

Migration and Gene Flow Among Domestic Populations of the Chagas Insect Vector *Triatoma dimidiata* (Hemiptera: Reduviidae) Detected by Microsatellite Loci

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ABSTRACT *Triatoma dimidiata* (Latreille, 1811) is the most abundant and significant insect vector of the parasite *Trypanosoma cruzi* in Central America, and particularly in Guatemala. *Tr. cruzi* is the causative agent of Chagas disease, and successful disease control requires understanding the geographic distribution and degree of migration of vectors such as *T. dimidiata* that frequently re-infest houses within months following insecticide application. The population genetic structure of *T. dimidiata* collected from six villages in southern Guatemala was studied to gain insight into the migration patterns of the insects in this region where populations are largely domestic. This study provided insight into the likelihood of eliminating *T. dimidiata* by pesticide application as has been observed in some areas for other domestic triatomines such as *Triatoma infestans*. Genotypes of microsatellite loci for 178 insects from six villages were found to represent five genetic clusters using a Bayesian Markov Chain Monte Carlo method. Individual clusters were found in multiple villages, with multiple clusters in the same house. Although migration occurred, there was statistically significant genetic differentiation among villages ($F_{RT} = 0.05$) and high genetic differentiation among houses within villages ($F_{SR} = 0.11$). Relatedness of insects within houses varied from 0 to 0.25, i.e., from unrelated to half-sibs. The results suggest that *T. dimidiata* in southern Guatemala moves between houses and villages often enough that recolonization is likely, implying the use of insecticides alone is not sufficient for effective control of Chagas disease in this region and more sustainable solutions are required.

KEY WORDS Chagas disease, *Triatoma dimidiata*, dispersal, vector control, genetic diversity

Understanding migration and gene flow of insect vectors is critical to effective control and elimination of vector-borne diseases. Of more than 140 species of triatomines, less than a couple dozen are responsible for the bulk of the transmission to humans of *Tr. cruzi*, the parasite that causes Chagas disease (Stevens et al. 2011). Chagas disease is the most serious parasitic disease in Latin America, with an estimated seven million people infected with *Tr. cruzi*, 60 million people living at risk of contracting the disease, and 23,000 deaths

annually (Hotez et al. 2008). The triatomine species that transmit *Tr. cruzi* to people differ in their epidemiological importance, and this difference is strongly influenced by vector movement (Lent and Wygodzinsky 1979). Immature nymphs can migrate, actively walking tens of meters, and adults of some species are known to fly up to 200 m, or even 2 km (Schweigmann et al. 1988). Triatomines can also migrate passively by carriage in wood, personal effects, agricultural products, or by birds (Lent and Wygodzinsky 1979). The field of population genetics can be used to understand the genetic structure of triatomine species and to quantify migration and gene flow, important parameters for designing control strategies effective against local species and populations.

Triatoma dimidiata (Latreille, 1811) is the major Chagas disease vector in Central America. Its range extends from southern Mexico throughout Central America, and into South America (Venezuela, Colombia, Ecuador, and northern Peru; Ramirez et al. 2005). *Triatoma dimidiata* varies morphologically, genetically, and biochemically over this geographic range with an unnamed sibling species identified in northern Guatemala (Petén); Yucatán, Mexico; and Belize (Panzera

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et al. 2006, Dorn et al. 2007, BARGUES et al. 2008, Dorn et al. 2009). *Triatoma dimidiata* shows variability in inhabiting domestic, peri-domestic, and sylvatic habitats (Zeledon et al. 1973, Nakagawa et al. 2003, Zeledon et al. 2005, Dorn et al. 2007, Blandon-Naranjo et al. 2010), and the tendency to colonize houses is of particular importance for transmission to people. In the hyperendemic area of southern Guatemala, as well as in Ecuador, only a single lineage of *T. dimidiata* occurs and it is found exclusively in domestic and peri-domestic habitats, often with high house infestation (Monroy et al. 2003b, BARGUES et al. 2008). Within southern Guatemala, the department of Jutiapa has had some of the highest house infestation rates in Guatemala (35% infestation), and bugs are not found in the surrounding remnant forest (Tabaru et al. 1999, Monroy et al. 2003b, Zeledon and Rojas 2006). In contrast, in northern Guatemala, Costa Rica, and Colombia, *T. dimidiata* remains in the sylvan environment, only rarely visiting houses (Monroy et al. 2003c; Zeledon and Rojas 2006). In the Yucatán, Mexico, *T. dimidiata* enters houses seasonally from April to June (Dumonteil et al. 2002, Monroy et al. 2003a).

