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Very Low Levels of Genetic Variation in Natural Peridomestic Populations of the Chagas Disease Vector *Triatoma sordida* (Hemiptera: Reduviidae) in Southeastern Brazil

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Abstract. Levels of genetic variation and population structure were determined for 181 *Triatoma sordida* insects from four populations of southeastern Brazil, through the analysis of 28 allozyme loci. None of these loci presented fixed differences between any pair of populations, and only two revealed polymorphism, accounting for low levels of heterozygosity ($H_e = 0.027$), and low genetic distances ($D < 0.03$) among populations. F_{ST} and Contingency Table results indicated the existence of genetic structure among populations ($F_{ST} = 0.214$), which were incompatible with the isolation by distance model (Mantel test: $r = 0.774$; $P = 0.249$).

INTRODUCTION

Triatoma sordida (Stål 1859) is the Chagas disease vector most frequently captured in the peridomestic environment in Brazil, particularly in areas where *Triatoma infestans* (Klug 1834) has been eliminated[1,2] by the Southern Cone Initiative, a successful insecticide-based vector control campaign launched in 1991, targeting this particular species.[3,4] However, because of its inability to form large colonies inside human habitations, and overall low levels of infection with the *Trypanosoma cruzi* parasite, *T. sordida* is considered to be of reduced vectorial relevance in both Brazil and Bolivia.[1,5,6]

With respect to *T. sordida* taxonomy, important genetic differences (allozymic and chromosomal) between populations from Brazil and Argentina,[7] led to the revalidation of *Triatoma garciabesi* (formerly *T. sordida* populations from Argentina), with further support from allozymes and morphology.[8] In addition, cryptic speciation in *T. sordida* populations from the Bolivian Chaco was revealed through the detection of diagnostic allozyme loci in sympatric samples.[9] Factors responsible for these speciation events (i.e., geographic isolation, ecologic segregation) are unknown. Likewise, little is known about which geologic or geographic structures or events might constitute effective gene flow barriers for triatomine populations.[10,11]

Although there are many studies on the application of molecular markers to research Triatominae taxonomic issues,[12–17] few (with an understandable bias toward *T. infestans*) are dedicated to most important aspects regarding the planning and execution of vector control initiatives: the assessment of the levels of genetic variation, population structure, and gene flow among insect populations.[18–23]

Thus, this work intends to address two issues: 1) whether there is cryptic speciation in Brazilian populations of *T. sordida* as reported for populations from Argentina and Bolivia; and 2) whether allozymes are suitable molecular markers for the assessment of genetic variability in natural Brazilian *T. sordida* populations, which would consequently enable population structure inferences.

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MATERIALS AND METHODS

Study areas. The four areas studied are located in the central and northern parts of Minas Gerais State, in southeastern Brazil (Figure 1, Table 1), an arid region where the *Cerrado*, *Caatinga*, and *Parana Forest* biogeographic provinces come into contact.[24] All four sampled areas were poor rural villages where *pau-a-pique* (wattle and daub) houses were common. This simple building technique is a crucial element in disease transmission as the walls are prone to the formation of cracks and crevices where domestic vectors hide. Subsistence agriculture and rudimentary poultry and livestock breeding (chicken coops and pigsties) were the main human activities observed. Geographic distances between locations ranged from 28 km (Espinosa and Mamonas) up to 425 km (Espinosa and Corinto) (Figure 1, Table 2).

Collection of insects. Insects were manually collected from the four sites during a 7-day expedition carried out in December 1996. Areas were selected based on a combination of two factors: 1) long time elapsed since last spraying (over 2 years); and 2) positive reports of triatomine occurrence gathered by the entomologic surveillance centers of each municipality. Ten houses (and peridomestic premises) were sampled from each site. Each house was searched for approximately 30 minutes (0.5 person-hour for the inside of houses, and 1 person-hour for the peridomestic premisses).

After collection, triatomines were morphologically identified based on Lent and Wygodzinsky[25] and stored in liquid nitrogen until genetic analyses.

