that those species living in a greater variety of less stable habitats (eg. *T. sordida*, *T. pseudomaculata)* have more types and higher number of sensilla on the pedicel. Moreover, results suggest that antennal sensilla patterns are sensitive indicators that can help to determine those species that may represent greatest risk to humans as vectors of *T. cruzi*.

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