

Inferring from the *Cyt B* Gene the *Triatoma brasiliensis* Neiva, 1911 (Hemiptera: Reduviidae: Triatominae) Genetic Structure and Domiciliary Infestation in the State of Paraíba, Brazil

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Abstract. The *Triatoma brasiliensis* genetic structure was analyzed using the *Cyt B* gene in different geographic locations and ecotopes after a short and long period after insecticide treatment. Four different localities (16–40 km apart) in the state of Paraíba, Brazil, were sampled. Analysis of molecular variance (AMOVA) showed that grouping populations according to the geographic location or ecotope resulted in a higher variance among populations within groups (Φ_{SC} ranging from 0.15 to 0.17) than among groups (Φ_{CT} ranging from 0.04 to 0.07). The percentage of variation was reduced among populations within groups and increased among groups ($\Phi_{SC} = 0.08$, $\Phi_{CT} = 0.16$) by grouping 1) the domiciliary populations from each village and 2) all wild populations. These data indicated that *T. brasiliensis* is genetically structured both ecologically and at a smaller geographic scale for domiciliary populations. Re-infestations after insecticide treatment were composed of distinct populations, pointing to variable population sources for domiciliary infestations.

INTRODUCTION

Chagas disease is caused by a flagellate protozoan parasite, *Trypanosoma cruzi*, transmitted to humans mainly by blood-sucking bugs, the triatomines. Approximately 25% of the inhabitants of Latin America are at risk of contracting *T. cruzi*. Until now, no vaccine or drug has been developed to eliminate the infection by this parasite in the chronic phase of the disease. Most of the efforts against Chagas disease are focused on the interruption of its natural transmission by combating domiciliated vectors using pyrethroid insecticides, because vector transmission is still considered the main way to get infected in Brazil.^{1–5}

The Brazilian National Health Foundation (Funasa) has used intense insecticide spraying since the 1990s. The most important achievement reached by this program has been the interruption of *T. cruzi* transmission by *Triatoma infestans*. However, after the successful vector control program for *Tri. infestans*, another vector, *Triatoma brasiliensis*, became the main vectorial threat in semi-arid areas of northeastern Brazil.⁶ This latter species exhibits remarkable chromatic variations observed across its geographic distribution. Multidisciplinary research based on morphology,⁷ biology,^{8,9} ecology,¹⁰ isoenzymes,¹¹ and mitochondrial DNA variation¹² showed that the four morphotypes of *Tri. brasiliensis* indeed constitute the *Tri. brasiliensis* species complex. A taxonomic review of this complex is in preparation, and the species of the complex are being described.^{13,14} The morphotype *Tri. brasiliensis* s.s. was considered the main concern in terms of Chagas disease transmission because this morphotype is the most widespread and has the highest rates of domiciliary captures and of natural infection by *T. cruzi* among the four members of the complex.¹⁵

The state of Paraíba, Brazil, was used as a model to esti-

mate the time taken by *Tri. brasiliensis* to re-infest houses after insecticide treatment and the effect of house improvements (basically by replacing houses made of mud by houses built of concrete) on this process.^{16,17} These studies showed that the temporal pattern of triatomine re-infestation after insecticide spraying is highly heterogeneous. Usually, the re-infestation varies from 6 months to 2 years, probably depending on eco-geographic factors. It was suggested that genetic factors also contribute to such variation in re-infestation time. The difficulty of controlling *Tri. brasiliensis* has been attributed to its capacity to occupy the intradomiciliary, peridomiciliary, and wild environments. Hence, it has been stressed^{16,17} that accurate determination of the foci responsible for re-infestation is a crucial challenge to improving control measures implemented by Brazilian health authorities.

Dispersal within and among spatially or ecologically structured groups of populations can be compared through several kinds of neutral genetic markers, and elements of the landscape that limit dispersal can be identified using population genetics inferences. For triatomines, molecular and morphometric tools have both pointed out populations that invade and colonize domiciles and also helped to elucidate the dynamics between insects from domiciliary and sylvatic environments.^{18–24}

One of the main questions of population genetic studies on triatomines is whether ecotopic populations distant from each other would exchange migrants more often than physically close ones differing by ecotope, as has been proposed by some authors.^{18,19,23,24} The genetic structure of *Tri. brasiliensis* had been studied using *cytochrome b mitochondrial* gene (*Cyt B*) variation, an approach that clearly detected subpopulations separated by long distances (~500 km apart).¹² However, the study referred to had not been designed to clarify important aspects regarding population structure over smaller distances, such as the gene flow between ecotopes.

This study was conducted in four sites (~16–40 km apart) in the state of Paraíba, Brazil, where only the morphotype *Tri. brasiliensis* s.s. is found, herein referred to as just *Tri. brasiliensis*. The study's area is known to have high *Tri. brasiliensis*

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re-infestation pressure after insecticide treatment.^{16,17} Mitochondrial DNA variation and ecologic observations were used to address questions regarding to the population genetic structure and gene flow between sylvatic and domiciliary populations of *Tri. brasiliensis*. In addition, we tried to recognize the origin of populations that colonize domiciles after control activities.

MATERIALS AND METHODS

Captures. The collection locations were between the coordinates 06°51'29.23"–07°00'25.31" W and 38°22'59.58"–38°00'49.08" S, an area that is made up of the municipality of São José da Lagoa Tapada and bordering cities. All sampled spots were within the biogeographic zone known as *Caatinga*, a mosaic of xerophytic, deciduous, semi-arid thorn scrubs, and forest.²⁵ Geographic locations and distances were obtained using GPS TrackMaker.²⁶

Captures were performed from 2001 to 2004 with attention to the records of the Program to Control Chagas Disease in the State of Paraíba, northeastern Brazil. The process of re-infestation was estimated to begin ~6 months after insecticide treatment. This observation was based on a large study monitoring the process of re-infestation in the entire state of Paraíba, Brazil.^{16,17} Based on these studies, we also selected the location with one of the highest recorded pressures for re-infestation after insecticide spraying.

We herein define the population collected from 6 months to 1.2 years after domiciliary insecticide spraying as the populations sampled within a "short period" after house spraying. These domiciliary populations were considered to be in the process of infestation re-establishment.^{16,17} Furthermore, we sampled the same geographic area within 2.9–3.5 years after the insecticide treatment, at which time we also sampled domiciles that had not been treated during this period. We considered these insects as populations collected after a "long period" after insecticide spraying (or populations of well re-established infestations).

Uninhabited (or wild) areas were sampled simultaneously with domiciliary collection. Although uninhabited environments are not treated with insecticide, these collections were also referred to as collected "within a short/long period after domiciliary insecticide spraying," to link the term to the time of collection.

