Am. J. Trop. Med. Hyg., 96(3), 2017, pp. 660–663
doi:10.4269/ajtmh.16-0717

Application of Inter-Simple Sequence Repeat Markers in the Analysis of Populations of the Chagas Disease Vector *Triatoma infestans* (Hemiptera, Reduviidae)

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Abstract. Here we apply inter-simple sequence repeat (ISSR) markers to explore the fine-scale genetic structure and dispersal in populations of *Triatoma infestans*. Five selected primers from 30 primers were used to amplify ISSRs by polymerase chain reaction. A total of 90 polymorphic bands were detected across 134 individuals captured from 11 peridomestic sites from the locality of San Martín (Capayán Department, Catamarca Province, Argentina). Significant levels of genetic differentiation suggest limited gene flow among sampling sites. Spatial autocorrelation analysis confirms that dispersal occurs on the scale of ~469 m, suggesting that insecticide spraying should be extended at least within a radius of ~500 m around the infested area. Moreover, Bayesian clustering algorithms indicated genetic exchange among different sites analyzed, supporting the hypothesis of an important role of peridomestic structures in the process of reinfestation.

INTRODUCTION

Triatoma infestans (Hemiptera, Reduvidae) is the main vector of Chagas disease in South America between the latitudes 10° and 46° S. The analyses of the spatial genetic structure of populations at fine scale can provide insight into the dynamic population and evolutionary process of *T. infestans* and a complementary approach to help improve vector control strategies.

Among the polymerase chain reaction (PCR)-based marker techniques, the microsatellites have been used to evaluate the genetic structure of natural populations of *T. infestans*. Analyses based on these markers strongly supported the existence of some type of stratification in *T. infestans* populations and support the hypothesis of vector population recovery from survivors of the insecticide-treated areas, highlighting the value of population genetic analyses in assessing the effectiveness of Chagas disease vector control programs.[1–7] Microsatellites have been widely used as genetic markers although drawbacks for their use are the time and cost required to characterize them. Inter-simple sequence repeats (ISSRs) have also been widely used as genetic markers because they permit the detection of DNA variation without the need to isolate and sequence specific DNA fragments.[8] Moreover, they are highly polymorphic, of easy application, and of low costs. However, there are no previous reports on the use of ISSRs for genetic characterization of populations of the species. In this study, we use ISSR markers to explore the spatial genetic structure at fine scale and dispersal patterns in populations of *T. infestans*.

MATERIALS AND METHODS

A total of 134 *T. infestans* specimens were captured in 11 peridomestic sites of nine houses from the locality of San Martín (Capayán Department, Catamarca Province, Argentina) (Table 1), which are located at geographical distances ranging from 39 m to 16.42 km.

Thirty primers were initially used for amplification of ISSRs by PCRs. Five of these primers, which allowed obtain reproducible and variable banding patterns among individuals, were selected for the study (see Supplemental Table 1). Reaction products were visualized after electrophoresis on a 1.5% agarose gel (1× Tris-acetate ethylenediaminetetraacetic acid buffer) containing 0.1 μg/mL of ethidium bromide and photographed under ultraviolet light. The images were analyzed using the software Gel-Pro-Analyzer (Media Cybernetics, Rockville, MD). The different ISSR amplified fragments (bands) were considered as different loci and each band was considered as "present = 1" and "absent = 0" for each individual.

The percentage of polymorphic loci (P), unbiased haploid genetic diversity (uH), and informative index (*I*, equivalent to Shannon index) was calculated at each locus and sampling site. An analysis of molecular variance (AMOVA) was carried out to obtain the variance components, Φ, similar to the *F* statistics of Wright.[9–11] Isolation by distance was examined by calculating the correlation coefficient between the pairwise Φ_{ST} values and geographical distances (as straight-line distance between all pairs of sampling sites) and was tested using the Mantel test after 1,000 permutations. All these analyses were performed using the program GENALEX version 6.502 (The Australian National University, Canberra, Australia).[12]

A Bayesian approach implemented in the program STRUCTURE version 2 (University of Chicago, Chicago, IL) was used to infer the number of populations (*K*) in the data set without prior information of the sampling locations.[13] The admixture model and correlated allele frequencies were assumed. A series of five independent runs for each value of *K* between 1 and 11 were conducted. Each run had a burn-in period of 100,000 and a 1,000,000 run length. The most likely number of clusters (*K*) was determined using the Δ*K* method, as well as by examining the plateau of the estimated probability of the data.[14]

