Genetic Structure of *Triatoma sordida* (Hemiptera: Reduviidae) Domestic Populations from Bolivia: Application on Control Interventions

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The genetic population of Triatoma sordida *group 1, a secondary vector of Chagas disease in Bolivia, was studied by multi-locus enzyme electrophoresis. A total of 253 nymphal and adult specimens collected from seven neighbouring localities in the Velasco Province, Department of Santa Cruz, were processed. The relatively low genetic variability was confirmed for this species (rate of polymorphism: 0.20). The absence of genetic disequilibrium detected within the seven localities was demonstrated. A geographical structuration appears between localities with distances greater than 20 km apart. Although* T. sordida *presents a relatively reduced dispersive capacity, its panmictic unit is wider than compared with* T. infestans*. Genetic distances between* T. sordida *populations were correlated with geographic distance. Gene flow between geographic populations of* T. sordida *provides an efficient framework for effective vigilance and control protocols.*

Key words: *Triatoma sordida* - multilocus enzyme electrophoresis (MLEE) - dispersal behavior - population genetics - Bolivia

Triatoma sordida (Hemiptera: Reduviidae) is a blood-sucking insect vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. It is widely distributed throughout central Brazil, eastern and central Bolivia, the Chaco region of Paraguay, and northwestern Argentina where it occurs primarily in a silvatic environment (Lent & Wygodzinsky 1979, Diotaiuti et al. 1995). In wide areas of the Southern Cone, *T. sordida* is more often found in peridomestic habitat and can also form domestic colonies (Forattini 1980, Dias 1988, Noireau et al. 1996).

T. sordida is currently considered as a possible substitute to the present domestic vector *T. infestans* in the transmission of *T. cruzi*. The process of domiciliation may also be primary without any relation to a previous eradication of the main vector (Dias 1988, Noireau et al. 1996). Owing to its inability to form large colonies, *T. sordida* holds a poor vectorial significance in domiciliary condition (Noireau et al. 1997). Nevertheless, its wide distribution, tendency to invade domestic environment, and vectorial competence in laboratory allow us to consider it a triatomine of potential epidemiological importance (Schofield 1994). The principal features of biology and ecology of *T. sordida* are quite well known (Forattini et al. 1982, Carcavallo & Martínez 1985). With reference to its dispersive pattern, this species shows a higher propensity for flight than does *T. infestans* (Schofield et al. 1991). Nevertheless, in spite of some works, the mobility of *T. sordida* with relation to artificial ecotopes was little studied (Forattini et al. 1971).

Previous works agreed with the existence of at least two biological species occurring within *T. sordida* (Panzera et al. 1997, Noireau et al. 1998). Consequently, the first step of any study about populations of this triatomine should be the genetic characterization of the biological species. The population genetics, which estimates the gene flow between geographic populations, provides an efficient framework for the study of the dispersive capacity of Triatominae and the design of effective vigilance and control protocols (Schofield et al. 1995, Dujardin et al. 1998). Using multilocus enzyme electrophoresis (MLEE), we explored the

Financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (grant 940209) and the French Ministry of Foreign Affairs.

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Received 30 October 1998
Accepted 13 January 1999

genetic variability and equilibrium in various domestic populations of *T. sordida* and estimated their level of genetic differentiation.

MATERIALS AND METHODS

Study areas - The field-work was carried out in Velasco Province located on the north of the Department of Santa Cruz, Bolivia. This Province forms part of the Chiquitania region and is made up of a mosaic of semi-humid woodland and wooded savanna (cerrado). The main climatic characteristics are: (i) a mean annual rainfall of 1,200 mm, including a dry season between May and September, (ii) moderate temperatures with an annual mean of 26°C and (iii) a mean relative humidity which fluctuates between 57 and 78%. Seven rural localities located in two areas were studied in 1995 (Noireau et al. 1997). The first area, situated around the town of San Ignacio de Velasco (16°23'S; 60°58'W), included three localities situated 20±9 km apart on an average: Cochabambita (CBA), Recreo (REC) and San Juan Bautista (SJB). The second area included four localities around the town of San Miguel (16°41'S; 60°59'W) situated 30±13 km apart on an average: Guapomocito (GUA), Cerrito (CER), Tacoigo (TAC) and Cotoca (COT). The dwellings investigated are mainly of wattle and daub construction and roofed with straw. A preliminary study performed in 1994 showed that *T. sordida* was the only triatomine species infesting houses in these localities (Noireau et al. 1996).