Spatial versus ecotopic and temporal genetic structures were analyzed independently. Because larger sample sizes were obtained after a long period had elapsed after insecticide spraying, only these samples were used to assess the general spatial versus ecotopic genetic structure.

Insects. Samples were labeled by locality and ecotope (intradomiciliary, peridomiciliary, ruderal, and sylvatic). Intradomiciliary ecotopes (I) are the indoor space of domiciliary units or home-like environment where humans live and/or work. Triatomines were generally found in mud wall crevices or under beds. The peridomiciliary ecotopes (P) were considered within a radius of 50 m from the domiciles, where domesticated animals slept or were raised. In peridomiciles, most of the triatomines were captured in chicken coops and in goat and pig corrals.

We considered the beginning of ruderal ecotopes (R) to be 50 m past the peridomicile radius, usually ranging from 1 to 2 km from houses. We took one R unit per village sampled. In

this environment, the native vegetation was substituted by new pastures or short-lived cropland. Local people, so-called "sertanejos," hunt to eat and to protect the crop and domesticated animals. Therefore, domesticated animals (e.g., goats, horses, and cows) were easily observed in this environment, whereas wild homeothermic animals were seldom seen among rock piles where triatomines were collected. The sylvatic ecotope (S) is also an uninhabited area, starting at least 200 m from any house. This ecotope differs from the ruderal environment mainly because the sylvatic ecotope has not been transformed into either pasture or cropland. Wild animals were most often seen where triatomines were collected. A rodent closely related to the guinea pig (the rock cave or *mocó*, *Kerodon rupestris*) was the most representative homeothermic vertebrate seen among the rocks.²⁷

Domiciliary captures were performed during the day, and dislodging (0.2% neopynamin) was used when needed. During the same period and with the same staff, we performed nocturnal and manual captures in uninhabited environments (R and S), where triatomines were captured in similar shelters (rock piles).

Only one sylvatic area was sampled, and except for this single sylvatic area, each locality was split into two sub-localities (or villages), referred to as 1 and 2 (Figure 1). We thus organized the samples according to the ecotope and sub-locality to define the sites. The sites were labeled by three characters: the first represents the locality label (A, B, or C), the second, the sub-locality (1 or 2), and the suffix, the ecotope (I, P, or R; Table 1). We used the suffix I when we merged intradomiciliary and peridomiciliary samples, also referred to as just domiciliary.

All immature triatomines collected were taken to the laboratory to be maintained under controlled temperature and humidity conditions ($27 \pm 2^\circ\text{C}$ and $60 \pm 3\%$ RH) and fed on mice once a week until imaginal molt. The adult specimens were identified according to their taxonomic characteristics.^{13,14,28,29}

DNA extraction and amplification. Insects were stored at -20°C until processed. Each sample was quickly frozen in nitrogen liquid to individually grind the insects' legs in an Eppendorf tube with a clean and sterile crusher. We used the Wizard Genomic Purification Kit (Promega, Madison, WI) for DNA extraction, following the manufacturer's recommended protocol for animal tissue. Standard polymerase chain reaction (PCR) techniques were used to amplify the gene fragments. A 510-bp region of the *Cyt B* mitochondrial gene was amplified using primers CYTB7432F, 50-GGACG(AT)GG(AT)ATTTATTATGGATC, and CYTBR, 50-ATTACTCCTCCTAGCTTATTAGGAATTG,^{12,30,31} by running reactions in an Eppendorf Master Gradient Thermocycler (Brinkman Instruments, Westbury, NY). We used the following cycles: denaturation at 94°C for 5 minutes and then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 47°C for 30 seconds, and extension at 72°C for 1 minute, followed by a 72°C extension for 7 minutes and holding at 4°C indefinitely. Amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide. Purified PCR products were sequenced using a Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA), according to the manufacturer's recommended protocol. Both strands were sequenced directly in both directions with amplification

