

A Preliminary Assessment of Genetic Differentiation of *Triatoma dimidiata* (Hemiptera: Reduviidae) in Guatemala by Random Amplification of Polymorphic DNA-Polymerase Chain Reaction

CLAUDIA I. CALDERÓN, PATRICIA L. DORN,¹ SERGIO MELGAR, JUAN JOSÉ CHÁVEZ,
ANTONIETA RODAS, REGINA ROSALES, AND CARLOTA M. MONROY

Laboratorio de Entomología Aplicada y Parasitología, Escuela de Biología, Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos, Edificio T-10 Ciudad Universitaria Zona 12, Ciudad de Guatemala, Guatemala

J. Med. Entomol. 41 (5): 882–887 (2004)

ABSTRACT The population genetics of *Triatoma dimidiata* (Latreille, 1811) from five different provinces in Guatemala, including three sylvan and three domestic populations, was investigated by random amplification of polymorphic DNA-polymerase chain reaction. There is a high degree of genetic variation in all of the *T. dimidiata* populations as evidenced by high levels of average expected heterozygosity and polymorphism. Domestic populations are more closely related to each other ($D = 0.05\text{--}0.085$, Nei's genetic distance) than are the sylvan ($D = 0.121\text{--}0.189$). Within the limited sample size of three populations, there was a correlation with geographic and genetic distance for the domestic populations, but not for the sylvan. Surprisingly, one of the sylvan populations was genetically very similar to the domestic populations. The F_{ST} demonstrated a high degree of differentiation at the country-wide level ($F_{ST} = 0.175$) and a moderate degree of differentiation within the sylvan ($F_{ST} = 0.135$) or domestic ($F_{ST} = 0.097$) populations. Although these results demonstrated that gene flow is limited between different provinces in Guatemala, hierarchical analysis showed that barriers between the Atlantic and Pacific drainage slopes were not biologically significant limiters of gene flow.

KEY WORDS *Triatoma dimidiata*, population genetics, random amplification of polymorphic DNA-polymerase chain reaction, sylvan, Guatemala

Triatoma dimidiata (LATREILLE, 1811) is one of the two main vectors of human Chagas disease (or American trypanosomiasis) in Guatemala, a parasitic infection caused by the hemoflagellate protozoan *Trypanosoma cruzi*. *T. dimidiata* is native to Latin America; its distribution encompasses the south of Mexico, throughout Central American and northern South America (Zeledón 1981). Unlike *Rhodnius prolixus* (Stal 1859), which seems to be almost entirely domestic and peridomestic in Central America, *T. dimidiata* is prevalent in domestic, peridomestic, and sylvan ecotopes (Monroy et al. 2003b), from sea level to $\approx 1,700$ m above sea level (Tabaru et al. 1999). Usinger (1944) suggested the division of *T. dimidiata* into subspecies because of its high morphological variability. This suggestion should be revisited based on recent molecular data obtained from ITS-2 sequences, which show that the Yucatecan *T. dimidiata* is significantly different from other populations of *T. dimidiata* (Marcilla et al. 2001).

T. dimidiata is widely distributed in Guatemala. It is present in 21 of 22 provinces (WHO 2002) with the highest rates of infestation in the southeastern provinces (e.g., 34.5% of the houses in regions of Jutiapa

and 25.4% in Santa Rosa) (Tabaru et al. 1999). The widespread distribution of this bug and apparent reinfestation after residual insecticide spraying present significant challenges to the Central American countries' initiative for the reduction of transmission of Chagas disease (Tabaru et al. 1999).

In spite of its major role as a Chagas vector, almost nothing is known about the population genetics of *T. dimidiata*. It seems that the variation in infestation and infection rates throughout the region are due to ecological and host factors and also could be related to differences in the populations of the vectors (Monroy et al. 2003a). An understanding of the epidemiology of the disease and effective control will be aided by an understanding of the genetic structure of *T. dimidiata*. For example, identification of distinct subpopulations of *T. dimidiata* would be indicative of genetic isolation that could have led subpopulations to differ in vector competence and/or sensitivity to insecticides.