Although information is available regarding the habitat preference of *T. dimidiata* in different localities (Zeledon et al. 1973, Monroy et al. 2003b, Zeledon et al. 2005, Dorn et al. 2007, BARGUES et al. 2008, Blandon-Naranjo et al. 2010), little is known if migration and gene flow differ among localities and habitats. Preliminary studies examining genetic differentiation among sylvatic locations 80–200 km apart, using Random Amplified Polymorphic DNA by the Polymerase Chain Reaction (RAPD-PCR), showed significant genetic differentiation ($D = 0.121\text{--}0.189$, Nei's genetic distance), indicating little gene flow (Calderon et al. 2004). However, the same study found domestic populations 40–100 km apart showed more gene flow, indicated by lower genetic differentiation ($D = 0.05\text{--}0.085$). On a smaller spatial scale of meters rather than kilometers, domestic populations showed migration among three households 80–160 m apart within the rural community of Aguazarca, Santa Rosa, Guatemala ($D = 0.013\text{--}0.022$) and between nearby villages Aguazarca and El Cuje (~25 km distant, $D = 0.0199$) by RAPD-PCR (Dorn et al. 2003). The low genetic differentiation indicates that gene flow, i.e., migration or passive transport, occurs among households as well as among villages. These results used RAPD markers, which can be inaccurate, and were based on only three houses and two villages (Dorn et al. 2003) or among three villages (Calderon et al. 2004). To gain a better picture of the ecology of *T. dimidiata*, we conducted further population genetic studies to determine if these observations apply elsewhere, in particular in an endemic area of Guatemala.

Microsatellite loci are valuable markers for understanding the genetic structure of vector populations (e.g., Anderson et al. 2002, Dumonteil et al. 2007, Pizarro et al. 2008). These loci are highly variable, differing in the number of repeats due to polymerase slippage during replication (Brinkmann et al. 1998). The large number of

alleles allows fine-scale discrimination of closely related individuals, providing the statistical power to understand gene flow and the genetic structure of populations (Hardy et al. 2003). In addition, microsatellite loci are more reproducible than RAPD-PCR markers and most microsatellite loci (except perhaps tri-nucleotide repeats) are noncoding, hence neutral, markers.

To understand migration and gene flow of *T. dimidiata*, seven microsatellite loci were examined in 178 individuals to analyze the population genetic structure and gene flow, within and among six villages, in southern Guatemala where Chagas disease is hyperendemic.

Materials and Methods

Specimen Collection. The 178 *T. dimidiata* specimens examined were collected by trained personnel from the Monroy laboratory and the Guatemalan Ministry of Health using the person/hour method (Monroy et al. 2003c) from houses in six villages in the Department (State) of Jutiapa, Guatemala, during August–September 2001 and January 2002 before fumigation (Fig. 1; Table 1). The study was approved for informed oral consent by two review boards: the World Health Organization Tropical Disease Research (WHO-TDR) and the Dirección General de Investigación de la Universidad de San Carlos. All residents provided informed oral consent, which was documented on a survey sheet prior to bug collection. Five villages, Calderas, El Carpintero, El Copante, El Tablón, and Tunillas, are located within the Municipality of San José Acatempa and one village, La Brea, is located within the Municipality of Quezada. All are rural communities with houses at least 50 m apart. The region is experiencing rapid deforestation, and the villages are nestled among forest remnants and agricultural crops on rolling hills. There are no major topographical barriers, such as mountains or large rivers, in the area.

The live insects were placed in vials with folded paper, and in most cases the house where the insect was collected was recorded. Insects were transported to the Universidad de San Carlos, Guatemala City, Guatemala, where the life cycle stage (first- through fifth-instar nymphs) or sex (for adults) and infection status with *Tr. cruzi* (assessed by microscopic examination of the contents of the intestines and rectum; [Pizarro et al. 2007]) were recorded. The legs of each bug were removed and placed in 95% alcohol and 5% glycerol and stored at -20°C . For each village, the number of insects analyzed, the average number of insects per house (for insects where the house of collection is known), and the number of houses examined are shown in Table 1.

Molecular Genetic Analysis. *DNA Isolation.* Insect legs were transported to Loyola University New Orleans and stored at -80°C until DNA was isolated using published methods (Dorn et al. 2003).

Microsatellite Amplification. PCR amplification of seven microsatellite loci (Table 2) was performed in 10 μl reactions: 5 μl Multiplex PCR Master Mix 2x (Qiagen, Valencia, CA), 2 μl H_2O , 1 μl Q-solution, 1 μl



Fig. 1. Map showing the location of the six villages in Jutiapa, Guatemala.