Spatial genetic structure was assessed using an approach to microspatial autocorrelation analysis implemented in the program GENALEX version 6.2.[12] This software calculates

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TABLE 1
Sampling site and sample size of *Triatoma infestans* from the locality of San Martín (Capayán Department, Catamarca Province,)

Capture site	Habitat	Sample size
BJ	Chicken coop	16
BR I	Goat corral	4
BR II	Shed	23
BR III	Chicken coop	16
BL	Goat corral	16
FD	Chicken coop	16
MA	Rabbit hutch	9
PF	Goat corral	12
PA	Shed	3
RE	Goat corral	4
RH	Shed	15

Numbers indicate different habitats from one dwelling.

the multilocus autocorrelation coefficient *r* among individual genotypes for a specified number of distance classes. The distance classes were selected to make the number of pairwise comparisons in each bin as even as possible to avoid noise in the confidence limits that can cause unequal sample size. To test whether the *r* correlation coefficient was significantly different from the null hypothesis of no spatial genetic structure, 1,000 random permutations were performed to determine upper and lower confidence intervals (CIs) for the null hypothesis.[15]

RESULTS AND DISCUSSION

A total of 90 polymorphic bands were detected across the 134 individuals captured from 11 sampled locations (Table 1). The percentage of polymorphic loci (P) per sampling site ranged from 35.56% in MA to 65.56% in BR II with an average value of 50.14%. The average unbiased haploid genetic diversity (uH) was of 0.18 and ranged from 0.14 in the samples from PF and FD to 0.25 in BR III. The average information index (*I*, equivalent to Shannon index) was of 0.25, with the lowest value in the sample from PF (I = 0.19) and the highest value in the sample from BR III (I = 0.34) (see Supplemental Table 2). In *Aedes aegypti* and *Culex quinquefasciatus* were detected higher levels of genetic diversity using ISSR markers, with an average value of uH of 0.31 and an average value of the Shannon index of 0.36, respectively.[16,17] However, although the levels of genetic diversity detected in this study were moderate, it is important to point out that the analysis of a greater number of primers will allow to explore a greater proportion of the genome, increasing the potential of ISSR markers for the analysis of *T. infestans* populations.

The overall Φ_{ST} value of 0.26 is significantly different from zero (P = 0.001), as are all pairwise Φ_{ST} values ($P < 0.001$), including the different sampled sites within the same house. The pairwise Φ_{ST} values ranged from 0.15 between the samples from BJ and PF to 0.39 between RH and PF (see Supplemental Table 3). There was not a significant association between geographical distance and genetic differentiation (Φ_{ST}) among sites (Mantel r = 0.09, P = 0.31). This pattern suggests restricted gene flow among sampling sites, where allele frequencies could drift independently without relation to the geographic distances separating them. These results are in agreement with previous works that suggested a high degree of subdivision in the population into breeding units with restricted possibilities of genic exchange.[1,2,4] In this regard, *T. infestans* is primarily restricted to domestic and peridomestic environments (such as chicken coops and pig or goat corrals) particularly in rural areas and usually remains in the same house or in its immediate vicinity during its lifetime.

Spatial autocorrelation analysis for the 134 individuals indicated that significantly positive autocorrelations were found in the first (70 m, r = 0.142, 95% CI: 0.008, −0.006) and third distance classes (1,000 m, r = 0.013, 95% CI: 0.007, −0.008), with an *x* intercept at 469 m. Significant negative values were observed in the second distance class (600 m, r = −0.047, 95% CI: 0.008, −0.008) and at distances equal to and greater than 14 km (r = −0.07, 95% CI: 0.006, −0.008) (Figure 1). In populations with restricted gene flow by distance, a positive correlation (r) is expected at the shorter distance classes. This correlation will become zero at distance classes where drift predominates over gene flow, declining to negative correlation, sometimes followed by oscillation of positive and negative values. In this respect, in concordance with one previous study carried out with microsatellite markers,[4] the scale of structuring detected in the spatial autocorrelation analyses suggests that dispersal typically occurs on the scale of approximately 469 m. The active dispersal inferred for *T. infestans* by autocorrelation is within the flight range indicated for this species,[18,19] and suggests that insecticide spraying should be extended at least within a radius of ~500 m around the infested area.