Most of the work on population genetics of triatomids has involved isoenzyme analysis, which has been extremely useful in understanding the genetic structure of South American triatomids, especially *Triatoma infestans* (Klug 1834). However, because isoenzyme analysis is often limited to the few polymorphic

¹ Department of Biological Sciences, Loyola University New Orleans, 6363 St. Charles Ave., New Orleans, LA 70131.

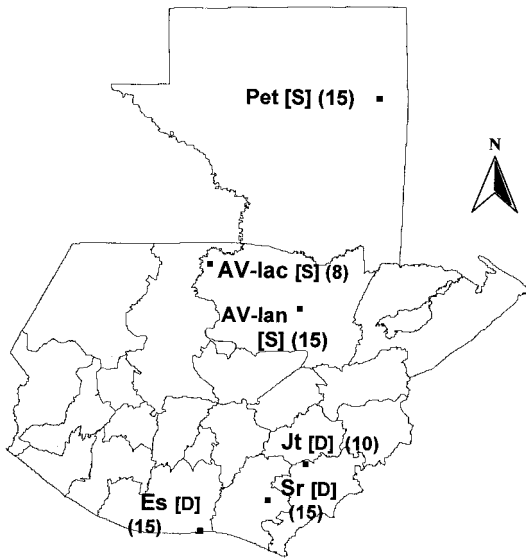


Fig. 1. Map of the Republic of Guatemala, indicating the six populations compared. Pet: Yaxjá, archeological site, Petén; AV-lac: National Park Laguna Lachuá, Alta Verapaz; AV-lan: Lanquín caves, Alta Verapaz; Jt: village El Carrizal, Jutiapa; Sr: village Agua Zarca, Santa Rosa; Es: village Puerto de San José, Escuintla; [S]: sylvan, [D]: domestic (number of individuals per locality).

loci available, García et al. (1998) proposed the application of random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) for both taxonomic and population genetic analyses of triatomids.

In previous work in our laboratory, RAPD-PCR results demonstrated that populations of *T. dimidiata* in houses within a village and among adjacent villages in Guatemala are panmictic (Dorn et al. 2003). To better understand the extent of panmixia and the genetic structure of *T. dimidiata* populations at the province level and between different ecotopes, we selected three sylvan and three domestic populations from different provinces in Guatemala. We investigated the amount of genetic variability within the domestic and sylvan populations and the degree of differentiation among these populations.

Materials and Methods

Specimen Collection. Seventy-eight *T. dimidiata* bugs were collected from six locations on the Atlantic (sylvan) and Pacific (domestic) slopes of Guatemala, between 1999 and 2001, to compare the genetic variability within and between populations (Fig. 1). Three sylvan populations from the Atlantic slopes were studied, including AV-Lachua, Yaxja, and AV-Lanquin. Eight bugs were collected within the national park Laguna Lachuá (15° 55'00" N, 90°40'30" W) in the municipality of Cobán, province Alta Verapaz (AV-Lachua population). The Yaxja population (17°09'15" N, 89°22'100" W) consisted of 15 bugs col-

lected from underground granaries in an ancient Mayan archeological site of the same name in Petén, in the municipality of Flores. The last sylvan site studied was Lanquín's dark and humid limestone caves (15°34'47" N, 89°59'30" W), where 15 bugs were collected. The other three sites represented the Pacific collections, which are domestic. Fifteen bugs were collected in San José (13°56'00" N, 90°14'28" W), province of Escuintla (13°56'00" N, 90°14'28" W). This population has never been treated with insecticides. The second domestic population was collected in province of Santa Rosa, at Aguazarca (14°09'23" N, 90°14'28" W), a hilly area where 15 bugs were collected. Finally, 10 bugs were collected in Jutiapa province, in the temperate village of El Carrizal (14°25'48" N, 89°57'28" W). It was not possible to compare domestic and sylvan populations from the same slope because sylvan populations have not been found on the Pacific slope of Guatemala and domestic populations are very rare on the Atlantic.

Each collected bug was placed in a separate vial and entered into the reference collection of the Laboratory of Applied Entomology and Parasitology (LENAP) at the University of San Carlos in Guatemala. Collection data included the life cycle stage or sex (for adults) and infection status with *T. cruzi*, which was analyzed by microscopy of the rectum and intestines (Dorn et al. 1999).