Insects - Manual collection of *T. sordida* was carried out in 88 houses of the seven selected localities during February 1995. Triatomine specimens caught in sleeping quarters of houses (wall or bed) were placed in plastic bottles containing filter paper and transported to the laboratory.

Isoenzyme electrophoresis - Nymphal instars and adults of both sexes were used. Alary muscles were dissected out and ground in 100 µl of an enzyme stabilizer (dithiothreitol, E-aminocaproic acid and EDTA, each at 2 mM). Extracts were stored at -70°C until used. MLEE was performed on cellulose acetate plates (Helena Laboratories, Beaumont, TX). The following 12 enzyme systems were assayed: diaphorase (DIA, EC 1.6.2.2.); aspartate aminotransferase (GOT, EC 2.6.1.1.); glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49.); glucose phosphate isomerase (GPI, EC 5.3.1.9.); a-glycerophosphate dehydrogenase (GPD, EC 1.1.1.8.); isocitrate dehydrogenase (IDH, EC 1.1.1.42.); leucine aminopeptidase (LAP, EC 3.4.11); malate dehydrogenase (MDH, EC 1.1.1.37.); malic enzyme (ME, EC 1.1.1.40.); aminopeptidase A (PEP-A with substrate L-leucyl-leucyl-leucine, EC 3.4.11.); phosphoglucomutase (PGM, EC 2.7.5.1.); and 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44). Conditions for electrophoresis and enzyme staining were as previously described by Noireau et al. (1998).

Statistical methods - Genetic interpretation was based on a previous description of *T. sordida* variability (Noireau et al. 1998). A locus was defined as polymorphic if the frequency of the rarest allele was ³ 0.02 in at least one of the samples. Genetic variability was estimated by the rate of polymorphism (P) and the mean number of alleles per locus (A). For the analysis of genetic equilibrium and spatial subdivision of *T. sordida* populations, only two possible alleles were taken into account: the most frequent one (a), and all other ones plotted together as a unique allele (N). Analysis of spatial subdivision of *T. sordida* populations was performed in triatomines issued from conglomerates (localities pooled by area) and whole population, using Nei's F statistics analysis (Nei 1987). Fit and Fis measure the departure from panmixia of individuals relative to the total population and its subpopulations, respectively. Fst measures the gene frequencies differences among subpopulations. Nei's standard genetic distances (Ds) were plotted against geographical distances between localities (21 pairwise comparisons) and the coefficient of determination assessed from a second order binomial curve. The Mantel test was used to confirm the statistical significance of the coefficient of determination (Sokal & Rohlf 1995).

RESULTS

Insects - A total of 253 nymphal and adult specimens of *T. sordida* were collected from seven localities. Data on the number of individuals processed by locality are summarized in Table I.

Isoenzyme variability - A single zone of enzymatic activity or band was scored for DIA, GOT, G-6-PDH, GPI, PGM and 6-PGDH, while two bands interpreted as two distinct loci were scored for GPD, IDH, LAP, MDH and ME. Four bands corresponding to four loci were scored for PEP-A. Thus, the studied set of enzymes represented a total of 20 gene loci. The allelic distribution at *Idh2* and *Mdh2* assigned our *T. sordida* populations to the group 1 as previously described by Noireau et al. (1998). *T. sordida* sample showed four polymorphic loci (*Mdh1*, *Pep4*, *Pgm* and *6-Pgdh*). The variable loci segregated for two (*6-Pgdh*) or four (*Pep4*, *Mdh1* and *Pgm*) alleles, according to locality of collection. Consequently, the genetic variability of *T. sordida* sample was apparently low (P = 0.20 and A = 1.35). Isoenzyme pattern was independent of the insect stage except for the *Gpd1* where only adults showed an activity. The obvious polymorphism at *Pep4* and *6-Pgdh* was not

