ASSESSMENT OF TRIATOMA DIMIDIATA DISPERAL IN THE YUCATAN PENINSULA OF MEXICO BY MORPHOMETRY AND MICROSATELLITE MARKERS

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Abstract. In the Yucatán Peninsula of Mexico, the main vector of Chagas disease is *Triatoma dimidiata*. Field studies suggest that natural transmission occurs through transient and seasonal invasion of houses by sylvatic/peridomestic triatomines, rather than through persistent domiciliated bug populations. We investigated the genetic structure of *T. dimidiata* populations, using morphometry and microsatellite markers, to assess dispersal of individuals in this triatomine species and to understand the dynamics of domestic infestation. We observed low phenotypic and genetic differentiation among populations from different villages, with an FST of only 0.0553, which suggested a weak but significant population structure at this level. Similarly low but significant differences were observed among populations from the same village but different biotopes (sylvatic, peridomestic, and domestic), with FST values ranging from 0.0096 to 0.0455. These data suggested elevated dispersed of bugs between biotopes (Nm = 5–25), which was confirmed by likelihood and Bayesian assignment tests. A proportion of bugs collected within domiciles were significantly assigned to peridomestic and sylvatic areas. This study showed that *T. dimidiata* has important dispersal capabilities that can explain the seasonal pattern of domicile infestation by peridomestic and sylvatic bugs. Therefore, dispersal should be taken into account in the design of effective vector control strategies.

INTRODUCTION

Chagas disease, or American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi*, and represents a major public health problem in Latin America, particularly in Mexico. Because of the limited efficacy of current therapeutic treatment and the lack of an effective vaccine, control of Chagas disease remains based on vector control using insecticides and on blood donor screening. Vector control programs in South America have focused on the interruption of natural transmission by attacking domiciliated vector populations using pyrethroid insecticides, and these have been enormously successful. The interruption of natural transmission is thus underway in the southern cone countries (Argentina, Brazil, Bolivia, and Chile). In the Yucatán Peninsula of Mexico, the main vector of Chagas disease is *Triatoma dimidiata*, an apparently ubiquitous species of triatomine found in a variety of environments. A thorough knowledge of key aspects of *T. dimidiata* biology and ecology is required for the implementation of successful and sustainable vector control programs. To this end, we previously characterized the geographic distribution of this vector and developed predictive models of vector-borne Chagas disease transmission risk to humans in the region. Also, our field data suggested that natural transmission occurs by transient and seasonal invasion of houses by sylvatic/peridomestic triatomines, rather than through established resident domiciliated bug populations. This transmission pattern implies high risk of re-infestation by nondomiciliated bugs after conventional indoor insecticide spraying. This was confirmed in a pilot study in which we documented the re-infestation of houses within only 3–4 months after insecticide application. Dispersal of *T. dimidiata* was later observed in northern Guatemala. Using mathematical modeling, we also provided theoretical evidence suggesting a major role of dispersal/immigration of bugs to explain seasonal infestation of domiciles by *T. dimidiata* in the Yucatan Peninsula (Gourbiere S and others, unpublished data).

Our previous work suggests that it is important to have a good understanding of *T. dimidiata* dispersal to design appropriate vector control strategies in the region. Recent studies on *T. dimidiata* from different regions, using morphometry, chemical and random amplified polymorphic DNA (RAPD) molecular markers, suggested only weak genetic structuring among populations and that this species has strong dispersal capabilities. However, although these techniques may be sufficient to detect clearly defined subpopulations separated by large distances (more than 120 km), they may have limited resolution to detect population structure over smaller distances and between biotopes. Alternatively, microsatellite markers have been extensively used for populations studies of many different species over a wide range of geographic scales. Several microsatellite loci have been identified in *Rhodnius pallescens* and *T. infestans*, making these markers an attractive molecular tool for the analysis of triatomine population structure and dispersal. We report a study of the genetic structure of *T. dimidiata* populations in the Yucatan Peninsula of Mexico, using morphometry and microsatellite markers, to assess dispersal of this triatomine species.