DNA Isolation. All bug's legs were removed and placed in 1.5- μ l microcentrifuge tubes containing 95% alcohol with 5% glycerol and stored at -4°C. DNA was isolated from the bug legs because they are free of parasite and blood meal contaminants. The process for DNA isolation was the same as described by Dorn et al. (2003), except for the modification in the last step, where the resulting pellet was resuspended and stored at -20°C until amplification.

RAPD-PCR. RAPD-PCR was performed with the four RAPD primers that had previously been shown to amplify triatomine DNA, as described by García et al. (1998) with slight modifications. Briefly, DNA was amplified in a 41- μ l reaction mixture containing: 1 \times PCR buffer (Applied Biosystems, Foster City, CA) 10 mM Tris-HCl (pH 8), 50 mM KCl, 3 mM MgCl₂, 40 pmol of selected decameric primer, and 200 μ M of each dNTP. Primers used included L1, L4, L5, and H3 (QIAGEN, Valencia, CA) (García et al. 1998). To each reaction mixture was added 2 μ l of DNA template and 1 U of *Taq*DNA polymerase for a final volume of 41 μ l. DNA was amplified using a GeneAmp PCR System 2400 thermal cycler (PerkinElmer Life and Analytical Sciences, Boston, MA) and the following cycle conditions: for L1, L4, and L5, 80°C for 15 min, 94°C for 1 min, 30°C for 2 min, 72°C for 1 min (two cycles); 94°C for 30 s, 40°C for 2 min, 72°C for 1 min (32 cycles); 95°C for 30 s, 40°C for 2 min, 72°C for 5 min (one cycle), hold at 4°C; and for H3, 80°C for 15 min, 94°C for 1 min, 30°C for 2 min, 72°C for 1 min (two cycles); 94°C for 30 s, 40°C for 2 min, 72°C for 1 min (32 cycles); 95°C for 30 s, 42.7°C for 2 min, 72°C for 5 min, (one cycle), hold at 4°C. The *Taq* was added once the thermocycler had reached 80°C. Negative controls containing all

components except DNA were included in all runs to detect contamination. In addition, some samples were tested in replicate to determine whether RAPD banding patterns were reproducible. We found that RAPD banding patterns were clearly reproducible in >60 comparisons (data not shown). Amplicons were run as stated in Dorn et al. (2003) for agarose gels.

The gel was digitized and the presence or absence of all bands was scored using GeneProfiler version 4.03 (Scanalytics, Merriemfield, WA). Because both homozygous dominant and heterozygous individuals show a band, Hardy-Weinberg equilibrium must be assumed when calculating genotypic frequencies. Nei's genetic distance was calculated from the loci with intermediate band frequencies (between 0.1 and 0.6) by using RAPDDIST (Tabachnick and Black 1997). These band frequencies were selected to obtain an accurate estimation of the genetic distance, without over- or underestimating the distance among populations by including very common or very rare alleles (Apostol et al. 1993). These frequency limits allow the selection of the most informative loci because these show bands at intermediate frequencies (between 0.2 and 0.8). An unweighted pair-group method with arithmetic average dendrogram was constructed with Phylip package version 3.5 (Felsenstein 1981). The fixation index F_{ST} , which measures the inbreeding effect caused by population structuring or reduction in heterozygosity due to genetic drift, was calculated using RAPDFST (Tabachnick and Black 1997). This program also calculates the effective number of mating migrants per generation (N_m) from the F_{ST} by using the formula: $F_{ST} = 1 / (4N_m + 1)$. In this study, the data obtained by the RAPD-PCR technique were converted to allelic frequencies with RAPDBIOS (Apostol et al. 1996) for subsequent analysis with BIOSYS-2 (Swofford et al. 1997) to analyze the genetic variability (mean expected heterozygosity per locus and percentage of polymorphic loci). Because RAPD-PCR markers are dominant, the heterozygosity must be calculated from q , the frequency of the recessive allele (lack of a band), assuming Hardy-Weinberg equilibrium. This calculated heterozygosity was then averaged over all loci to give the mean expected heterozygosity. The variance was partitioned into its component parts in a hierarchical manner at three levels: the total population of *T. dimidiata* sampled, each slope population (Atlantic and Pacific), and each individual population.