TABLE I

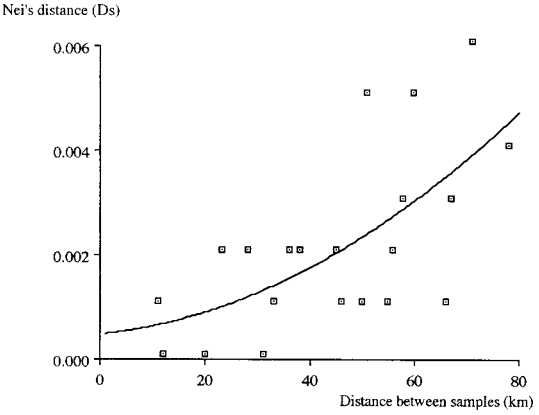
Genotypes in *Triatoma sordida* according to the locality of collection

Locality	No. of insects analyzed	*Pgm*			*Mdh1*		
		aa	a/N	NN	aa	a/N	NN
CBA	41	39	1	0	37	4	0
REC	20	19	1	0	17	2	0
SJB	48	48	0	0	41	4	1
Total area 1	109	106	2	0	95	9	2
GUA	36	24	9	1	23	11	2
CER	29	29	0	0	17	9	1
TAC	37	36	1	0	17	17	3
COT	42	37	5	0	20	14	7
Total area 2	144	126	15	1	77	51	13
TOTAL	253	232	17	1	172	60	15

a/a: homozygotes for the prevailing allele; a/N: heterozygotes between the prevailing allele and any other one; N/N: homozygotes for alleles other than the prevailing one.

amenable to a reliable allelic interpretation (heterozygote patterns not clearly defined) and these loci were not considered for further genetic interpretation.

Genetic structure of T. sordida *populations* - The distribution of genotypes in *T. sordida* according to the locality of collection is showed in Table I. Within samples of *T. sordida* from each locality, departure from Hardy-Weinberg equilibrium was tested by the F statistics (Fis). Observed genotype frequencies at *Pgm* and *Mdh1* were consistent with random mating expectations (Table II). In contrast, the pooled samples proceeding from the seven localities showed evidence of heterozygote deficiency at *Mdh1* locus with significant value of Fit ($P < 0.01$). The difference between local and total uni-locus statistics suggested spatial structuring of the population also known as the Wahlund effect (Cockerham 1973). Because our collections were made over the same month (February 1995), we can discard a variation of genotypic proportions with time. The spatial partition was confirmed by significant results of Fst among populations from the area 2 and the pooled populations from both areas (Table II). The calculated index of Nei's standard genetic distance was highly correlated to geographical distance between localities (R = 0.65; $P < 0.01$), conforming with the isolation distance model of Richardson et al. (1986) irrespective of topography (Figure). The Mantel test (5040 runs) confirmed the statistical significance of the determination coefficient ($P = 0.007$).



Genetic relationships between populations of *Triatoma sordida* according to the geographical distance between them. Each point represents a single pairwise Nei's standard genetic distance (Ds) between localities according to distance. These indices are computed from allelic frequencies. The line represents the second order binomial, with a coefficient of determination of 0.65.

TABLE II

F statistics[a] at two polymorphic loci (*Pgm* and *Mdh1*) among *Triatoma sordida* populations from the two surveyed areas

		Pgm			*Mdh1*		
		Fis	Fit	Fst	Fis	Fit	Fst
Area 1	CBA - REC - SJB	-0.005	0.014	0.019	0.095	0.105	0.011
Area 2	GUA - CER - TAC - COT	0.012	0.092	0.081[b]	0.075	0.101	0.028[c]
Area 1 + 2	CBA - REC - SJB - GUA - CER - TAC - COT	0.010	0.096	0.086[d]	0.079	0.170[d]	0.098[b]

a: Fis and Fit represent the indices of fixation of individuals relative of the different subpopulations and total population, respectively. Fst is the gene frequencies difference among populations. Values were calculated according to Bilton (1992); *b*: $P < 0.001$; *c*: $P < 0.05$; *d*: $P < 0.01$.