MATERIALS AND METHODS

Field work. Triatomine bugs were collected from 18 sites separated by as little as 1 km, and up to 280 km, corresponding to 14 villages and 3 biotopes (Figure 1). Different subsets of these collections were used for analysis at different geo-
The study area was assessed by analyzing population genetic structure using Hardy-Weinberg equilibrium for all populations and loci after Bonferroni correction. Population genetic structure was evaluated at two levels, between villages and between biotopes within villages, respectively, using F statistics. We calculated FST, a measure of divergence among such populations, FIS, a measure of inbreeding relative to an S population and its allele frequency, and Nm, the number of migrants per generation between subpopulations. A Mantel test on linearized FSTs and log geographic distances was used to evaluate isolation by distance. As with morphometric data analysis, we further analyzed genotype data with assignment tests to detect potential migrants between populations and habitats. We used the likelihood method implemented in Arlequin version 2.001,27 as well as a Bayesian approach implemented in Immanc 1.5.30 Both methods assume that populations are in Hardy-Weinberg equilibrium and that loci are in linkage equilibrium. The approach implemented in Arlequin version 2.001 uses allelic frequencies in each population to calculate the probability that an individual genotype belongs to a certain population. If an individual genotype is more likely to belong to a population other than that from where it was sampled, the individual is considered a migrant. The Bayesian approach implemented in Immanc 1.5 uses a Bayesian derivation to calculate the probability density of population allele frequencies from sample population frequencies. The probability that a given genotype belongs to one or the other

FIGURE 1. Map of the study area in Mexico. Collection sites and villages are indicated. Hunucma N = Hunucma Norte; Hunucma S = Hunucma Sur. Domestic bugs were collected at all 14 villages and in Dzidzileche and Tetiz, where peri-domestic and sylvatic bugs were also collected.

Insect genotyping and analysis. DNA was extracted from 1–3 legs of the collected bugs using standard phenol-chloroform extraction and precipitation. A total of eight microsatellite loci were amplified by polymerase chain reaction (PCR) from 50–100 ng of DNA, using the primers and PCR conditions described by Anderson and others.22 and PCR products were analyzed on an ABI310 automatic sequencer using GenScan software (Applied Biosystems, Foster City, CA).

All genotype data were analyzed using Arlequin version 2.001 software.27 We first assessed compliance with Hardy-Weinberg equilibrium for all populations and loci after Bonferroni correction. Population genetic structure was evaluated at two levels, between villages and between biotopes within villages, respectively, using F statistics.28 We calculated FST, a measure of divergence among such populations, FIS, a measure of inbreeding relative to an S population and its allele frequency, and Nm, the number of migrants per generation between subpopulations.16,29 A Mantel test on linearized FSTs and log geographic distances was used to evaluate isolation by distance. As with morphometric data analysis, we further analyzed genotype data with assignment tests to detect potential migrants between populations and habitats. We used the likelihood method implemented in Arlequin version 2.001,27 as well as a Bayesian approach implemented in Immanc 1.5.30 Both methods assume that populations are in Hardy-Weinberg equilibrium and that loci are in linkage equilibrium. The approach implemented in Arlequin version 2.001 uses allelic frequencies in each population to calculate the likelihood that a given genotype belongs to a certain population. If an individual genotype is more likely to belong to a population other than that from where it was sampled, the individual is considered a migrant. The Bayesian approach implemented in Immanc 1.5 uses a Bayesian derivation to calculate the probability density of population allele frequencies from sample population frequencies.31 The probability that a given genotype belongs to one or the other
As for morphometric analysis, the population is calculated using a Monte Carlo re-sampling procedure simulating a large number of random multilocus genotypes based on allele frequencies directly estimated from the reference population samples. We used the program to calculate the probability of an individual’s genotype being a current migrant or the progeny of a migrant mated with an individual from the local population. We used 10,000 replications for each analysis as recommended by the authors and a significance value of 0.05.30 As for morphometric analysis, the agreement between bug collection sites and genotype assignments was evaluated by Kappa statistics.26