Results

RAPD-PCR results using primers H3, L1, and L5 showed mostly strong, consistent bands of similar intensity (data not shown). In contrast, primer L4 showed a strong monomorphic band around 500 bp and weaker additional bands. Amplification of *T. dimidiata* genomic DNA generated a total of 241 bands. Thirty-one bands with intermediate allele frequencies (between 0.1 and 0.6) were selected for further analysis.

Table 1. Geographic and Nei's genetic distance between populations within the same ecotope

Location	Ecotope	Geographic distance (km)	Nei's genetic distance
Jut/Sr	D/D	43.0	0.050
Esc/Sr	D/D	60.6	0.081
Esc/Jut	D/D	102.0	0.085
AV-Lac/AV-Lan	S/S	83.4	0.173
AV-Lan /Pet	S/S	187.3	0.189
AV-Lac /Pet	S/S	195.6	0.121

AV-Lan, Alta Verapaz-Lanquín; AV-Lac, Alta Verapaz-Lachuá; D, domestic; Esc, Escuintla; Jut, Jutiapa; Pet, Peten; Sr, Santa Rosa; S, sylvan.

Nei's Genetic Distance. Nei's genetic distance (D), calculated by pairwise comparisons between the same ecotope (domestic versus domestic or sylvan versus sylvan) showed a trend in which domestic populations are genetically more similar to each other than are sylvan populations (Table 1). In domestic populations, genetic distance seems to change with geographic distance; however, this is not the case in sylvan populations (Table 1).

Comparing the different ecotopes (domestic versus sylvan) two of the sylvan populations seem genetically distinct from the domestic populations (Table 2). The dendrogram constructed using Nei's genetic distance, by using a bootstrap analysis with 1000 iterations, shows a clear separation of the sylvan Petén and AV-Lanquín from the domestic populations. However, the sylvan population from AV-Lachuá, clusters with the domestic clades (Fig. 2). In contrast to the comparisons within ecotopes, there is no correlation between genetic and geographic distance between all populations pooled together ($\rho = 0.086$, $P = 0.761$).

Allele Frequency Variance among Populations. Restricted gene flow among subpopulations has resulted in structuring of the total population. A high degree of differentiation at the country-wide level (among all populations, $F_{ST} = 0.175$), has resulted in a calculated 1.2 mating migrants per generation (N_m). A moderate degree of differentiation, $F_{ST} = 0.135$ ($N_m = 1.6$) and 0.097 ($N_m = 2.3$) was observed for the Atlantic (sylvan)

Table 2. Nei's genetic distance and geographic distance between populations of different ecotopes

Location	Ecotope	Geographic distance (km)	Nei's genetic distance
Esc/AV-Lan	D/S	200.3	0.254
Esc/AV-Lac	D/S	220.5	0.084
Esc/Pet	D/S	387.3	0.167
Jut/AV-Lan	D/S	127.1	0.224
Jut/AV-Lac	D/S	182.2	0.041
Jut/Pet	D/S	309.2	0.125
Sr/AV-Lan	D/S	160.0	0.213
Sr/AV-Lac	D/S	201.1	0.040
Sr/Pet	D/S	345.9	0.118

AV-Lan, Alta Verapaz-Lanquín; AV-Lac, Alta Verapaz-Lachuá; D, domestic; Esc, Escuintla; Jut, Jutiapa; Pet, Peten; Sr, Santa Rosa; S, sylvan.