The high degree of isolation detected among different sampling sites was consistent with results obtained by the program STRUCTURE. Seven different genetic clusters were identified (Figure 2). Individuals from MA, BR II, BR III,

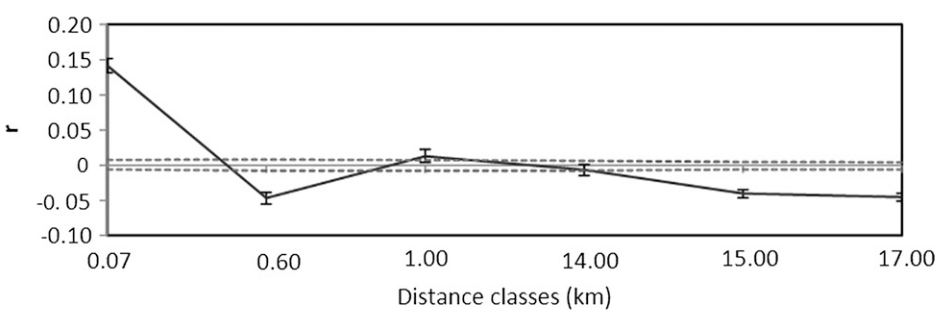

FIGURE 1. Autocorrelogram plot of the genetic correlation coefficient (r) as a function of distance. The permuted 95% confidence intervals (dashed lines) and bootstrapped 95% confidence error bars are also showed. This figure appears in color at www.ajtmh.org.

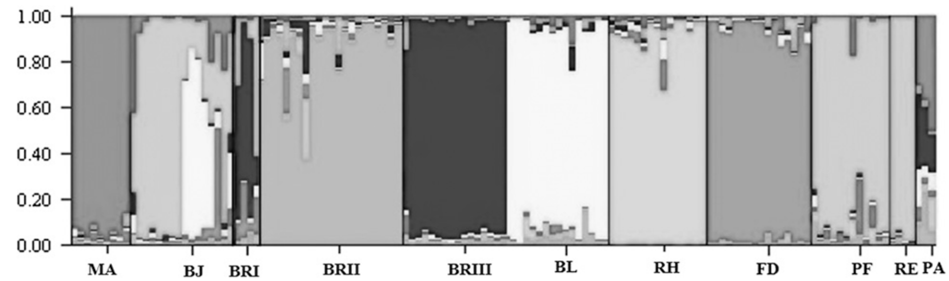

FIGURE 2. Results of STRUCTURE analyses for the sampled locations from San Martín (Capayán Department, Catamarca Province, Argentina). A model of K = 7 was most supported. Each column represents an individual where cluster membership assignment is on the y axis. This figure appears in color at www.ajtmh.org.

BL, RH, FD, PF, and RE formed nearly homogeneous clusters. In contrast, samples from BJ, BR I, and PA were found to consist of a mixture of clusters, which share different percentage of ancestry (relative proportion of the genome of an individual originating from each inferred cluster) with the rest of the capture sites analyzed. The individuals from two goat corrals (PF and RE) were grouped into a cluster with more than 86% of ancestry shared among them, and the sample from BJ (chicken coop) shares with this cluster around 30% of the ancestry. The sites PF and BJ, which are geographically close, showed the lowest level of genetic differentiation (Φ_{ST} = 0.15), suggesting gene flow between the sites. Similarly, the individuals from BJ share 23% of ancestry with the individuals obtained in other geographically close goat corral (BL). Moreover, the bugs from another goat corral (BR I), share about 55% of ancestry with those from a chicken coop from the same house (BR III), and the insects captured in PA (shed) share 40% of ancestry with those from MA (rabbit hutch). It is known that insecticide spraying in animal corrals has limited effectiveness. Particularly, goat corrals have been known to support abundant population of *T. infestans*. These environments has higher prevalence of *T. infestans* before and after residual spraying with insecticides than other peridomestic environments and may increase the risk of reinfestation in the whole area.[20] The shared ancestry among different sampling sites suggest genetic exchange among individuals from these places, supporting the hypothesis that peridomestic structures would be involved in the reinfestation process. These results highlight the importance of entomological surveillance of peridomestic environments in the insecticide-treated areas.