RESULTS

Population structure between villages. We initially evaluated *T. dimidiata* population structure across the Yucatan Peninsula by comparing bugs collected from different villages. Univariate analysis of head and wing measurements and Guillaumin profiles of 336 domestic bugs from a subset of 10 villages showed significant differences between bugs from the various collection sites for some morphometric variables. Discriminant analysis showed marked sexual dimorphism with females being significantly larger than males (Wilks’ Lambda = 0.671, $P < 0.0001$) and bugs from different sex were thus analyzed separately. Discriminant analysis was also able to detect significant differences between bugs from the 10 collection sites (Wilks’ Lambda = 0.400, $P < 0.0001$ for males and Wilks’ Lambda = 0.331, $P < 0.001$ for females), although there was considerable overlap of the respective canonical maps (Figure 2A). There was a significant correlation between the first canonical factor centroids (an indicator of size) and the longitude of the villages (Figure 2B, $r^2 = 0.42$, $P = 0.002$), which suggested a significant gradient in size from west to east and a significant phenotypic structure across the peninsula. A similar level of phenotypic differentiation was observed using wing measurements. Combining head and wing measurements did not improve the level of differentiation detected and provided similar results.

Population genetic structure was also assessed using microsatellite markers, and 295 bugs from 12 collection sites were genotyped. Of the eight microsatellite loci included in our study, two did not give reproducible amplifications (Tdms1 and Tdms19), and one was monomorphic (Tdms3); these were thus discarded from the study. One additional locus gave a disproportionate excess of homozygotes (Tdms16), which suggested a high frequency of null alleles, and was also removed from the analysis. Thus, four loci were included in our final analysis to assess population structure (Tdms4, Tdms9, Tdms11, and Tdms22). These microsatellite loci were highly polymorphic, with a minimum of 4 and up to 21 alleles per locus (Table 1). A large number of alleles had not been described previously in *T. dimidiata* populations.22 After Bonferroni correction, Hardy-Weinberg expectations were significantly rejected in only 2 of 48 cases of populations and loci.

The FST estimates were computed for a subset of domestic populations from nine villages (Table 2). These indicated a rather low but significant level of genetic differentiation across the Yucatan peninsula. The maximum FST was 0.0553, between Tres Reyes and Hunucma Norte, which are separated by a distance of 250 km. However, there was no evidence of isolation by distance between populations ($P = 0.402$, by Mantel test on linearized FSTs and log geographic distance), and comparable FST values were obtained between very close villages (e.g., 0.0367 between Hunuca Norte and Sur, separated by only a few kilometers). Microsatellite markers were thus able to detect a finer genetic structure than morphometric analysis. The inbreeding coefficient $F_{is}$ indicated low inbreeding in all collection sites (average $F_{is} = 0.040$), except in Tixcacalcualup, where it reached 0.104 (Table 1). Overall, both morphometric and microsatellite data suggested important dispersal capabilities of *T. dimidiata* over large geographic distances (up to 280 km), which resulted in detectable but weak genetic structure and population differentiation in the Yucatan Peninsula.

Population structure between biotopes. We then conducted an analysis to determine if we could detect substructuring between domestic, peri-domestic, and sylvatic populations. Morphometric analysis of male bugs indicated significant morphologic differences between bugs from the different

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**Figure 2.** Discriminant analysis of morphometric data of populations from different villages in Mexico. Analysis of head measurements of bugs from the indicated villages are shown. **A.** Canonical maps indicating significant differences between male populations (Wilks’ Lambda = 0.400, $P < 0.0001$). Only a subset of five villages is shown for clarity. **B.** Correlation between the centroid of the domestic populations for 10 villages and the village’s longitude ($r^2 = 0.42$, $P = 0.002$ for male and female). Open circles = females, black circles = males.
biotopes from Tetiz (Wilks’ Lambda = 0.401, P = 0.015), but not from Dzidzilche (Wilks’ Lambda = 0.686, P = 0.27) (Figure 3), and analysis of female bugs indicated a significant difference between biotopes in Dzidzilche but not in Tetiz (Wilks’ Lambda = 0.557, P = 0.006 and Wilks’ Lambda = 0.812, P = 0.66, respectively). These data suggested a weak level of phenotypic differentiation between biotopes in both villages. We also looked at the assignment of bugs to sub-populations/biotopes based on the canonical scores of the discriminant analysis. For both Dzidzilche and Tetiz, when we used either head or wing measurements, only 43–82% of bugs could be correctly assigned to the biotope where they had been collected (Table 3), which resulted in a low agreement between assignments and collection sites (Kappa statistics = 0.370 ± 0.075 and 0.349 ± 0.085 for Dzidzilche and Tetiz, respectively). This analysis confirmed the weak or borderline level of population differentiation between the respective biotopes (Table 3). Conversely, the important proportion of miss-assigning bugs suggested significant dispersal between biotopes. Approximately 27–36% of bugs collected in the domiciles seemed to originate from the peridomicile, and 7–24% were assigned to the sylvatic biotope (Table 3). Furthermore, several peri-domestic and even sylvatic bugs appeared to come from the domiciles, which suggested dispersal in multiple directions.