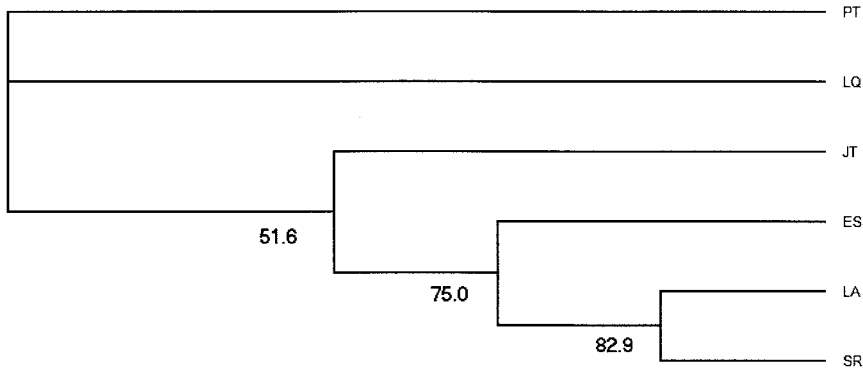


Fig. 2. Consensus unweighted pair-group method with arithmetic average dendrogram based on Nei's genetic distance, calculated using 31 RAPD-PCR loci (with 1000 iterations, by using Rapddist). This figure shows the relations between populations from all the study sites. (Numbers next to the nodes represent the percentage of bootstrap replicates.) LQ, Lanquín, Alta Verapaz; PT, Yaxjá, Petén; JT, Jutiapa, Santa Rosa; LA, National Park Laguna Lachuá, Alta Verapaz; and SR, village Agua Zarca, Santa Rosa.

van) and Pacific (domestic) slope populations, respectively (Hartl and Clark 1997).

Little genetic differentiation was observed among the Atlantic compared with domestic populations, $F_{ST} = 0.036$ ($N_m = 6.8$).

Heterozygosity and Hierarchical Analysis. The mean expected heterozygosity in domestic populations was calculated and found to be very high and similar to that found in the sylvan populations (Table 3). Values among individual populations were also similar and ranged from 0.283 to 0.355 in sylvan populations and from 0.310 to 0.348 in domestic populations. The percentage of polymorphic loci was also very high from a low of 73.3% in Petén to a high of 100% for AV-Lachuá. A hierarchical analysis of populations (Wright 1978) showed that the majority of the variance was found within the individual populations as opposed to between the Atlantic and Pacific slopes (Table 4).

Discussion

Genetic Distance. Previous work in our laboratory indicated that there was little genetic distance among populations of *T. dimidiata* found in houses within and among adjacent villages (Dorn et al. 2003). In this study, we find a three- to five-fold greater distance among domestic populations from different provinces than was found among houses or adjacent villages.

Table 3. Expected heterozygosity and percentage of polymorphism in each population

Pop	Mean Heterozygosity	% of Polymorphic loci
S Petén	0.283	73.33
S AV-Lanquín	0.352	93.33
S AV-Lachuá	0.355	100.00
D Jutiapa	0.310	76.67
D Santa Rosa	0.342	90.00
D Escuintla	0.348	83.33

Genetic distance among the sylvan populations is even greater, more than twice that of the domestic populations from different provinces, and an even higher genetic distance is evident between all the provincial populations.

Population genetic studies for other triatomid species are largely based on isoenzyme analyses. Among domestic and sylvan *T. infestans* populations genetic distance from these studies were estimated at $D = 0.001-0.004$ (Dujardin et al. 1987) and among nine colonies from three different countries at $D = 0.001-0.011$ (García et al. 1995). Sylvan "dark morph," compared with the domestic *T. sordida* (Stal 1859), resulted in a greater genetic distance estimate, $D = 0.037$ (Noireau et al. 2000). However, because results based on isoenzymes may not be directly comparable with those based on RAPD-PCR markers, further work is required, using other triatomids and *T. dimidiata*, with the same technique to gain a comprehensive understanding of the differences in genetic distance among populations of different Triatomine species.

The "isolation by distance model," first proposed by Dujardin et al. (1988) and confirmed for *T. infestans* (Breniere et al. 1998), may partially explain the increasing genetic distance with geographic distance for *T. dimidiata* domestic populations (separated by <100 km). A correlation between genetic and geographical distances was observed for populations of *Triatoma brasiliensis* Neiva 1911 by using RAPD-PCR (Borges et al. 2000). However, no such correlation is evident for the *T. dimidiata* sylvan populations tested here (all at >80-km separation). Higher genetic distance among

Table 4. Hierarchical analysis: variance components and F-statistics combined across loci

Comparison	Variance component	F_{xy}
Slope-total	-0.39942	-0.036
Population-slope	2.01037	0.174
Population-total	1.61096	0.145

sylvan (compared with domestic) populations could be due to greater habitat heterogeneity and therefore more diverse selective pressures present in different environments, which include ancient Mayan granaries (Petén), caves (AV-Lanquín), and forest (AV-Lachúa). It is known that the process of domestication, presumably due to selection under homogeneous and stable microhabitat inside the houses, results in a reduction of the genetic variability (Schofield et al. 1999).