Table 1. *T. dimidiata* collection information for six villages in Jutiapa, Guatemala

Feature	Calderas	El Tablón	El Carpintero	La Brea	Tunillas	El Copante
No. insects (<i>n</i>)	30	30	30	30	30	28
n/house ^a average	2.7	3.0	2.2	2.1	2.5	2.6
n/house range	1–6	1–6	1–5	1–7	1–5	1–6
No. of houses	6	9	13	13	6	7
House unknown	14	3	2	0	15	12
Latitude (N)	14° 13'16"	14° 14'35"	14° 14'12"	14° 13'44"	14° 19'43"	14° 15'00"
Longitude (W)	90° 04'52"	90° 07'50"	90° 06'30"	90° 03'40"	90° 03'50"	90° 06'40"
Meters above sea level	1240	1310	1245'	1140	1250	1350

^a Only for insects where the house of collection is known.

primer mix, and 200 ng of DNA. The reactions were multiplexed in two groups. The loci in group 1 (and their fluorescent dye) included—TDMS09 (HEX), TDAK01 (FAM), TDAK17 (FAM), and TDAK20 (NED). Group 2 included—TDMS22 (HEX), TDAK04 (NED), and TDAK49 (FAM). TDMS22 is a tetra-nucleotide repeat; the other six loci are di-nucleotide repeats. The amplification consisted of 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 52°C for 90 s, 72°C for 60 s, and a final extension at 72°C for 10 min, using a Techne Thermocycler (TC-512, Burlington, NJ). The PCR product was diluted 1/20 with water, and 0.5 µl of diluted product from each insect was mixed with 14.6 µl of formamide and 0.40 µl of LIZ 500 size standard (Applied Biosystems, Foster City, CA). The sizes of the resulting fluorescently labeled DNA fragments were determined using either an Applied BioSystems 3100-Avant Genetic Analyzer (University of Vermont Cancer Center, DNA Analysis Facility) or Applied BioSystems 3730xl DNA Analyzer (Cornell University, Life Sciences Core Laboratories Center) and data recorded in .fsa format data files.

Fragment Analysis. The fsa data files were viewed using GeneMapper version 3.7 (Applied Biosystems, Foster City, CA) to identify and classify the alleles present per locus and per insect.

Statistical Methods. *Summary Statistics for Each Locus and Village.* For each locus, the allele size range, average number of alleles per village, and percent of individuals with missing data were summarized. For each village, genetic diversity was estimated using Nei's unbiased estimator of H_S (Nei and Chesser 1983) and unbiased allelic richness (El Mousadik and Petit 1996) using FSTAT (Goudet 1995, 2002).

Estimating Population Genetic Structure. Two approaches were used to estimate the genetic structure. First because the villages and houses represent discrete physical units, multilocus versions of Wright's F statistics (Wright 1978) were used in a hierarchical AMOVA (Arlequin ver. 3.51, Excoffier and Lischer 2010) to test for significant population structure among villages (F_{RT}) and among houses within villages (F_{SR}). F_{IS} values are not presented because they cannot be accurately estimated given the small number of individuals per house; however, relatedness (Lynch and Ritland 1999) of insects within houses and within villages

was calculated using GenALEX version 6.3 (Peakall and Smouse 2006). To facilitate interpretation of F statistics based on microsatellite loci, we calculated the maximum possible differentiation among villages $F_{RT(\max)}$ (Hedrick 2005). This allows standardization of F values because for loci with more than two alleles, the magnitude is dependent on the amount of genetic variation ($F_{RT} < 1 - H_R$, where $1 - H_R$ is the average within-subpopulation homozygosity [Hedrick 2005]); the maximum value may be much lower than 1 for highly variable loci. Letting p_{ij} be the frequency of allele i in subpopulation j , the maximum F value is:

$$F_{RT(\max)} = 1 - \frac{H_{T(\max)} - H_S}{H_{T(\max)}}$$

where $H_{T(\max)} = 1 - \frac{1}{k^2} \sum_i \sum_j p_{ij}^2$ and

$H_S = 1 - \frac{1}{k} \sum_i \sum_j p_{ij}^2$. The standardized F value is:

$F'_{RT} = F_{RT}/F_{RT(\max)}$. In addition, isolation-by-distance was tested (GENEPOP ver. 4.1.4, Watts et al. 2007, Rousset 2008). The statistic $\hat{\epsilon}$ was used to estimate the neighborhood size (Watts et al. 2007) as the slope of the regression of $\hat{\epsilon}$ on $\ln(\text{distance})$.