Allozyme electrophoresis. Horizontal allozyme electrophoresis was carried out using two different support media, to obtain the best resolution possible for the enzyme systems tested: 1% agarose gels[26]; and cellulose acetate plates.[27] The head and thorax of each specimen were homogenized in 150 μL of lysis buffer (0.50 M Tris HCl, 0.026 M EDTA, 0.010 M DTT, 0.010 M ε-amino-n caproic acid). The enzyme and buffer systems researched, and the support media used are summarized in Table 3. After electrophoresis, gels were stained according to standard procedures.[28]

Data analysis. Genotype frequencies were obtained by direct genetic interpretation of bands on the gels. From these, gene frequencies, fits to Hardy-Weinberg equilibrium, genetic variation (percentage of polymorphic loci and heterozygosity), inbreeding indices (F_{IS}, F_{ST}[29]), Contingency Tables, and unbiased genetic distances (D[30]) were obtained with the BIOSYS program version 1.7.[31] The significance of F_{ST} (H_o: $F_{ST} = 0$) was

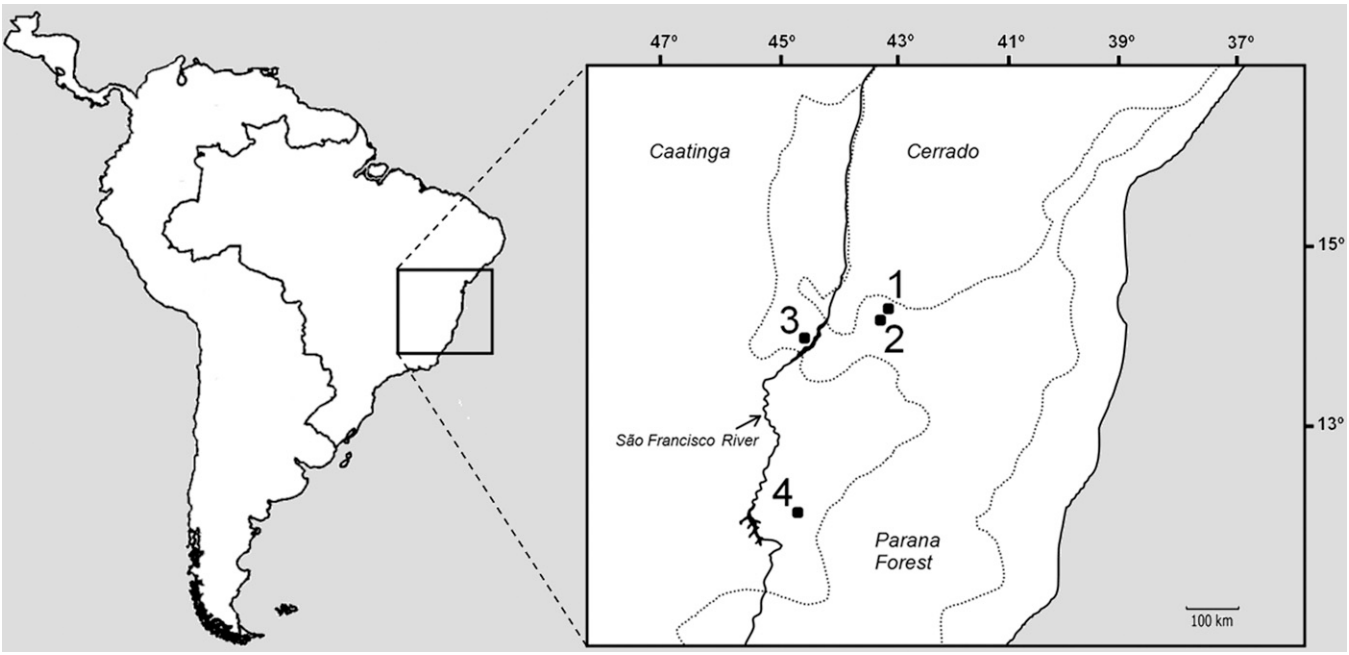

FIGURE 1. Sampling sites of *Triatoma sordida* populations in Minas Gerais State, southeastern Brazil. (1) Espinosa; (2) Mamonas; (3) Januária; (4) Corinto. Dotted lines represent the boundaries of the *Cerrado*, *Caatinga*, and *Parana Forest* biogeographic provinces. Note that the Januária population is located on the west bank of the wide São Francisco River.

tested according to Waples's χ^2 test[32]: $\chi^2 = 2NF_{ST}$ *(k-1)*; *d.f. = (k-1)(s-1)*; where *N* is the total number of individual sampled, *k* is the number of alleles at the locus, and *s* is the number of populations.