Clustering patterns in the Nei's distance dendrogram was somewhat surprising (Fig. 2). For example, the sylvan Lachúa population clusters with the domestic populations whereas the other two sylvan populations are distinct. The separation of sylvan Lanquín from other Guatemalan domestic populations also is supported using head metric characters by traditional morphometrics (D. M. Bustamante, personal communication). (Lachúa was not tested in the morphometry study.) The close association of AV-Lachúa with the domestic populations may be due, at least in part, to the small sample size (reflected in low bootstrap values), or perhaps to the high amount of human migration into this area. Large numbers of refugees from Mexico were resettled in the area around Lachua Park and a major transit route exists nearby. It is possible that triatomids originating in domestic habitats could have been passively transported to AV-Lachúa in the belongings of the migrants, although there is no direct evidence of this. Passive transport also could explain the greater similarity of the domestic populations to each other.

The domestic Jutiapa population occupies a branch on the dendrogram separate from the other two domestic populations. Jutiapa shows some of the highest infestation rates in Guatemala and bugs are not found in surrounding forest. Therefore, this population may be more reproductively isolated than others that are still cocirculating in domestic and sylvan habitats. The behavior of *T. dimidiata* is significantly different in different localities, e.g., *T. dimidiata* from the Yucatán seems to be mostly sylvan and enters houses only during particular months for reproduction (Dumontel et al. 2002). Therefore, the level of domestication will affect the degree of genetic distance among these populations.

Population Structuring. The fixation indices support division of the Guatemalan *T. dimidiata* population into subpopulations. Populations within a particular slope (Atlantic or Pacific) showed a moderate degree of structuring, $F_{ST} = 0.135$ and 0.097 , respectively, and at the country-wide level a great degree of structuring, $F_{ST} = 0.175$. This is in marked contrast to what was found within a village or between adjacent villages where very little genetic differentiation is found ($F_{ST} = 0.025$ and 0.019 , respectively) (Dorn et al. 2003). These higher amounts of population structure (between slopes) are still less than that seen by RAPD-PCR among different species of triatomids (Wright's $F_{ST} = 0.29$).

The hierarchical analysis showed that with respect to gene flow, the Atlantic and Pacific drainage slopes

are not biologically significant as barriers to gene flow. Future studies analyzing regions defined by their topography and other parameters such as rainfall and vegetation type also may contribute to understanding the distribution of triatomids and defining risk for Chagas disease transmission (Carcavallo 1999, Dumonteil et al. 2002, Gorla 2002).

Genetic Variability. *T. dimidiata* in Guatemala shows a high amount of genetic variability as evidenced by the high levels of polymorphism (73–100%) and high average expected heterozygosity (0.283–0.355), as is often seen with RAPD-PCR (Dorn et al. 2003). These rates were uniformly high for all populations, showing that at least in this sampling of six populations from across Guatemala, there has not been a significant reduction in genetic variability, not even in the domestic populations due to genetic drift. This is similar to the results obtained in an earlier study analyzing *T. dimidiata* within a village and among adjacent villages (Dorn et al. 2003). Due to the difficulty of obtaining large numbers of (especially sylvan) specimens (Harry et al. 1992, Dujardin et al. 1998, Zeledón et al. 2001), these conclusions must be considered preliminary and will require confirmation with a greater number of samples.

Acknowledgments

We thank the residents of the villages and the park rangers for cooperation with the study. Special thanks to Donald P. Hauber for critical review of the manuscript. We also appreciate the technical assistance of Patricia Landaverde, Franklin Herrera, Barbara Moguel, and the Enfermedades Transmitadas por Vectores (vector-borne diseases) technicians of the Ministry of Health of Guatemala. The manuscript was significantly improved through the helpful comments of anonymous reviewers. Research was funded by Central American Network for Tropical Disease Research (Ne-Tropica) grant to C.M.M. and the National Institutes of Health Grant R15 AI45523 awarded to P.D.