The second approach to assessing genetic population structure used Bayesian Markov chain Monte Carlo (MCMC) simulation (Pritchard et al. 2000) to estimate the number of genetic clusters and then determined the geographic distribution of the genetic clusters among villages and among houses within a village. The number of genetic clusters among the sample of all 178 insects was determined with the software STRUCTURE version 2.3.2 (Pritchard et al. 2000) using the recommended conservative settings: correlated allele frequencies (migration or shared ancestry may lead to similar frequencies in different populations) and admixture (allows that some insects might be progeny of migrants). A single run of the algorithm starts with an initial random association of alleles in K clusters and iteratively rearranges the alleles so that variation within clusters is minimized and variation among clusters is

Table 2. Genetic information for the seven microsatellite loci analyzed in 28–30 *T. dimidiata* from each of six villages in Jutiapa, Guatemala

Locus ^a	Accession number	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)	Na	null alleles (%)
TDMS09	AF487985	cat tgc aat cgt gtc gaa at	tgc cca aaa ttt teg tgt ct	157–205	10.2	1.7
TDMS22	AF487989	tat tat ggt tgc cgg tat taa gg	cca ccc aag ttt tac tat cca	98–160	5.8	0
TDAK01	AY695416	gaa aft agc tca aga aca ctc c	acc aaa gca tgc caa gta gg	169–215	11.0	11.2
TDAK04	AY695418	age gat ttg tet cct ttg c	ttc ttt teg gtc ceg tag tg	130–208	13.5	2.2
TDAK17	AY695419	tct tcc aac cat ttt teg ttt tg	cgt cta cta aag gat cta tca aat tt	120–142	8.5	0
TDAK20	AY695420	tgt cgt cca agt tta ttt gct c	tga ttt ett cta ttt cgt ttg gaa	119–188	11.8	6.2
TDAK49	AY695423	tat cat aga ccc aca cgg t	taa atg gca ggt tgg att cg	144–178	9.8	1.1

The locus name, based on previous publication or information in GenBank for unpublished loci, GenBank accession number, nucleotide sequences for forward and reverse primers, size of the amplified fragment in base pairs (bp), the average number of alleles per village (Na), and percent of individuals with null alleles for each locus are indicated.

^a TDMS are from (Anderson et al. 2002), TDAK (http://www.ncbi.nlm.nih.gov/nucore/ACCESSION_NUMBER).

maximized. Each replicate compares the simulated clusters to a random clustering and estimates the likelihood of the data $\ln(K)$ for the specified K (number of clusters). We simulated 10 independent replicates for each value of K from 2 to 6. Each replicate included a burn-in period to ensure the results did not vary depending on the starting configuration. Previous studies (Evanno et al. 2005) recommend a burn in of at least 20,000 iterations followed by 20,000 iterations. We used a burn in of 100,000 iterations followed by 100,000 iterations, although preliminary analyses showed values $>20,000$ did not significantly change the results. The appropriate number of clusters was determined with the statistic ΔK (Evanno et al. 2005); however, previous work has shown that with a hierarchical island population structure (i.e., houses within villages), STRUCTURE will detect the uppermost level of structure, in this case villages (Evanno et al. 2005). The distribution of genetic clusters within and among villages was visualized using CLUMPP ver. 1.1.2 and DISSTRUCT ver. 1.1 (Rosenberg 2004, Jakobsson and Rosenberg 2007) to clarify relationships.

Test for Biased Dispersal and Variation in Infection Prevalence. We tested for biased dispersal between males and females and between *Tr. cruzi*-infected and uninfected insects. Two parameters for biased dispersal were analyzed with FSTAT Version 2.9.3.2 (Goudet et al. 2002) using 10,000 randomizations. First, F_{ST} which should be lower in the group with greater

dispersal, was compared between the pairs described above. The second test compares the variance of assignment index, corrected for among-population variation in gene diversity (vA_{IC}). Within a village, the dispersing group will include both residents (with common genotypes) and immigrants (with rare genotypes), so that vA_{IC} will be larger for the dispersing group (Goudet et al. 2002, Prugnolle and de Meeus 2002). In addition to testing for biased dispersal, we used a nested ANOVA to test for a difference in the prevalence of insect infection with *Tr. cruzi* among houses within villages and among villages.

Results

Summary Statistics for Each Locus and Village. As expected for microsatellite loci, all seven loci are highly polymorphic (6–14 alleles per locus, per village, Table 2). The genetic variability of these loci is quite similar among the six villages; allelic richness varies from 9–11 and Nei's H_S ranges from 0.74 to 0.85 (Fig. 2).