As before, population substructuring was also assessed using microsatellite markers. There seemed to be some differences in allele frequencies between subpopulations, and a few private alleles were detected in populations from each biotope (Figure 4). The FST statistics for Dzidzilche and Tetiz are shown in Table 4. In Dzidzilche, there was no significant genetic differentiation between peri-domestic and domestic populations, which suggested a high number of migrants per generation (Nm = 25.8). In contrast, bug populations from domestic and peri-domestic habitats were significantly differentiated from those in the sylvatic habitat, and the estimate of migrants between those biotopes was comparatively lower (Nm = 5–6). However, this differentiation was not observed in Tetiz where domestic bugs were not significantly differentiated from sylvatic ones (Table 4).

We further analyzed dispersal between sub-populations from Dzidzilche using genotype assignment tests. The likelihood method resulted in assignment plots showing V-shaped distributions of bugs for all biotope pairs, which is characteristic of poorly differentiated populations. Nonetheless, 70–78% of the bugs collected in Dzidzilche could be correctly assigned to their corresponding biotopes, which indicated good agreement between assignments and actual collection sites (Table 5, Kappa statistic = 0.609 ± 0.073). Bayesian assignments resulted in a somewhat improved accuracy, with 77–95% of the bugs correctly assigned (Table 5, Kappa statistic = 0.796 ± 0.057). In spite of good agreement obtained between genotype assignments and field collection sites, approximately 10–22% of the bugs collected in the domiciles seem to be immigrants from the peridomicile and sylvatic area. Also, dispersal in multiple directions seems to occur because several peridomestic and sylvatic bugs were assigned to the domiciles (Table 5), as observed with morphometric analysis.

**DISCUSSION**

We investigated the genetic structure of *T. dimidiata* as an indicator of dispersal because understanding dispersal is criti-
cal for the design and implementation of effective vector control strategies. Morphometric analysis has been widely used to evaluate triatomine population structure because of its ease of use. However, it may lack the power to distinguish between closely related subpopulations. Furthermore, morphometric studies essentially describe phenotypic variation, which does not necessarily imply that the same pattern of variation would be observed at the genetic level. Microsatellites have been extensively and successfully used for population genetics studies of a wide variety of species, and over a variety of geographic scales, but to our knowledge, this is the first study in triatomines, even though various microsatellite loci have been described in several triatomine species.

Our results indicate that there was an overall good agreement between morphometric and molecular data, although the latter seemed more sensitive to population structure, particularly at the level of biotopes from single villages. At the level of the Yucatan Peninsula, both methods indicated a weak but significant differentiation of *T. dimidiata* populations. Morphometric analysis was able to detect a west-east gradient in bug size, and genotypic data indicated some degree of genetic differentiation shown by low but significant FSTs between populations from different villages, but no significant isolation by distance. These results are in good agreement with previous population genetic studies of *T. dimidiata* that showed a weak population structure over large distances.

Previous morphometric analysis showed significant differences between populations from central Mexico and Guatemala, and populations from the Peten region of North-

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Biotope</th>
<th>n</th>
<th>Domestic</th>
<th>Peridomestic</th>
<th>Sylvatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetiz</td>
<td>Domestic</td>
<td>14</td>
<td>8 (57%)</td>
<td>5 (36%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td></td>
<td>Peridomestic</td>
<td>39</td>
<td>11 (28%)</td>
<td>17 (43%)</td>
<td>11 (28%)</td>
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<tr>
<td></td>
<td>Sylvatic</td>
<td>22</td>
<td>0 (0%)</td>
<td>4 (18%)</td>
<td>18 (82%)</td>
</tr>
<tr>
<td></td>
<td>Domestic</td>
<td>41</td>
<td>20 (49%)</td>
<td>11 (27%)</td>
<td>10 (24%)</td>
</tr>
<tr>
<td>Dzidzilche</td>
<td>Peridomestic</td>
<td>38</td>
<td>8 (21%)</td>
<td>25 (66%)</td>
<td>5 (13%)</td>
</tr>
<tr>
<td></td>
<td>Sylvatic</td>
<td>18</td>
<td>5 (26%)</td>
<td>1 (5%)</td>
<td>12 (66%)</td>
</tr>
</tbody>
</table>