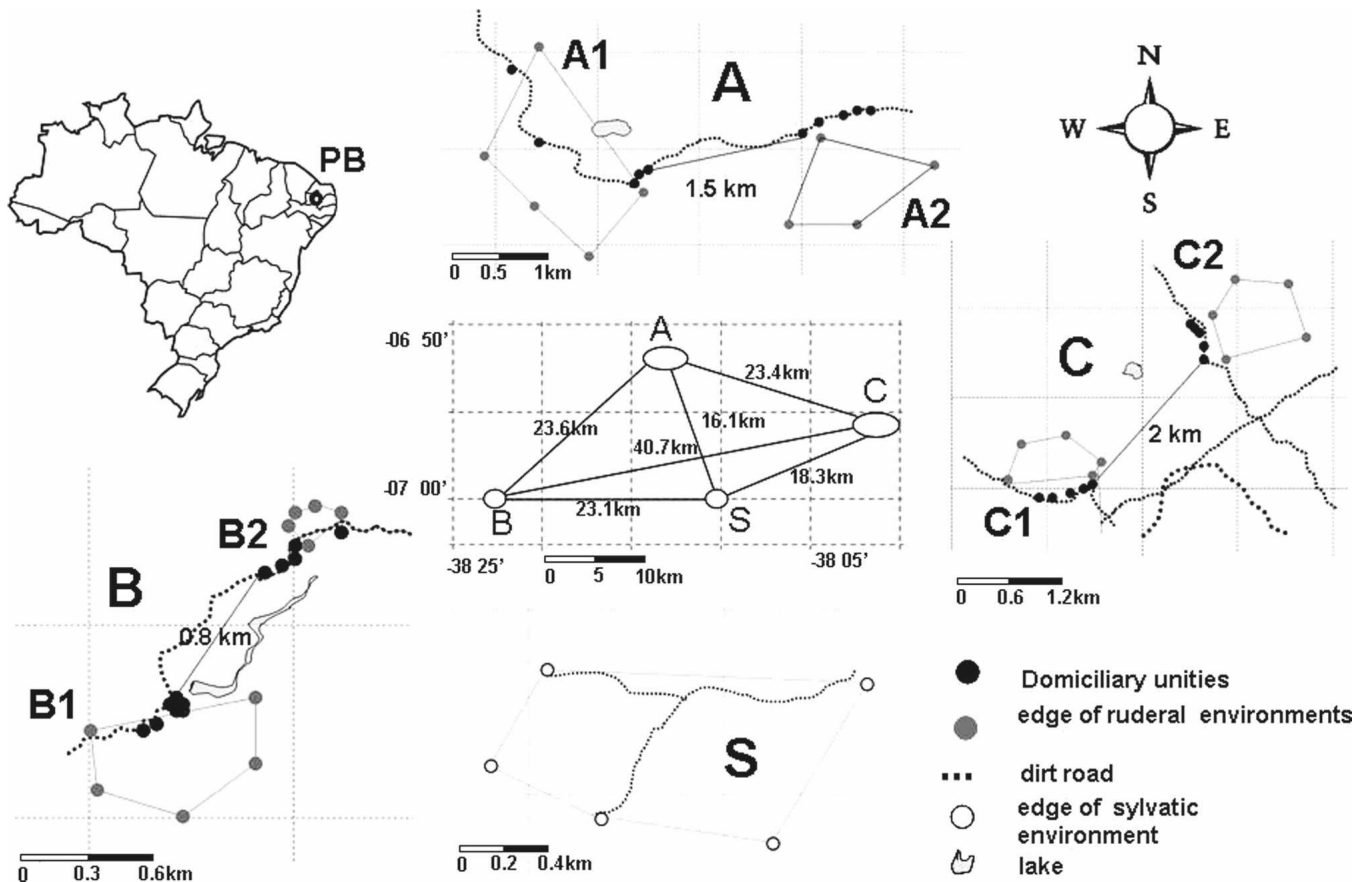


FIGURE 1. Collection sites for *Triatoma brasiliensis* specimens in the four localities (A, B, C, and S) in the state of Paraíba (PB), Brazil. Except for S (the sylvatic spot), each locality was divided into two sub-localities (1 and 2).

primers, using an Applied Biosystem model 3100 automated DNA sequencer. The consensus sequences were edited and assembled using the SEQMAN program³² and aligned using Alignment Clustal available through Mega3.³³

Neutrality and demography. We tested the null hypothesis of neutrality using the Tajima D statistic,³⁴ implemented by the software DNASP 4.10.4.³⁵ Because panmixis is a key assumption of Tajima D statistics, an initial test included all individuals sampled across the study area. The Tajima D was tested for all samples sorted by ecotope and after that by geographic location, which allowed more robust inferences based on multiple tests at finer scales. We also tested the hypothesis that geographic and ecotopic populations are not panmictic, further implementing neutrality tests on each proposed site individually. Values of the Tajima D are also used to assess evidence for demographic events.³⁴

Genetic structure. Variation among and within collections was partitioned by a hierarchical analysis of molecular (co)variance (AMOVA)³⁶ using Arlequin version 3.01.³⁷ Significance of the fixation indices was determined using a non-parametric approach.³⁶ F_{ST} was calculated among all populations and between all pairs of populations. The significance of departures from zero of Φ statistics and genetic variance components were tested using 1,000 permutations.³⁸ Nesting was imposed in the two ways because it has been hypothesized to limit the gene flow: physical distance and ecotopic barriers. First, we tested the null hypothesis that population genetic structure was not associated with ecotopes by grouping each

site according to its geographic location. Second, we grouped sites according to ecotopes. Comparison between proportions of variance explained by each partition provides information on the relative role of geography versus ecology in *Tri. brasiliensis* population structure.

Localities A, B, and C are outside the probable flight range of triatomines. We tested the isolation by distance (IBD) by regressing pairwise F_{ST} on pairwise log-transformed geographic distances among populations and significance was determined by Mantel tests (1,000 permutations) using Arlequin. Additionally, we looked for evidence of IBD using matrices of pairwise linearized F_{ST} ($F_{ST}/[1 - F_{ST}]$)^{39–41} for the correlation by Euclidean distance. We assume the insects could actively fly throughout any two spots between sites within localities, and the same approach was applied to ecotopic populations within localities to detect evidence for ecotopic boundaries.

We constructed a haplotype network using the software TCS 1.21⁴² based on the statistical parsimony method.⁴³ Reticulations were resolved according to common theoretical predictions about network structure.^{44,45} To visualize differences between geographic versus ecotopic groups, the network was coded in two ways, each depending on the structure of interest.

Influence of temporal factors and control activities on the genetic structure. A second level of sampling was designed to study the temporal differences on the genetic structure of *Tri. brasiliensis*. We considered the population of insects collected

TABLE 1
Sample size (N), haplotype diversity (Hd), and numbers of each haplotype (I-XXIX) found in each site

Sites	AII	AIP	AIR	A2I	A2P	A2R	BII	BIP	BIR	B2I	B2P	B2R	CII	CIP	CIR	C2I	C2P	C2R	S
N	16 (10)	11 (21)	15 [67]	15	12	14	11	18	16	11	14	14	7	10	14	8	9	11	37
Hd	0.78 (0.62)	0.84 (0.69)	0.90 [0.85]	0.77	0.80	0.92	0.18	0.40	0.88	0.69	0.58	0.91	0.67	0.93	0.88	0.68	0.81	0.84	0.93
I			4 [7]	1	1	2				2	1	2						1	2
II	5	3 (1)	1	6	2	1			1										
III	5 (2)	(8)	2 [10]	4	1					4	9	5							
IV	4 (6)	3 (9)	1 [2]	3	4	1	10	14	4	5	2	2				4		1	1
V			[7]				1		1					2	5				1
VI	1 (2)	(1)	1	1									4	1	1	1	3	4	
VII	1												1	2	1	3	3	1	
VIII													2	1	1				
IX		1	[6]			2		1	2		2	2		1	1				
X		(1)												1	1		1		
XI																	2		
XII														1	1		1		
XIII		3 (1)	1 [19]		4	1		2	4					1	1		2	3	
XIV		1	2 [2]			3		1											
XV			[1]						1										
XVI																		1	3
XVII									2			1							1
XVIII			3 [9]			2			1						1				1
XIX															1				8
XX									1										1
XXI																			1
XXII																			2
XXIII																			3
XXIV																			3
XXV																			3
XXVI																			1
XXVII																			2
XXVIII																			2
XXIX																			1

The first eight and the following six lines represent the samples found for intradomiciles and peridomiciles, respectively. The values for samples collected within a short period (0.5–1.2 years) after insecticide treatment are in parentheses. Ruderal collections for this same period are in brackets. Shaded haplotypes were private for ruderal or sylvatic environment. The first letter (A, B, C, or S, see Figure 1) refers to the locality. The following number refers to the sub-locality and the last letter to the ecotope where the samples were collected: I, intradomiciliary; P, peridomiciliary; R, ruderal; S, sylvatic.

within a short period after house spraying (from 0.5 to 1.2 years) “in the process of re-establishment.” The populations collected within 2.9–3.5 years after insecticide treatment (the populations used to make inferences on the overall genetic structure) were considered “well re-established” populations. Previous studies^{16,17} have shown that this last time frame was sufficient for the re-establishment of domiciliary infestations.