Estimating Population Genetic Structure. Hierarchical AMOVA (Table 3) indicates small, but statistically significant, differences among villages ($F_{RT}=0.049$) and among houses within villages ($F_{SR}=0.110$). Standardization of F_{RT} by $F_{RT(max)}=0.217$ gives a value of $F'_{RT}=F_{RT}/$

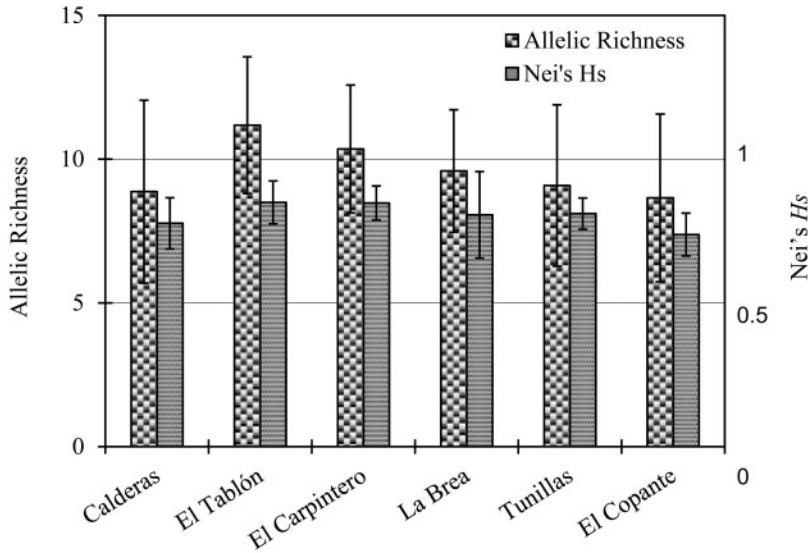


Fig. 2. Similarity of the genetic variability of seven microsatellite loci in *T. dimidiata* from six villages in Jutiapa, Guatemala, based on allelic richness and Nei's H_S .

Table 3. Hierarchical AMOVA shows significant population structure among villages (F_{RT}) and among houses within villages (F_{SR})

Source of variation	df	Sum of squares	Variance components	Fixation Index	<i>P</i>
Among villages (R)	5	53.16	0.13	$F_{RT}=0.049$	< 0.001
Among houses within villages (S)	24	127.30	0.29	$F_{SR}=0.110$	< 0.001
Total	213	582.98	2.77		

$F_{RT(max)} = 0.226$. Average relatedness among insects was at the level of second cousins ($r = 0.031$) within four villages and higher in two villages (close to the level of first cousins, $r = 0.125$; Fig. 3). Some migration among houses within villages is apparent because relatedness was significantly greater than zero in 60% of the houses with three or more insects (12/20). In eight of these houses, the relatedness was at the level of half-sibs ($r = 0.25$) or higher. In only one of the 20 houses is there evidence that all the insects could be from the same parents (i.e., standard error of the mean includes

$r = 0.5$, the level of full sibs). The isolation-by-distance analysis (Fig. 4) indicates that related individuals are spatially near each other; however, the correlation is not very strong: $\hat{e} = 0.0719 + 0.0165 \times \ln(\text{distance})$, 95% confidence interval for slope [0.0061–0.0292]. The estimate of the neighborhood size is $Nb = 60$.

With hierarchical island structure, such as houses within villages, we expect the Bayesian MCMC simulations from STRUCTURE to detect the highest level of structure i.e., villages (Evanno et al. 2005). The STRUCTURE results (Fig. 5) found five genetic