To research whether geographic distances were directly correlated to genetic distances between pairs of *T. sordida* populations, we used a Mantel test, with 1,000 replicates,[33] with the TFPGA program version 1.3.[34]

RESULTS

Triatoma sordida was the sole species present in the studied area and no insects were found inside human habitations. The majority of insects (71%) were captured from chicken coops (or other places used by hens to nest). The remaining insects were captured from wood piles and pigsties. This effort resulted in a total of 181 insects collected from the four geographic populations (see Table 1 for sample sizes per population).

All insects were typed for 28 allozyme loci. The 18 enzyme systems were tested for both electrophoresis support media (Table 3). None of the studied loci presented fixed differences between any pair of populations (i.e., was diagnostic, *sensu* Ayala[35]), and only two loci (*Acon-2* and *Pgd*) revealed polymorphism (Table 4).

As a consequence of the high number of monomorphic loci, values of unbiased genetic distances among populations were very low ($D < 0.03$).[30] Neither *Acon-2* nor *Pgd* presented significant departures from Hardy-Weinberg expectations in any of the four populations analyzed (exact test: $P > 0.05$; $F_{IS} = 0$, $P > 0.05$; Table 4). Pairwise F_{ST} comparisons between populations, as well as Contingency Tables, resulted in significant results (Table 2), indicating the existence of genetic structure.

No significant correlation was observed between geographic distances and genetic differentiation among southeastern Brazilian populations of *T. sordida* (Mantel test: $r = 0.774$; $P = 0.249$).

TABLE 1

Sampling sites (with respective geographic coordinates), and sample sizes (*N*) of the four *Triatoma sordida* populations studied

Site	Coordinates	*N*
(1) Espinosa	14°55′38″ S/42°48′59″ W	68
(2) Mamonas	15°03′00″ S/42°56′58″ W	34
(3) Januária	15°29′15″ S/44°21′40″ W	42
(4) Corinto	18°21′47″ S/44°27′40″ W	37

DISCUSSION

We found no fixed genetic differences (i.e., diagnostic loci) among the studied populations, and very low levels of genetic variability after the analysis of 28 allozyme loci ($P = 7.1\%$; $H_e = 0.027$). Regardless of the low levels of polymorphism observed, significant estimates of genetic structure were detected among the four *T. sordida* populations analyzed from southeast Brazil ($F_{ST} = 0.214$; $P < 0.05$).

Although hidden taxonomic variation within *T. sordida* from Argentina and Bolivia was disclosed with the use of allozyme markers,[7,9] we were unable to detect cryptic speciation in the four Brazilian populations studied, even after the analysis of a large number of allozyme loci. The reduced allozyme variability determined in the present work for Brazilian *T. sordida* populations corroborates earlier reports for other species,[18,36–38] and indicates what seems to be a rule for Chagas disease vectors.

Low heterozygosity levels were also detected for field-collected *T. sordida* Groups 1 and 2 from the Bolivian Chaco ($H_e = 0.050$ and 0.065, respectively[9]), and from colony specimens of the closely related *T. garciabesi* from Argentina ($H_e = 0.070$),[8] and are compatible with the levels found in the present study ($H_e = 0.027$). On the other hand, García and others[39]

TABLE 2

Genetic differentiation (F_{ST}) and geographic distances (in parentheses*), above diagonal, and χ^2 values (Contingency Table†), below diagonal, among *Triatoma sordida* populations studied from southeastern Brazil