* Bug assignment to the different biotopes was determined from probabilities calculated from the discriminant scores of a separate analysis of male and female bugs. The combined number of male and female bugs assigned to each biotope is presented for each collection site and in parenthesis as a percentage of bugs collected in each site. **Bold** numbers indicate correct assignments. Agreement between assigned biotopes and collection sites was low (Kappa statistics = 0.370 ± 0.085 and 0.349 ± 0.075 for Dzidzilche and Tetiz, respectively).
The use of assignment tests, such as those used in this study, avoid this problem. Because highly polymorphic microsatellite loci tend to underestimate levels of genetic divergence as estimate by FST (probably due to size homoplasy), thus diminishing their power to detectable isolation by distance. Highly polymorphic microsatellite loci tend to discriminate among samples.

Population substructure is more difficult to assess when we focus on a smaller geographic area such as single villages. Using morphometric analysis, we were able to detect some significant differences between bugs from domestic, peridomestic, and sylvatic collection sites. The presence of some alleles is restricted to a particular biotope (private alleles).

The number of bugs assigned to each biotope is presented for each collection site, and in parenthesis as a percentage of bugs collected in each site. Assignment tests were performed using a likelihood and bayesian method, respectively. Bold numbers indicate correct assignments. Agreement between assigned biotopes and collection sites was high (Kappa statistics = 0.609 ± 0.073 and 0.796 ± 0.057 for likelihood and bayesian method, respectively).

Genotype assignment tests also appear to provide a clearer picture that conventional FSTs. It has been suggested that these tests, and particularly those based on Bayesian statistics, can still identify immigrants even when there is very low genetic differentiation between populations and no detectable isolation by distance. It is important to note that some of the loci used in this study were very highly polymorphic. Highly polymorphic microsatellite loci tend to underestimate levels of genetic divergence as estimate by FST (probably due to size homoplasy), thus diminishing their power to discriminate among samples.

The use of assignment tests, such as those used in this study, avoid this problem. Because of the low statistical significance of morphometric differentiation between populations from distinct biotopes that we observed, it is likely that immigration is overestimated by this method. However, likelihood and Bayesian assignment tests provided an identification of immigrants and non-immigrants that is much more statistically robust. Of particular interest is the observation that 10–22% of the bugs collected in the domiciles were immigrants from the peridomicle and sylvatic areas. It is also important to note that some domestic bugs emigrate to the peridomicle and sylvatic areas as well, resulting in dispersal in multiple directions.

Finally, these results are in agreement with our hypothesis of seasonal infestation of houses by flying adults and limited colonization of domiciles (Gourbiere S and others, unpublished data).
These data explain the short-term efficacy of insecticide spraying for the control of domestic infestation by *T. dimidiata* in the Yucatan Peninsula. It is thus crucial to take this particular behavior of *T. dimidiata* into account for effective control, and our results point towards potential alternative strategies aimed primarily at reducing bug dispersal into the domiciles with physical barriers such as mosquito screens. In addition, we observed an important role for the peridomicile as a transit area between sylvatic and domestic biotopes. This is in agreement with previous observations that peridomicile infestation and colonization is critical for reinfection of domiciles after control of several triatomine species. Accordingly, reducing the suitability of the peridomicile for triatomine infestation by removing potential bug refuges (cleaning and possibly insecticide spraying) may also improve *T. dimidiata* control. Lastly, the apparent elevated dispersal of *T. dimidiata* over large distances, likely involving a combination of diffusion-like local dispersal and passive human- or animal-mediated dispersal over larger distances, stresses the need for integrated control measures over large geographic areas to prevent rapid reinfection. Alternatively, this high dispersal may represent a key advantage for the introduction of paratransgenic control of *T. cruzi* transmission because it would facilitate the spread of *T. cruzi* resistance in natural *T. dimidiata* populations. This species thus represents an excellent model for further exploration of this novel control strategy. Testing some of these alternative strategies in field studies would provide confirmation of their usefulness for *T. dimidiata* control.

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