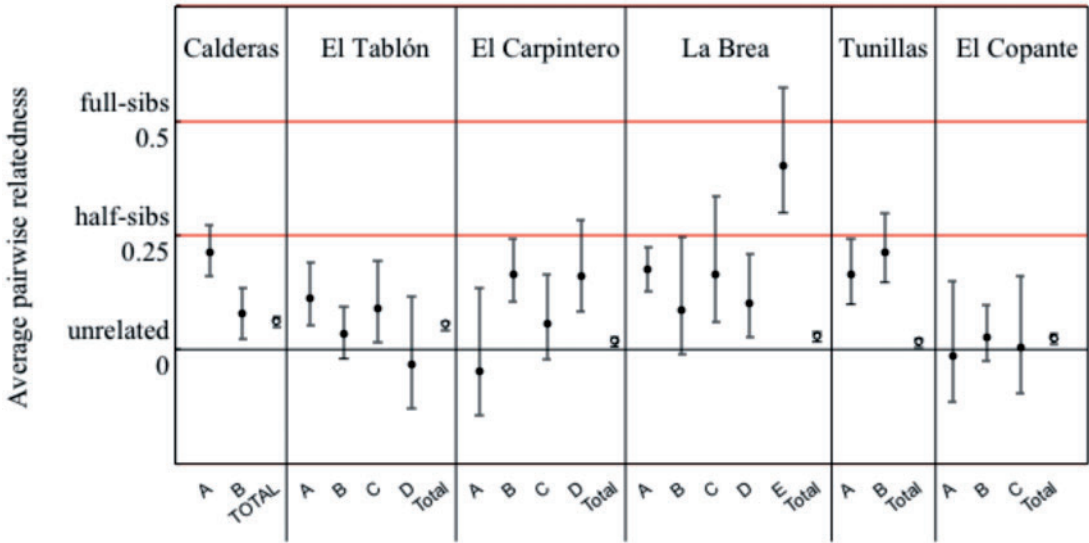


Fig. 3. Variability in average pairwise relatedness among houses with >2 insects (A–E, ●) and for the 28–30 insects from each village (Village, ○). Values are \pm SEM. Note values can be negative due to sampling error.

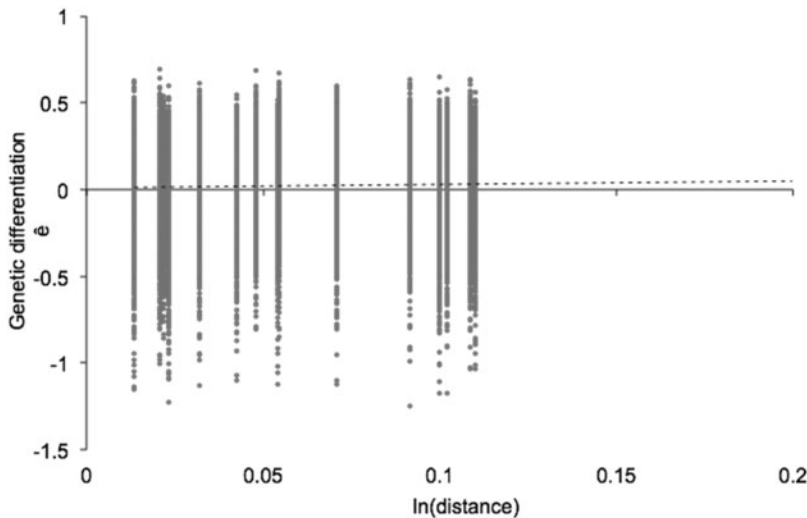


Fig. 4. Linear regression (.....) showing that genetic differentiation between insects increases with geographical distance. Data are pairwise genetic distances among six villages (28–30 insects per village). Linear regression: $\hat{e} = 0.0719 + 0.0165 * \ln(\text{distance})$; 95% confidence interval for slope [0.0061, 0.0292].

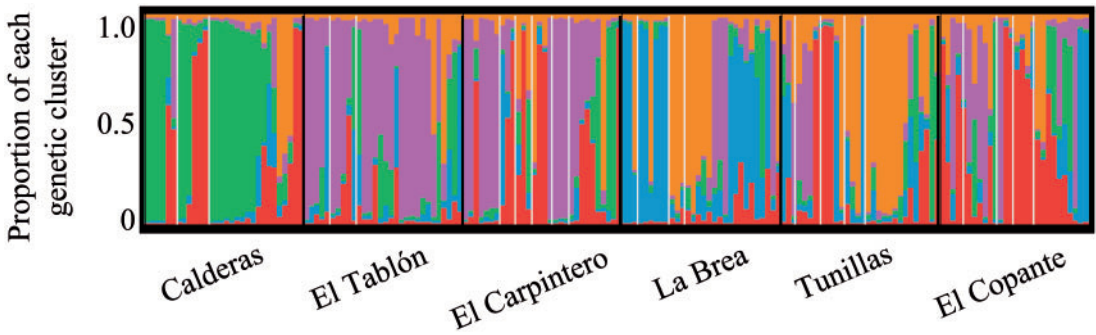


Fig. 5. Distribution of five genetic clusters (indicated by colors) among villages, based on Bayesian MCMC simulation. Vertical black lines separate the six villages, indicated below the figure. White lines separate the houses. Each insect is represented by a thin vertical line partitioned into colored segments indicating one of $K=5$ genetic clusters. For example, the first house in Calderas contained four insects from the green cluster, an orange–green–blue admixture, and an orange–purple admixture. Only houses with more than two insects are delineated; the remaining insects are pooled in the rightmost partition for each village.

clusters, one less than the number of villages (Supporting Information [Supp Fig. 1](#) [online only]). Although a single cluster dominates many villages, migration of *T. dimidiata* is inferred because all of the clusters are found in multiple villages and all villages have more than one cluster. The replicate STRUCTURE runs nearly always grouped the adults into the same clusters (correlation among the 10 structure runs = 98.9% for $K=5$ [i.e., five clusters]). Many individual insects are of one cluster (i.e., more than half of most of the vertical lines are one color; [Fig. 5](#)), especially in Calderas, yet many insects from Tunillas and El Copante are genetic admixtures. Although each cluster is present in all six villages, within a village often one cluster (Calderas: green, El Tablón: purple, and Tunillas: orange) or sometimes two or more clusters (El Carpintero: red and purple, La Brea: blue and orange, and El Copante: red blue and purple) tend to predominate. Most houses had insects from more than one cluster (e.g., Calderas) while in two houses all the insects were from the same genetic cluster (La Brea orange and El Copante red). We take this evidence of multiple clusters per house and multiple houses per cluster to indicate migration and gene flow among houses and among villages.