Haplotype diversity was measured for each population, and F_{ST} pairwise differences were calculated among all populations and between the pairs of populations collected in both periods after insecticide spraying. Taking into account signals of genetic variation, we nested combinations of groupings for an AMOVA using domiciliary and ruderal populations collected during both periods to assess the spatio/ecotopic versus temporal variation on the genetic structure, in regard to vector control activities.

RESULTS

Captures. Two hundred twenty-six samples collected in three inhabited localities (A, B, and C that were each divided into two, resulting in six sub-localities) and 37 samples from the single sylvatic (S) site (in the south) were available to analyze the general genetic structure. Furthermore, we collected 98 samples in A1 within a short period after insecticide spraying that were used to analyze the influence of temporal factors and control activities on the genetic structure.

Considering the material available to analyze the general genetic structure, we collected insects in the three ecotopes per sub-locality, allowing us to define a total of 18 sites with sample sizes ranging from seven to 18 individual sequences per site (Table 1). The sub-localities had a sample size ranging from 28 (C2) to 45 (B2). The totals analyzed per sub-locality in intradomiciliary and peridomiciliary ecotopes ranged from 9 to 18 and 7 to 16 samples, respectively. Ruderal sites were represented by 11–16 insects. We could not find any other spot exhibiting sylvatic characteristics among the A, B, and C localities (Figure 1). The sylvatic collection might herein correspond to a spatial and/or an ecotopic population.

In both R and S ecotopes, the triatomines captured during nocturnal fieldwork appeared to be searching for food or copulation outside rock piles. Despite the environmental similarity, bugs were easily collected in R sites in all localities, whereas approximately five times more effort (hour per captured bug) was required to obtain the same number of bugs in S as in R.

Thirty-one samples, all from the locality A1, were found in domiciliary ecotopes within a short period after insecticide spraying. On this same occasion and in the same sub-locality, 67 samples were collected in the ruderal environment (Table 1).

DNA sequences. Twenty-nine haplotypes were identified across the sampling range. Polymorphic sites are shown in Table 2. We identified substitutions at 34 of the 510 nucleotide sites; of these, 32 were transitions, 2 were transversions, and 4 were non-synonymous substitutions. As commonly observed in insect mtDNA, nucleotide frequencies were AT-biased (frequencies of T = 0.31, C = 0.25, A = 0.31, and G = 0.13).

Neutrality and demography. High average haplotype diversity (H_d) values were recorded in the four localities, ranging from 0.75 (overall B) to 0.93 (overall S). The sylvatic popu-

lation showed the highest haplotype diversity of all populations at the levels of 1) total sample, 2) total for each locality, 3) ecotopic populations (overall I, P, and R), and 4) sites (Table 1).

Tajima D values for A, B, C, and S localities were -0.40 , -0.66 , -1.21 , and -1.62 , respectively. Except for the sylvatic and overall ruderal population (both $P = 0.04$), no significant deviation from neutrality (all $P > 0.10$) was identified among the sequences at any level (overall $D = -1.28$). Except for the B1I site ($D = -1.43$), high positive D values were associated with intradomiciliary ecotopes (overall $D = 1.15$). Overall intradomiciliary populations showed the following D values for each locality: A = 0.51, B = 0.01, and C = 1.73. Positive values were notably high for intradomiciliary populations in the sub-localities of B2, C2, and C1 ($D = 1.56$, 1.53, and 1.46, respectively). High negative D values were observed for ruderal sites ranging from -0.23 to -1.38 in A2R and C1R, respectively (all $P > 0.10$).

Genetic structure. To examine the spatial versus ecotopic genetic structure, we used 263 samples exhibiting 29 haplotypes collected a long period after house spraying (Table 1, I–XXIX). The total number of sequences for well re-established colonies from intradomicile, peridomicile, ruderal, and sylvatic environments were 68, 74, 84, and 37 samples, respectively. The intradomiciliary insects were represented by only eight haplotypes (ranging from I to VIII), being also found in peridomiciliary and ruderal environments. Haplotypes I–XIV were found in domiciliary and ruderal environments, and of these haplotypes, I and V were found in all ecotopes. Twenty-three and 16 haplotypes were identified in the ruderal and sylvatic environments, respectively. The haplotype XV was private for ruderal environment. Most of the private haplotypes in ecotopes were found in the sylvatic environment: three samples of haplotype XXIV and XXVI, two samples of haplotypes XXVII and XXVIII, and the two unique singletons found for haplotypes XXV and XXIX (Table 1).

The haplotype network was relatively superficial, with a maximum of seven mutational steps between haplotypes (Figure 2). We determined the frequency of each haplotype occurrence among localities (Figure 2a), as well as by ecotope (Figure 2b). Haplotype IV was found in all inhabited localities and the most frequent in intradomiciliary and peridomiciliary ecotopes, where this haplotype represented 38% and 25%, respectively, of the total sample collected in those environments (Table 1; Figure 2).

The lowest F_{ST} value among overall localities was between A and B (0.02), and the highest one (0.25) was observed between B and S (all $P < 0.02$). The highest F_{ST} values observed among the overall ecotopic populations were 0.29 and 0.20 (for I versus S and P versus S, respectively, both $P < 0.0001$). The F_{ST} values between ecotopic populations I versus P (0.02), P versus R (0.04), and R versus S (0.08) were lower (all $P < 0.03$).

The F_{ST} values between intradomiciliary and peridomiciliary sites indicated a low differentiation (all $F_{ST} < 0.08$, except for C2I versus C2P; $F_{ST} = 0.2$) in the same sub-locality. To improve the robustness of results, we repeated the F_{ST} calculations by merging the domiciliary sites (I and P) from each sub-locality. This allowed us to improve the statistical significance to make inferences about different domiciliary, ruderal, and sylvatic population sources for domiciliary colonization.