References Cited

- Apostol, B. L., W. C. I. Black, P. Reiter, and B. R. Miller. 1996. Population genetics with RAPD-PCR markers: the breeding structure of *Aedes aegypti* in Puerto Rico. *Heredity* 76: 325–334.
- Apostol, B. L., W. C. Black IV, B. R. Miller, P. Reiter, and B. Beaty. 1993. Estimation of the number of full sibling families at an oviposition site using RAPD-PCR markers: applications to the mosquito *Aedes aegypti*. *Theor. Appl. Genet.* 86: 991–1000.
- Borges, E. C., J. P. Dujardin, C. J. Schofield, A. J. Romanha, and L. Diotaiuti. 2000. Genetic variability of *Triatoma brasiliensis* (Hemiptera: Reduviidae) populations. *J. Med. Entomol.* 37: 872–877.
- Breniere, S. F., M. F. Bosseno, F. Vargas, N. Yaksic, F. Noi-reau, S. Noel, J. P. Dujardin, and M. Tibayrenc. 1998. Smallness of the panmictic unit of *Triatoma infestans* (Hemiptera: Reduviidae). *J. Med. Entomol.* 35: 911–917.
- Carcavallo, R. U. 1999. Climatic factors related to Chagas disease transmission. *Mem. Inst. Oswaldo Cruz* 94 (suppl 1): 367–369.
- Dorn, P., D. Engelke, A. Rodas, R. Rosales, S. Melgar, B. Brahney, J. Flores, and C. Monroy. 1999. Utility of the