Testing Biased Dispersal and Variation in Infection Prevalence. There was no evidence of biased dispersal based on *Tr. cruzi* infection status (uninfected vs infected: $F_{ST} = 0.08$ vs 0.07 , $P > 0.05$; $vAIC = 15.38$ vs 14.85 , $P > 0.05$) or sex (female vs male: $F_{ST} = 0.07$ vs 0.05 , $P > 0.05$; $vAIC = 16.52$ vs 19.30 , $P > 0.05$). Further, the overall prevalence of *Tr. cruzi* infection was 34%; however, neither villages nor houses within villages differ in infection prevalence (likelihood ratio chi-square: villages = 8.88, $df = 4$, $P > 0.05$; houses within villages = 24.38; $df = 19$, $P > 0.05$).

Discussion

In this study, we show that in southern Guatemala, populations of the major Chagas disease vector, *T. dimidiata*, contain distinct genetic clusters that are found in multiple houses within the same village,

suggesting migration and gene flow among houses. This conclusion is based on both multilocus versions of Wright's F statistics (among houses within villages: $F_{SR} = 0.110$, $P < 0.001$, [Table 3](#)) and Bayesian maximum likelihood clustering ([Fig. 5](#)). Gene flow is also supported because average relatedness among insects was on the order of full or half-sibs in over half the houses with more than three insects ([Fig. 3](#)). In addition, the lack of aggregation of infected insects within households or villages supports the conclusion that insects are migrating, but tests for biased dispersal indicated that migration is not influenced by *Tr. cruzi* infection and is similar among males and females.

Although cryptic species are one possible explanation for the observed genetic differentiation (i.e., five clusters), there is no evidence of cryptic species in this hyperendemic area of southern Guatemala ([Panzera et al. 2006](#), [Dorn et al. 2007](#), [Bargues et al. 2008](#), [Dorn et al. 2009](#)). The single lineage of *T. dimidiata* in this region is found exclusively in domestic and peri-domestic habitats, often with high house infestation ([Monroy et al. 2003b](#), [Bargues et al. 2008](#)). A more likely explanation for the observed genetic structure is migration among houses, which leads to several clusters within a house. This explanation would include cases with a mated female moving to a house and producing several offspring.

Three lines of evidence support population structure among villages. First F statistics calculated from AMOVA show statistically significant genetic differentiation among villages ($F_{RT} = 0.05$, standardized $F'_{RT} = 0.23$, $P < 0.001$, [Table 3](#)). Second we detected weak, but statistically significant, isolation by distance ([Fig. 4](#)). Third Bayesian MCMC simulation detected five genetic clusters and although all six villages contained all five genetic clusters, one or two clusters predominate in most villages ([Fig. 5](#)).

All six villages had similar estimates of allelic diversity ([Fig. 2](#)), showing the villages do not differ dramatically in factors that affect allele frequencies and distribution, including *T. dimidiata* population size and gene flow, or that differences in these factors cancel

each other out. For most loci, there were few if any samples with null alleles (i.e., no amplification, Table 2); five of the seven loci had null allele frequencies $\leq 2.2\%$. Although null alleles can cause individuals to be incorrectly assigned in Bayesian clustering methods, simulations have shown the bias to be small, lowering the proportion of incorrectly assigned individuals to only about one percent (Carlsson 2008). Null alleles can also inflate estimates of genetic differentiation by detecting excess homozygotes (Carlsson 2008); however, analysis of our data both with and without the locus with the highest frequency of null alleles produced very similar estimates of F_{RT} (0.049 and 0.051).