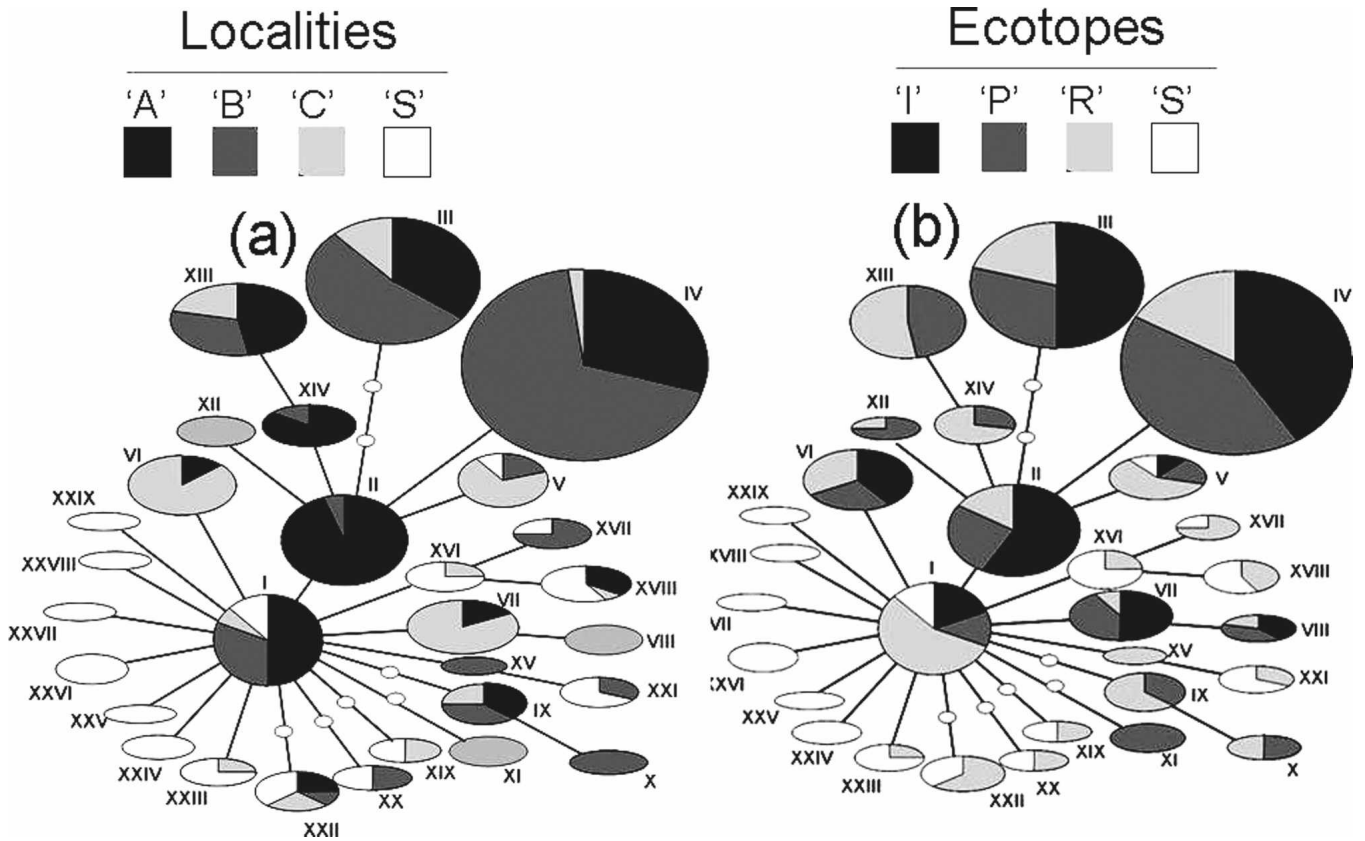


FIGURE 2. Maximum parsimony network showing the relationship among the 29 *Triatoma brasiliensis* haplotypes based on the *cytochrome b* mitochondrial gene. The left network (a) is coded in localities and the right one (b) in ecotopes. For an explanation of the localities, see Figure 1. The ecotopes are intradomiciliary (I), peridomiciliary (P), ruderal (R), and sylvatic (S).

We constructed a neighbor-joining tree of pairwise F_{ST} distances (Figure 3), showing an association between the domiciliary populations from the sub-localities 1 and 2 within the localities A and C, however, without statistical significance ($P = 0.9$ and 0.36 , respectively). We found that domiciliary populations B1IP and B2IP have been significantly differentiated ($F_{ST} = 0.37$, $P < 0.0001$), but B1IP was significantly related to the ruderal site within the sub-locality ($F_{ST} = 0.28$, $P < 0.0001$). Domiciliary populations from both B sub-localities were also significantly related to the domiciliary populations from A (all $F_{ST} < 0.22$, $P < 0.01$).

The only F_{ST} values > 0.1 among ruderal sites were observed for C2R with B2R and with C1R (0.17 and 0.11 , $P = 0.009$ and 0.01 , respectively). No correlation among ruderal populations could be observed taking into account the physical distances (Figure 3; Table 3).

Despite the low differentiation between domiciliary and ruderal populations within sub-localities (except for B1), no consistent pattern could be traced for this relationship, because domiciliary populations were, in most cases, associated with domiciliary or ruderal populations from other sub-localities (Figure 3).

AMOVA showed that grouping all sites according to geographic location resulted in a higher percentage of variance within localities (14% ; $\Phi_{SC} = 0.15$, $P < 0.0001$) than among them (7% ; $\Phi_{CT} = 0.07$, $P = 0.02$; Table 4). A similar pattern was obtained if we grouped sites by ecotope, regardless of the geographic location ($\Phi_{SC} = 0.17$, $P < 0.0001$; $\Phi_{CT} = 0.04$, $P = 0.07$; Table 4).

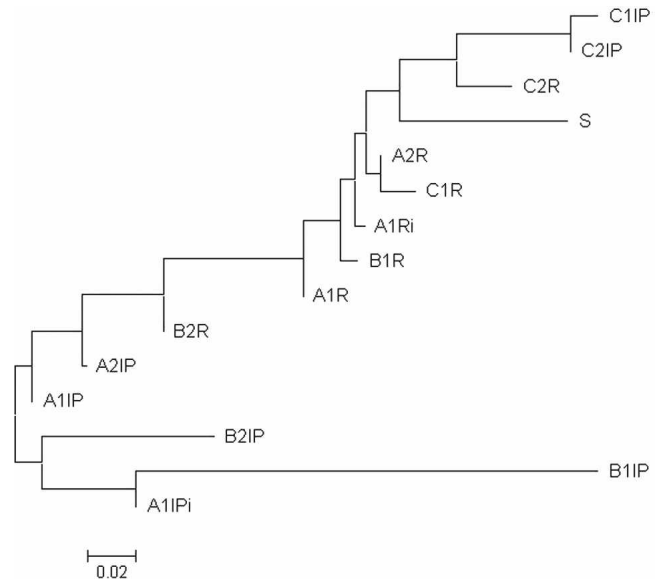


FIGURE 3. Neighbor-joining tree derived from linear pairwise F_{ST} distances of *Triatoma brasiliensis* populations. The collection spots are shown in Figure 1. The suffix IP refers to samples collected for well reestablished intradomiciliary plus peridomiciliary infestations, and R to the ruderal ecotope. The code S refers to the sylvatic population. Suffix (i) indicates the samples collected within a short period (0.5 to 1.2 years) after insecticide spraying for A1IPi and simultaneous collections for ruderal (A1Ri). The values used to build the tree are shown in Table 3.