Populations	1	2	3	4
(1) Espinosa	–	0.007‡ (28)	0.049§ (179)	0.227§ (425)
(2) Mamonas	2.785‡	–	0.043‡ (156)	0.184§ (399)
(3) Januária	17.116§	12.893§	–	0.356§ (322)
(4) Corinto	84.116§	50.940§	108.496§	–

* km.
† *d.f.* = 2, $P < 0.05$, after Bonferroni series.[47]
‡ Not significant, $P > 0.05$.
§ $P < 0.05$, after Bonferroni series.

reported a high percentage of polymorphic loci for colony insects of four *Triatoma* species, with values ranging from 52.9–58.3%. However, if such values are corrected for differences in sample sizes by taking into account the 95% criterion (i.e., loci for which the frequency of the most common allele exceeds 95% are considered monomorphic), the percentage of polymorphic loci reported will be considerably reduced (ranging from 7.1% to 41.7%). The *T. sordida* specimens used in that study were from an F3 colony started with 67 nymphs from Campo Duran, Salta, Argentina, and produced an H_e value of 6.2%.[39]

The native *Panstrongylus megistus* (Burmeister 1835) originally abundant in houses of central and eastern Brazil seems to have been progressively displaced by *T. infestans* during the past century.[2] As *T. infestans* further dispersed northwards, it apparently displaced competitively not only *P. megistus* but *T. sordida* as well,[40] arguably because of its higher efficiency in obtaining bloodmeals.[41]

Triatoma infestans was, until the 1980s, the main domestic vector species present in the studied area (and in the rest of the country for that matter). Vector control campaigns launched in 1991 as part of the Southern Cone Initiative targeting this particular species were very successful,[3] and by 1995 *T. infestans* had disappeared from the region. After nationwide successful control achievements, the Brazilian Ministry of Health was awarded the International Elimination of Transmission of Chagas Disease Certificate from the Pan American Health Organization.[4]

As a consequence of the elimination of *T. infestans*, other secondary vector species such as *T. sordida* became more frequently detected in the studied region. However, Brazilian *T. sordida* seems only capable of colonizing chicken coops, whereas *T. infestans* colonizes both chicken coops and houses effectively. For this reason, the nationwide adopted strategy for insecticide application against *T. infestans* always aimed at both intradomestic and peridomestic structures. This action is likely to have had an impact on the existing sympatric *T. sordida* peridomestic populations.

Although this study was not originally designed to evaluate the effect insecticide control campaigns against *T. infestans* had on *T. sordida* populations, the observation that all four *T. sordida* peridomestic populations analyzed presented the same two alleles for the same two polymorphic loci seems to favor the interpretation that *T. sordida* populations were in fact affected by the control actions. Moreover, it suggests that present day peridomestic populations from southeastern Brazil could represent a recent recolonization event derived from a residual peridomestic focus. Otherwise, if *T. sordida* populations were not affected by control actions, the most likely result would have been the detection of high and randomly distributed variability across examined loci. Nonetheless, as *T. sordida* is autochthonous in Brazil, it is worthwhile to speculate on the role that natural sylvatic populations (supposedly unaffected by the control actions) would have played in the recolonization of treated areas. Given the low levels of genetic variation observed, it seems unlikely that sylvatic *T. sordida* populations would have served as genetic diversity reservoirs during insecticide control efforts (assuming that sylvatic populations are genetically more variable than peridomestic populations).

TABLE 3

Enzyme systems (with respective number of scored loci) and support media used in this study*

Enzyme	Enzyme code (EC) no.	Abbreviation	Medium	No. of loci
Aconitase	4.2.1.3	ACON	Agarose	2
Diaphorase	1.6.2.2	DIA	Acetate	1
α-Esterases	3.1.1.1	α-EST	Agarose	2
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH	Agarose	2
Glucose-6-phosphate isomerase	5.3.1.9	PGI	Agarose	1
Glutamate dehydrogenase	1.4.1.4	GDH	Agarose	1
Glutamate oxaloacetate transaminase	2.6.1.1	GOT	Acetate	1
α-Glycerophosphate dehydrogenase	1.1.1.8	α-GPD	Agarose	2
Hexokinase	2.7.1.1	HK	Acetate	2
Isocitrate dehydrogenase	1.1.1.42	IDH	Agarose	1
Leucine aminopeptidase	3.4.11.1	LAP	Acetate	2
Malate dehydrogenase	1.1.1.37	MDH	Acetate	2
Mannose 6-phosphate isomerase	5.3.1.8	MPI	Agarose	1
Malic enzyme	1.1.1.40	ME	Acetate	1
Peptidases (pro-phe)	3.4.1.1	PEP	Acetate	3
Phosphogluconate mutase	5.4.2.2	PGM	Agarose	1
Phosphogluconate dehydrogenase	1.1.1.44	PGD	Acetate	1
Superoxide dismutase	1.15.1.1	SOD	Acetate	2