Although our results cannot be directly compared with previous studies for which standardized F statistics are not provided, these results are similar to previous studies in southern Guatemala that show weak, but statistically significant, population structure (Dorn et al. 2003). Microsatellite markers have revealed less structure (maximum pairwise $F_{ST} < 0.05$, range 0.01–0.05) among 14 villages up to 250 km apart in Yucatán, Mexico (Dumontel et al. 2007). The degree of genetic differentiation among villages detected in this study ($F_{RT} = 0.05$, standardized $F'_{RT} = 0.23$; Table 3) is higher. This higher among-village differentiation in southern Guatemala compared with Yucatán may be due to the insects occupying sylvatic as well as domestic and peri-domestic ecotopes in Yucatán, but only domestic and peri-domestic ecotopes in southern Guatemala. Higher genetic differentiation in southern Guatemala was also suggested in a study characterizing microsatellite diversity in *T. dimidiata* (Anderson et al. 2002). In Jutiapa, 7 of 30 genotypes were heterozygotes; in contrast, 18 of 32 genotypes from Yucatán were heterozygotes, supporting the idea of more population structure in southern Guatemala.

The insects in Yucatán and Jutiapa are very different, and some in Yucatán are thought to be members of a cryptic species (Dorn et al. 2007, Bargues et al. 2008). Yucatán populations are sylvatic, with seasonal entry into houses and high migration. Individuals from sylvatic populations in northern Guatemala are similar to those in Yucatán; they rarely enter houses, show seasonal migration, and disperse as both nymphs and adults, as evidenced by infestation of experimental chicken coops (Monroy et al. 2003c). Our results show that the basically domestic population in southern Guatemala also migrates among houses and villages but to a lesser extent. The utility of microsatellite-based population genetic studies is revealed by the ability to detect lower gene flow among houses and villages in a largely domestic population.

So although it is domesticated, our results show that *T. dimidiata* is mobile in southern Guatemala. As a domestic vector in this region, rather than moving between sylvatic and domestic ecotopes, it moves among houses. Even though migration between houses may incur a fitness cost, we can think of two possible advantages to migration. First, if individuals remained in the same house, inbreeding would ensue; migration effectively avoids endogamy. Evidence in support of this comes from a nearby southern Guatemalan village

where a single house had 41 *T. dimidiata* families. There were few individuals per family (average family size of 2.17 individuals), and 46.3% of the families were represented by just one individual (Melgar et al. 2007). Second, flight is associated with poor nutritional status (Lehane et al. 1992). If higher bug densities within a house result in lower nutritional status, increased migration can prevent the number of bugs in a house from becoming too high.

Population genetics theory evaluates drift, migration, mutation, natural selection, and nonrandom mating, as possible explanations for population structure. With finite population size, drift should lead to fixation of different alleles in different subpopulations (houses or villages). The presence of the same genetic cluster in different houses and different villages indicates migration among houses within villages and among villages. The amount of genetic structure within villages ($F_{SR} = 0.110$) is significant with respect to population genetics and can lead to population differentiation (Wright 1978); however, there is still sufficient gene flow and migration to explain the observed house recolonization (Tabaru et al. 1999).

Source-sink dynamics may help understand the pattern of variation. At the time the insects were collected, Tunillas and El Carpintero had high numbers of houses and high infestation indices (203 houses, 48% infestation and 147 houses, 37% infestation, respectively), and thus may be a source of insects. El Tablón, with 58 houses and 25% infestation, may show low variation because of genetic drift from the small size of the insect population; however, Copante with 38% of 34 houses infested did not show similar low genetic variation and may be a sink. Calderas, with 43% of 104 houses infested, may show low genetic variation because it is more isolated, many of the families are related to law enforcement and anecdotally, it appears the general population interacts with them less. La Brea with 124 houses and 33% infestation supports the conclusion that the insects travel long distances between La Brea and/or Tunillas and Carpintero. However, these possibilities suggested by the data require further study.

Control Implications

The pattern of genetic variability detected in this study suggests that use of insecticides alone is not a sustainable control method for *T. dimidiata* for this region because reinfestation is probable. A previous study in this region reported reinfestation rates of 50% only 20–45 mo after insecticide spraying and that fumigation increased migration (Hashimoto et al. 2006). Because our study indicates gene flow and migration among houses and villages occurs, if fumigation increases migration further, it might increase the number of houses infested.

In Jutiapa and southern Guatemala, house improvements combined with insecticide spraying are quite effective in controlling *T. dimidiata* in spite of the more limited dispersal capabilities of these domesticated populations (Monroy et al. 2009). In contrast, in

Yucatán and northern Guatemala, where house improvements are not effective, alternative strategies, such as creating a more effective barrier between the inside and outside environments using screens and mosquito nets and reducing the amount of clutter and bug refugia, are necessary to reduce infestation rates (Dumonteil et al. 2004, Zeledon and Rojas 2006, Bustamante et al. 2009).

Our results on the population genetic structure of *T. dimidiata* help to illuminate the role of migration and gene flow in Chagas disease transmission and provide important information for the design of effective control strategies in southern Guatemala.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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