TABLE 3
 F_{ST} pairwise population comparisons for domiciliary, ruderal, and sylvatic populations from four localities (see Figure 1)

	A1IP	A2IP	B1IP	B2IP	C1IP	C2IP	A1R	A2R	B1R	B2R	C1R	C2R	S	A1IPi	A1Ri
A1IP	—														
A2IP	-0.036	—													
B1IP	0.202	0.217	—												
B2IP	0.098	0.106	0.372	—											
C1IP	0.216	0.221	0.503	0.313	—										
C2IP	0.112	0.113	0.439	0.170	0.005	—									
A1R	0.061	0.056	0.385	0.190	0.112	0.054	—								
A2R	0.115	0.118	0.390	0.275	0.131	0.122	-0.010	—							
B1R	0.083	0.084	0.286	0.254	0.129	0.123	0.012	-0.020	—						
B2R	0.046	0.050	0.343	0.014	0.145	0.046	0.041	0.089	0.085	—					
C1R	0.119	0.120	0.387	0.253	0.083	0.090	0.037	0.010	0.034	0.085	—				
C2R	0.224	0.230	0.544	0.359	0.057	0.091	0.057	0.057	0.055	0.167	0.108	—			
S	0.271	0.265	0.474	0.380	0.156	0.177	0.071	0.072	0.108	0.195	0.101	0.096	—		
A1IPi	0.019	0.024	0.184	0.034	0.285	0.162	0.153	0.232	0.185	0.061	0.217	0.311	0.349	—	
A1Ri	0.095	0.090	0.317	0.213	0.116	0.088	0.017	0.008	0.022	0.068	0.034	0.057	0.094	0.190	—

All values except those in bold were significant at $\alpha = 0.05$. Shaded populations were collected within a short period after insecticide spraying. The first code (A, B, and C) followed by the number (1 or 2) refers to the village. The subsequent characters refer to the ecotope: IP, collections from well re-established intradomiciliary plus peri-domiciliary colonies; R, ruderal; S, sylvatic collections. Suffix i (shaded populations) refers to collection within a short period (within 0.5–1.2 years) after insecticide spraying. Ruderal population collected for this same period was also shaded.

To test another AMOVA, we considered the indications (F_{ST} values) of population differentiation. Domiciliary populations (I and P) from the same sub-localities (or villages) were closely associated, and all populations from the ruderal environment seemed to not be differentiated from each other but did differ from the sylvatic population. We therefore nested the populations, taking into account the synanthropic level and spatial distribution of domiciliary sites as follows: 1) domiciliary (I plus P) sites from each village, 2) all ruderal sites across the sampling area, and 3) the sylvatic population. Grouping the samples in this manner showed that the percentage of variation among sites within groups decreased to 7% ($\Phi_{SC} = 0.08$, $P < 0.0001$) of total of variation further increased to 15% the magnitude of variation among groups ($\Phi_{CT} = 0.16$, $P < 0.0001$). The highest percentage of genetic variation was distributed within the sites (77.7%, $P < 0.0001$). Overall Φ_{ST} was significant ($P < 0.0001$) and detected as high at 0.22 (Table 4).

Isolation by distance. F_{ST} values > 0.15 might indicate great genetic differentiation.⁴⁶ Pairwise differences showed a rea-

sonable range of F_{ST} values, all statistically significant (ranging from 0.02 to 0.25, $P = 0.01$ and < 0.0001) for variation among overall sampling for the four geographic localities. Because the genetic structure indicated that ruderal populations are panmictic, we analyzed all samples excluding ruderal sites. At both scales, there were very low relationships between F_{ST} and geographic distances. The correlation between genetic and geographic distances within localities was also weak (Figure 4). A Mantel test confirmed that the matrixes had very weak correlation (all $r < 33\%$, $P > 0.24$).

Influence of temporal factors and control activities on the genetic structure. Because of the small sampling size for populations from intradomiciliary and peridomiciliary ecotopes within a short period after insecticide spraying ($N = 31$ samples from A1), we merged all the samples to define the A1IPi population. This approach allowed us to improve the robustness of the results. Ten samples exhibited haplotype III, and 15 and 2 insects represented haplotypes IV and VI, respectively. A total of four insects, one each representing haplotypes I, VII, XI, and XIII, were also collected. In addi-

TABLE 4

AMOVA variance components, percentage variation explained at each hierarchical spatial/ecotopic level, and fixation indices for sites grouped according to locality (Figure 1), sites grouped according to ecotopes (intradomiciliary, peridomiciliary, ruderal, and sylvatic), and peridomiciliary and intradomiciliary sites grouped from each village, all ruderal populations, and the sylvatic population

Source of variation	df	Variance components	Percentage of variation	Fixation indices	P
According to locality					
Among geographic populations	3	0.10 Va	7.10	$\Phi_{CT} = 0.07$	0.02 (LS)
Among ecotopic sites within localities	15	0.21 Vb	13.72	$\Phi_{SC} = 0.15$	0.00
Within sites	245	1.19 Vc	79.18		
Total	263	1.50 Vt		$\Phi_{ST} = 0.21$	0.00
According to ecotopes					
Among ecotopic populations	3	0.058 Va	3.87	$\Phi_{CT} = 0.04$	0.09 (NS)
Among geographic sites within ecotopes	15	0.24 Vb	16.32	$\Phi_{SC} = 0.17$	0.00
Within sites	245	1.19 Vc	79.77		
Total	263	1.49 Vt		$\Phi_{ST} = 0.20$	0.00
From each village, all ruderal populations, and the sylvatic population					
Among domiciliary sites from each village, all ruderal, and the sylvatic site	7	0.24 Va	15.54	$\Phi_{CT} = 0.16$	0.00
Among sites within groups	11	0.10 Vb	6.72	$\Phi_{SC} = 0.08$	0.00
Within sites	244	1.18 Vc	77.74		
Total	262	1.52 Vt		$\Phi_{ST} = 0.22$	0.00

$\Phi_{CT} = Va/Vt$; $\Phi_{SC} = Vb/(Vb + Vc)$; $\Phi_{ST} = 1 - (Vc/Vt)$; NS, not significant; LS, low significance.