- polymerase chain reaction in detection of *Trypanosoma cruzi* in Guatemalan Chagas' disease vectors. *Am. J. Trop. Med. Hyg.* 60: 740–745.
- Dorn, P. L., S. A. Melgar, V. Rouzier, A. Gutierrez, C. Combe, R. Rosales, A. Rodas, S. Kott, D. Salvia, and C. Monroy. 2003. The Chagas vector, *Triatoma dimidiata* (Hemiptera: Reduviidae), is panmictic within and among adjacent villages in Guatemala. *J. Med. Entomol.* 40: 436–440.
- Dujardin, J. P., C. La Fuente, L. Cardozo, and M. Tibayrenc. 1988. Dispersing behaviour of *T. infestans*: evidence from a genetical study of field populations in Bolivia. *Mem. Inst. Oswaldo Cruz* 83 (suppl 1): 435–440.
- Dujardin, J. P., M. Tibayrenc, E. Venegas, L. Maldonado, P. Desjeux, and F. J. Ayala. 1987. Isozyme evidence of lack of speciation between wild and domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. *J. Med. Entomol.* 24: 40–45.
- Dujardin, J. P., M. Munoz, T. Chavez, C. Ponce, J. Moreno, and C. J. Schofield. 1998. The origin of *Rhodnius prolixus* in Central America. *Med. Vet. Entomol.* 12: 113–115.
- Dumonteil, E., S. Gourbiere, M. Barrera-Perez, E. Rodriguez-Felix, H. Ruiz-Pina, O. Banos-Lopez, M. J. Ramirez-Sierra, F. Menu, and J. E. Rabinovich. 2002. Geographic distribution of *Triatoma dimidiata* and transmission dynamics of *Trypanosoma cruzi* in the Yucatan peninsula of Mexico. *Am. J. Trop. Med. Hyg.* 67: 176–183.
- Felsenstein, J. 1981. PHYLIP: phylogeny inference package computer program, version 3.5c. Seattle, WA.
- García, A. L., H. J. Carrasco, C. J. Schofield, J. R. Stothard, I. A. Frame, S. A. Valente, and M. A. Miles. 1998. Random amplification of polymorphic DNA as a tool for taxonomic studies of triatomine bugs (Hemiptera: Reduviidae). *J. Med. Entomol.* 35: 38–45.
- García, B. A., J. M. Soares Barata, and A. Blanco. 1995. Enzyme polymorphism among *Triatoma infestans* (Hemiptera: Reduviidae) colonies. *J. Med. Entomol.* 32: 126–133.
- Gorla, D. E. 2002. Variables ambientales registradas por sensores remotos como indicadores de la distribución geográfica de *Triatoma infestans*. *Ecol. Austral.* 12: 117–127.
- Harry, M., I. Galindez, and M. L. Cariou. 1992. Isozyme variability and differentiation between *Rhodnius prolixus*, *R. robustus*, and *R. pictipes*, vectors of Chagas disease in Venezuela. *Med. Vet. Entomol.* 6: 37–43.
- Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics. Sinauer, Sunderland, MA.
- Marcilla, A., M. D. Bargues, J. M. Ramsey, E. Magallon-Gastelum, P. M. Salazar-Schettino, F. Abad-Franch, J. P. Dujardin, C. J. Schofield, and S. Mas-Coma. 2001. The ITS-2 of the nuclear rDNA as a molecular marker for populations, species, and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. *Mol. Phylogenet. Evol.* 18: 136–142.
- Monroy, C., A. Rodas, M. Mejia, R. Rosales, and Y. Tabaru. 2003a. Epidemiology of Chagas disease in Guatemala: infection rate of *Triatoma dimidiata*, *T. nitida* and *R. prolixus* (Hemiptera, Reduviidae) with *Trypanosoma cruzi* and *Trypanosoma rangeli* (Kinetoplastida, Trypanosomatidae). *Mem. Inst. Oswaldo Cruz* 98: 305–310.
- Monroy, M. C., D. M. Bustamante, A. G. Rodas, M. E. Enriquez, and R. G. Rosales. 2003b. Habitats, dispersion and invasion of sylvatic *Triatoma dimidiata* (Hemiptera: Reduviidae: Triatominae) in Petén, Guatemala. *J. Med. Entomol.* 40: 800–806.
- Noireau, F., B. Bastrenta, S. Catala, J. P. Dujardin, F. Panzera, M. Torres, R. Perez, C. Galvao, and J. Jurberg. 2000. Sylvatic population of *Triatoma infestans* from the Bolivian Chaco: from field collection to characterization. *Mem. Inst. Oswaldo Cruz* 95: 119–122.
- Schofield, C. J., L. Diotaiuti, and J. P. Dujardin. 1999. The process of domestication in Triatominae. *Mem. Inst. Oswaldo Cruz* 94: 375–378.
- Swofford, D. L., R. B. Selander, and W. Black IV. 1997. Biosys-2 computer program, version 1/June/1997. Fort Collins, CO.
- Tabachnick, W., and W. Black IV. 1997. Population Genetics in vector biology, pp. 417–437. In J. Crampton, C. Beard and C. Louis [eds.], *The molecular biology of insect disease vectors: a methods manual*. Chapman & Hall, New York.
- Tabaru, Y., C. Monroy, A. Rodas, M. Mejia, and R. Rosales. 1999. The geographical distribution of vectors of Chagas' disease and populations at risk of infection in Guatemala. *Med. Entomol. Zool.* 50: 9–17.
- Usinger, R. 1944. The Triatominae of North and Central America and the West Indies and their public health significance. *Public Health Bull.* 81.
- [WHO] World Health Organization. 2002. Control of Chagas disease, WHO Technical Report Series 905. World Health Organization, Geneva, Switzerland.
- Wright, S. 1978. Evolution and the genetics of populations. University of Chicago Press, Chicago, IL.
- Zeledón, R. 1981. El *Triatoma dimidiata* (Latreille, 1811) y su relación con la enfermedad de Chagas. Editorial Universidad Estatal a Distancia, San José, Costa Rica.
- Zeledón, R., J. A. Ugalde, and L. A. Paniagua. 2001. Entomological and ecological aspects of six sylvatic species of triatomines (Hemiptera, Reduviidae) from the collection of the national biodiversity, Institute of Costa Rica, Central America. *Mem. Inst. Oswaldo Cruz* 96: 757–764.

Received 18 August 2003; accepted 28 May 2004.