*The Tris-citrate pH 8.0 (0.25 M Tris, 0.06 M citrate, pH 8.0)[48] buffer system was used with both electrophoresis support media.

TABLE 4

Gene frequencies and fits to Hardy-Weinberg expectations (F_{IS}), for the two polymorphic loci for four *Triatoma sordida* peridomestic populations from southeastern Brazil, and observed (H_o) and expected (H_e) mean heterozygosities for all 28 loci studied*

		Population			
Locus	Allele	Espinosa	Mamonas	Januária	Corinto
Acon-2	1	**0.546**	**0.574**	**0.821**	0.162
	2	0.454	0.426	0.179	**0.838**
	(*N*)	(65)	(34)	(42)	(37)
	F_{IS}	0.108†	0.352†	0.281†	0.217†
Pgd	1	0.221	0.328	0.230	**0.757**
	2	**0.779**	**0.672**	**0.770**	0.243
	(*N*)	(68)	(32)	(37)	(35)
	F_{IS}	0.237†	0.235†	0.325†	0.160†
H_o		0.025	0.024	0.016	0.019
H_e		0.030	0.034	0.023	0.023

*Most frequent alleles per locus are in bold. (*N*): number of individuals analyzed.
†Not significant, $P > 0.05$.

Population structure inferences, although preliminary because of the small number of polymorphic loci detected, revealed significant results for most pairwise comparisons made, indicating the existence of genetic differentiation. A Mantel test used to assess whether populations exhibited a pattern compatible with the isolation by distance model (IBD), was statistically non-significant ($r = 0.774$; $P = 0.249$), in contrast to findings for *T. sordida* Group 1 populations from Bolivia that were compatible with the IBD model.[19] Unfortunately, no data was ever generated for either *T. sordida* Group 2, or *T. garciabesi* to allow their testing for IBD.

Alternative hypotheses to explain the pattern of genetic structure found should take into account the possible influence current geographic barriers might have on the restriction of gene flow between the studied populations. Januária is the sole population located west of the São Francisco River (Figure 1), which presently has an estimated 730 m in breadth. Interestingly, what became evident from the genetic structure estimates, was that Januária and Corinto populations are more differentiated ($F_{ST} = 0.356$) than, for example, the geographically most distant sites compared (Mamonas versus Corinto: $F_{ST} = 0.184$; Espinosa versus Corinto: $F_{ST} = 0.227$). Although it is possible that large rivers could act as barriers to gene flow for triatomines, this hypothesis has not been addressed using appropriate sampling designs.[11]

Population level studies require information on a large number of polymorphic loci to allow for the formulation of robust population structure inferences, and the very low levels of variation here reported represent a clear limitation in that regard. Microsatellite markers, which have already been isolated and characterized for five triatomine species,[42–46] present advantages over the traditional allozymes, because of their faster evolution rate (and thus expected higher variability), and the non-requirement of fresh (or frozen) samples for the laboratory procedures. Therefore, they will most likely replace the traditional allozymes as the markers of choice for triatomine population level investigations in the near future. The development of microsatellite loci for *T. sordida* will enable the testing of the allozyme-based genetic structure here detected.

It would be important for the better understanding of the taxonomy of this species complex to determine the phylogenetic relationship that the *T. sordida* samples studied here have with respect to the cryptic species detected in Argentina and Bolivia. This would enable the characterization and subsequent identification of the epidemiologically relevant targets to be combated in each country.

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