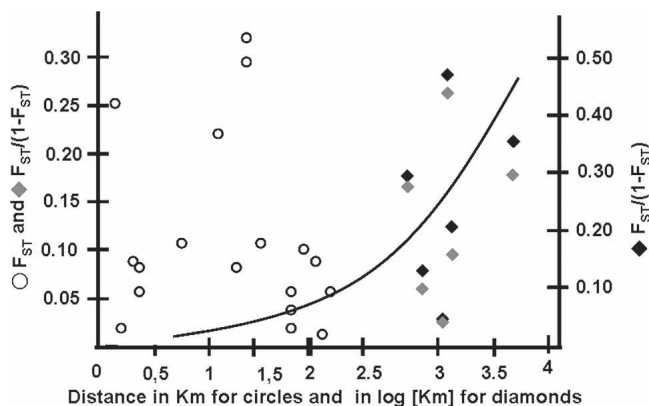


FIGURE 4. Each spot represents the genetic differentiation of one unique pair of *Triatoma brasiliensis* populations correlated with (Euclidean) distance. The left scale (gray diamonds) indicates the $F_{ST}/(1-F_{ST})$ values between populations from each locality (A, B, C, and S; see Figure 1). The same domiciliary sampling was used for the $F_{ST}/(1-F_{ST})$ values indicated on the right axis (black diamonds); ruderal samples were excluded from this analysis. Geographic distances were not log-transformed for circles, representing geographic and genetic correlation within localities.

tion, we sampled the ruderal environment ($N = 67$) in this same geographic location and period and identified 10 different haplotypes (Table 1).

Genetic diversity values for well re-established groups of domiciliary populations from A and C were high ($0.67 < H_d < 0.93$). Lower values ($0.18 < H_d < 0.69$) were obtained for well re-established groups from B and for the population collected within a short period after insecticide spraying (A1IPi). The F_{ST} values of A1IPi insects with well re-established groups from C (domiciliary populations) with all ruderal and sylvatic populations were significantly high (F_{ST} ranging from 0.15 to 0.35, all $P < 0.0001$). This A1IPi population exhibited the lowest differentiation observed for the well re-established B1IP population ($F_{ST} = 0.18$, $P < 0.0001$), and non-significant F_{ST} values (all $F_{ST} < 0.04$, P ranging from 0.13 to 0.19) with others also well re-established domiciliary populations from A and B localities collected a long period after insecticide spraying.

The low F_{ST} values (all < 0.07 , P ranging from 0.04 to 0.69) between ruderal populations collected for the two different periods regarding short (A1Ri) and long (all others ruderal sites) periods after domiciliary insecticide spraying indicated a low influence of temporal factor (~ 2.5 years) in the genetic structure for this environment (Table 3).

AMOVA showed that when we nested one group with the domiciliary population collected within a short period after insecticide spraying (A1IPi) with the domiciliary A (A1IP and A2IP) and B (B1IP and B2IP) populations collected a long period after insecticide spraying, and further nested another group composed of all ruderal populations (including A1Ri) and well re-established domiciliary C populations (C1IP and C2IP), 15% of variation between these two groups was observed. In addition, only 8% of variation among populations within groups was detected ($\Phi_{CT} = 0.15$ and $\Phi_{SC} = 0.09$, for both $P < 0.002$). The overall Φ_{ST} was at 0.22 ($P < 0.0001$). The high variation within populations (78%) can be explained by the high haplotype diversity observed, particularly for the ruderal populations.

DISCUSSION

The data presented here showed that, within the area studied, *Tri. brasiliensis* is genetically structured both ecological and at a small geographic scale for the domiciliary populations. A reasonable degree of population fragmentation was also suggested by variation from uninhabited areas (ruderal and sylvatic). The pattern of re-colonization was analyzed by comparing *Cyt B* gene variation in ruderal and domiciliary populations and by comparing populations collected a short time and long time after insecticide spraying. Re-infestations after insecticide treatment are composed of distinct populations, indicating variable population sources from both ruderal and domiciliary sites.

Our results confirmed previous studies regarding the applicability of *Cyt B* sequence data for population genetics studies in *Tri. brasiliensis* and showed that this tool can be also used to obtain information regarding gene flow over smaller distances, such as ecotopic populations. Twenty-four haplotypes were previously recorded in a much wider geographic range (the states of Pernambuco, Paraíba, Rio Grande do Norte, and Piauí).¹² In our study, only 14 haplotypes were recorded in the domiciliary environment, and of these, 6 had been previously described.¹² A significant amount of haplotypes ($N = 15$) was herein identified in uninhabited environments (sylvatic and ruderal), where the sample sizes were extended in our study.

Based on ecologic niche modeling⁴⁷ and nested clade analysis,¹² it was assumed that the three morphotypes recorded for this species complex were a result of fragmentations and population expansions that had occurred in the past across the geographic distribution of the morphotype studied here. Here we recorded the same central haplotype in the network,¹² which was also characterized by few mutational steps between haplotypes within this morphotype. Haplotype I was strongly supported to be the ancestral haplotype, because of its representation in a significant proportion of individuals in geographic and ecotopic populations and its central location in the network (Table 1; Figure 2).

Tajima D was significantly negative for the sylvatic population ($D = -1.62$) and overall ruderal ($D = 1.29$), suggesting that selection pushed the frequency spectrum of polymorphisms towards an excess of rare variants. However, in contrast to ruderal populations, we did not observe abundant colonies for the sylvatic population. Other authors²³ have also described the abundance of wild populations of *Tri. brasiliensis*. Because a significant Tajima D could also originate from demographic events, such as population bottleneck or population expansion, our results can be interpreted as a signal of past demographic bottleneck eventually associated with natural selection on mitochondrial genes^{34,48} for these natural populations. Under a neutral model context (based on non-significant D values), most of the ruderal sites also exhibited high negative D values, in contrast to domiciliary populations (with the exception of B1I) that showed high positive D values. This may also indicate population fragmentation and distinct demographic events in wild and domiciliary populations.⁴⁸

Genetic structure. AMOVA indicated that, in the synanthropic populations within the sub-localities, the genetic structure was significantly associated with particular genetic profiles for domiciliary populations within each village. How-

ever, ruderal populations did not exhibit significant differentiation, allowing us to nest all populations in the same group. High haplotype diversity values were also recorded for the ruderal populations.