Received September 1, 2016. Accepted for publication December 2, 2016.

Published online January 23, 2017.

Note: Supplemental tables appear at www.ajtmh.org.

Financial support: This research was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (FONCyT-Argentina), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina), and Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba (SECyT-UNC-Argentina).

Disclosure: Alicia R. Pérez de Rosas and Beatriz A. Garcia are Career Investigators of CONICET.

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SUPPLEMENTAL TABLE 1

Sequences of 30 ISSR primers initially screened for amplification in *Triatoma infestans*

Primer	Sequence (5′–3′)
BOA1*	$(GT)_8YG$
BOA2	$(GT)_8A$
BOA3	$(CA)_8T$
BOA4	$(AC)_8C$
BOA5*	$(TC)_9G$
ISSR1	$(AG)_8Y$
ISSR2	$(AC)_8G$
ISSR3	$(CA)_8RT$
ISSR4	$(GT)_8YA$
ISSR5	$(GACA)_4$
ISSR7	$(AC)_8YT$
ISSR9	$GT(GGT)_2GGC$
ISSR10*	$(CAC)_4RC$
ISSR11*	$(CA)_6RG$
AEISSR1	$(GA)_8C$
AEISSR2	$(CA)_8G$
AEISSR4	$(GTT)_5T$
AEISSR6	$(CCA)_5$
RHEA1	$(TG)_{10}CG$
RHEA2	$(TG)_{10}CG$
RHEA4	$(AC)_{10}AG$
PRIMER2	$(CAA)_6$
PRIMER3	$(GA)_9$
CT	$C(TCC)_5T$
HER1	$(CA)_8$
HER2	$(CT)_9$
PA1	$(CT)_8AG$
PA2	$(CT)_8GG$
PA3*	$(CA)_7CTCTT$
PA4	$(CTT)_5TT$

ISSR = inter-simple sequence repeats; R = A or G; Y = C or T.

*ISSR primers selected to population genetic analyses of *T. infestans*. Polymerase chain reaction amplifications were carried out in 25 μL of a solution containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, each dNTP at 200 μM, each primer at 1 μM, genomic DNA (10–50 ng), and 1 U of GoTaq (Promega, Madison, WI). Thermal profiles consisted of an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of 30 seconds at 94°C (denaturation), 1 minute at 47°C (annealing), and 90 seconds at 72°C (extension), with a final extension step of 5 minutes at 72°C.

SUPPLEMENTAL TABLE 2

Summary of genetic variation at eight collection sites for *Triatoma infestans*

Capture site	P (%)	*I*	uH
BJ	47.78	0.23	0.16
BR II	65.56	0.30	0.20
BR III	62.22	0.34	0.25
BL	61.11	0.29	0.20
FD	43.33	0.20	0.14
MA	35.56	0.20	0.15
PF	43.33	0.19	0.14
RH	42.22	0.23	0.16
Overall	50.14	0.25	0.18

I = information index; P = polymorphic loci; uH = unbiased haploid genetic diversity. In BR I, RE, and PA, three or four insects were captured. These samples were excluded for the analysis of genetic diversity because it depends on sample size.

Supplemental Table 3

Levels of genetic differentiation (Φ_{ST}, below the diagonal) and geographic distances among sample sites (km, above the diagonal)

	MA	BJ	BR III	BR IV	BL	RH	FD	PF
MA	0	0.63	15.53	15.50	0.70	0.05	1.03	0.76
BJ	0.21***	0	14.97	14.95	0.07	0.59	1.66	0.16
BR III	0.29***	0.24***	0	0.04	14.91	15.48	16.42	14.91
BR IV	0.27***	0.25***	0.20***	0	14.88	15.45	16.42	14.88
BL	0.24***	0.24***	0.27***	0.19***	0	0.66	1.74	0.12
RH	0.33***	0.35***	0.27***	0.27***	0.21***	0	1.07	0.73
FD	0.31***	0.29***	0.32***	0.27***	0.26***	0.32***	0	1.79
PF	0.28***	0.15***	0.26***	0.22***	0.30***	0.39***	0.27***	0

In samples sites BR I, RE, and PA, three or four insects were captured. These samples were excluded for the analysis of genetic differentiation among sites because it depends on sample size.

***$P < 0.001$.