DISCUSSION

All analyzed domiciliary specimens from the Velasco region pertain to the first group of *T. sordida* as described by Noireau et al. (1998). Up to now, the whole of *T. sordida* populations domiciliated in Bolivia form part of this group which seems to present a higher ability to colonize houses (Noireau et al. 1998). Moreover, this group has a wider distribution through the Southern Cone countries than *T. sordida* group 2 which seems to be restricted to the Chaco.

In our study, the obvious polymorphism observed at *Pep4* and *6-Pgdh* was not amenable to a reliable allelic interpretation when the *Gpd1* activity was exhibited only in adult stages (Noireau et al. 1998). Consequently, allozyme frequencies at only two loci (*Pgm* and *Mdh1*) were scored as convenient genetic markers for the populations of *T. sordida* group 1. The relatively low genetic variability for this species is similar to *T. infestans* but apparently lower than values reported for *T. sordida* group 2 and *T. guasayana* with the same electrophoretic procedure (Dujardin et al. 1998, Noireau et al. 1998). From *T. sordida* natural populations from the State of Minas Gerais, Brazil, Monteiro et al. (1997) reported a very low level of gene variation (6.7% of the 30 loci were polymorphic). According to Forattini (1980), the Brazilian phytogeographic region of "cerrado", which includes Minas Gerais, would be the endemic center of the *sordida* species. The arguments of a restricted distribution (Chaco) and higher isoenzyme variability for *T. sordida* group 2 lend support to the hypothesis that this one would represent the ancestral species of the complex from which *T. sordida* group 1 derived and does not support the assumption of *T. sordida* origin center in the "cerrado" (Forattini 1980).

For *T. infestans*, Dujardin et al. (1998) suggested that the panmictic unit had a variable extent according to the region while Brenière et al. (1998) showed a strong departure from genotypic equilibrium within localities and concluded in favour of a smaller panmictic unit. Monteiro (1997) showed that *T. sordida* peridomestic populations collected 50 to 450 km apart seemed to be very structured, indicating a low dispersal capacity for this species. The absence of genetic disequilibrium detected for this species within the seven localities and two areas suggests that the panmictic unit would be wider than for *T. infestans* and formed by a group of neighbouring localities. The geographical structuration appears for the whole studied localities as indicated by significative departure of Hardy-Weinberg equilibrium at *Mdh1* locus. The Fst test proves that the structuration is caused by gene frequencies differences among localities issued from area 2 which are more distant apart than localities from area 1 (30 ± 13 km vs. 20 ± 9 km, respectively). On the basis of allozyme data, genetic distances between populations were found to be generally correlated with geographic distance (Figure). Geographical differences would explain the genetic heterogeneity with no need to infer climatical or ecological differences.

To be effective, the design of control protocols against a triatomine species should consider its population structure (Schofield et al. 1995). Although the low enzymatic polymorphism rate observed in *T. sordida* limits the interpretation of gene flow between populations, our data as a whole are compatible with a wider panmictic unit for *T. sordida* compared with *T. infestans*, the main vector of Chagas disease in South America, and suggest the higher flight ability of the first mentioned species (Schofield et al. 1991). The demonstration of a geographical structuration between localities more distant than 20 km apart points out the relatively reduced dispersive capacity of *T. sordida*.

This study clearly suggest that insecticidal control interventions against *T. sordida* will encounter more difficulty than those against *T. infestans*. While this species has silvatic foci which may lead to a reinfestation of houses, the wider panmictic unit of *T. sordida* favours also the reappearance of domestic vectors due to immigrants from neighbouring localities in case of poor geographic insecticide coverage.

ACKNOWLEDGEMENTS

To S Garcia for technical assistance in the field. To J Ireland for reviewing the manuscript.

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