All haplotypes found in intradomiciles were also found in both the peridomiciliary and ruderal ecotopes, highlighting the role played by these environments as sources of invasive populations. Peridomiciliary environments do not usually include artificial lights that can attract bugs.⁴⁹ On the other hand, in addition to proximity to the ruderal environment, weather may reduce the period of the insecticide's residual effect.⁵⁰ This might explain the higher haplotype diversity observed in peridomiciliary compared with intradomiciliary ecotopes.

The highest haplotype diversity recorded for *Tri. infestans* Cyt B was 0.67,⁵¹ which can be considered low if compared with the highest haplotype diversity of 0.93 found in this study. This can be considered significant in terms of vector control, given that higher variability has been associated with higher difficulty of controlling vectors.^{51–53}

Light traps were used⁴⁹ to estimate that approximately one wild *Tri. brasiliensis* might be reaching houses every two nights attracted by artificial light in this same biogeographic zone (the *Caatinga*). Initial levels of founding genetic diversity are known to influence the likelihood that invasive populations become successful, because genetic diversity may also mediate population fitness and susceptibility to adversities,^{54–56} such as treatments with insecticide.

The domiciliary populations from A and C that exhibited high genetic diversity also showed low F_{ST} values with all ruderal populations ($F_{ST} < 0.15$, except for A domiciliary populations with C2R). On the contrary, the B domiciliary population that exhibited lower genetic diversity also showed high F_{ST} values with ruderal populations. This suggests that domiciliary populations A and C were mainly colonized by ruderal populations, whereas domiciliary B populations originally resulted from another population source, likely domiciliary ecotopes, because they were less genetically diverse.

We observed that intradomiciliary and peridomiciliary sites within villages were genetically related (all $F_{ST} < 0.14$). Similar results were obtained for this same species using random amplified polymorphic DNA (RAPD) and morphometric markers.²³ Domiciliary versus ruderal populations within both of the C sub-localities, as well as A2, exhibited low F_{ST} values (< 0.12 , all $P < 0.05$), suggesting significant gene flow between ecotopes within a sub-locality. All C populations also exhibited the lowest F_{ST} values (all < 0.19 , $P < 0.0001$) with the sylvatic population, pointing toward a reasonable influence of this environment. For B sub-localities, however, domiciliary and ruderal populations were not significantly related within the sub-localities. For *Triatoma dimidiata* in the sylvatic and domiciliary environments in the Yucatan Peninsula of Mexico,⁵² variable patterns of relationship between domiciliary, peridomiciliary, and wild ecotopes were also shown by microsatellite analysis.

Triatoma brasiliensis is phylogenetically related to *Tri. infestans*,⁵⁷ which can fly 1.5–2 km from its source.⁵⁸ The distances between our localities were likely longer than the possible flight range of *Tri. brasiliensis*. However, both the Euclidean distance and the Mantel test indicated that the genetic differentiation observed for domiciliary populations has no correlation with physical distance. The lack of correlation be-

tween genetic and geographical distances also within localities strengthens the role played by ecotopes to limit the gene flow mainly because domiciliary and ruderal populations are not always genetic associated, despite the physical proximity. On the other hand, domiciliary populations in the same locality are often genetically associated, despite the longer physical distance.

The higher genetic interchanging among ruderal populations might be explained by environmental observations in the field. First, except for the physical distance, no other possible barrier to gene flow was detected. Second, ruderal populations are not subject to drastic bottleneck events, such as insecticide treatment directed at vector control, which has been suggested to promote genetic drift.⁵³ A similar panmictic pattern has also been postulated for wild populations of *Triatoma sordida*.^{20,21}

Influence of temporal factors and control activities on the genetic structure. A domiciliary population (B1IP) showed particularly high differentiation from all other populations, with all F_{ST} values significant and > 0.18 . This differentiation was caused by the low genetic diversity exhibited for this population, because one haplotype (IV) represented 48% of the total sample. However, the only domiciliary population that could be found within a short period after house spraying (A1IPi, originating from the A1 locality) exhibited the lowest F_{ST} value observed for B1IP collected a long period after house spraying. In the event these two populations share a related origin, we assume that the B1IP genetic profile has likely been maintained by population expansion after a bottleneck event and less successful ruderal invasions.

Domiciliary well re-established populations from A and B, as well as the population collected within a short period after insecticide spraying (in the process of re-establishment of infestation), seemed to be genetically related. AMOVA indicated that these domiciliary populations (from the A and B localities) collected a long period after insecticide spraying could compose a robust group with the populations collected within a short period after insecticide spraying (A1IPi). Overall results led us to assume that there were connections between A and B domiciliary populations, maybe through persistent foci between these localities that were not eliminated. Analysis of mtDNA sequences of the 12S and 16S ribosomal RNA genes in different natural populations of *Tri. infestans* from Argentina also suggested that the recovered insecticide-treated populations in some localities originated from survivors in the same area.⁵⁹ Because of the high variability for domiciliary populations in A a long period after insecticide spraying and the detection of positive captures in a short period after insecticide spraying, this locality might have acted as a source of founders for neighboring domiciliary populations. The dispersion between neighboring domiciles is a potential epidemiologic issue, because it might be the result (or increase the potential) of specialization to domiciliary environments.⁶⁰

Vector control implications. The combination of distinct geographic, ecologic, and temporal (or seasonal) patterns of domiciliary infestation represents a complex scenario for designing effective vector control strategies. This profile has been suggested for *Tri. dimidiata* in Mexico by morphometry and microsatellite markers.⁵² We identified different patterns of domiciliary colonization and dispersion, exhibiting different sources for re-infestations. Thus, we suggest that the het-

erogeneity observed in the genetic structure of *Tri. brasiliensis* must be taken into account for control measures. The only population found within a short period after insecticide spraying was genetically related to populations collected a long time after insecticide spraying in the same and other localities. Hence, we recommend particular attention to localities exhibiting a higher pressure of re-infestation. Ecologic studies are also required to identify factors favorable for domiciliary invasions, especially from ruderal foci.

A historical overview of the epidemiology of Chagas disease in northeastern Brazil indicates that two of the most important Chagas disease vectors (*Tri. infestans* and *Panstrongylus megistus*) became vulnerable to control after the event of domestication (adaptation to the domiciliary environment).^{6,61} There are also indications that this specialization allowed the enlargement of the vector's geographical distribution. The *Tri. brasiliensis* morphotype studied here exhibits the widest geographical distribution among the four members of this species complex.¹⁵ Despite being the most domiciliated morphotype, it still shows strong persistence in infesting houses after the control activities.¹⁵⁻¹⁷ Thus, modern molecular techniques can be useful tools for improving the control measures, monitoring vectorial, and understanding the events related to Chagas disease epidemiology.

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