Smallness of the Panmictic Unit of *Triatoma infestans* (Hemiptera: Reduviidae)

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ABSTRACT The population genetic structure of *Triatoma infestans* (Klug), the principal vector of the causative agent of Chagas disease in Bolivia, was investigated by enzyme electrophoresis at 15 loci, of which 3 were polymorphic. A total of 1,286 adults and nymphs was collected from 19 localities of the Cochabamba (high endemicity) and La Paz (low endemicity) departments. Previous results were confirmed, including a low level of polymorphism (0.20), low genetic distance between geographic areas, and a population structure compatible with an isolation by distance model. However, a high proportion (26.3%) of the surveyed localities showed a significant excess of homozygotes, disputing previous conclusions that considered the village as the probable panmictic unit. The excess of homozygotes was reduced when smaller subunits, such as individual houses or chicken coops, were considered, indicating a Wahlund effect.

KEY WORDS *Triatoma infestans*, Chagas disease, population structure, population genetics, Bolivia

THE VECTORS of *Trypanosoma cruzi* (Chagas), the causative agent of Chagas disease in Latin America, are bugs of the subfamily Triatominae. Among them, *Triatoma infestans* (Klug), a vector highly adapted to human habitats, is widespread in the 7 southernmost American countries. Historical reconstruction (Schofield 1988), combined with genetic analysis (Dujardin et al. 1994, 1998), indicated that domestic populations of *T. infestans* may have originated in central Bolivia, where sylvatic populations are found among rock piles in association with wild guinea-pigs (Torrico 1946, Dujardin et al. 1987, Bermudez et al. 1993). Since 1991, this vector has been the target of a massive eradication campaign known as the Southern Cone Initiative (Schofield 1992). Key factors in such a program include accurate knowledge of the biological characteristics of the vector (Schofield and Dujardin 1997).

In Bolivia, where *T. infestans* is the only important vector, Chagas disease is a major cause of morbidity and mortality (WHO 1991). Previous population genetic studies on specimens from Bolivia, Peru, Chile, Argentina, Brazil, and Uruguay did not find any evidence for sibling species within *T. infestans* and concluded that a massive founder effect may explain the overall similarity among countries (Dujardin et al. 1994, 1998; Garcia et al. 1995). From Bolivia to Uruguay, genetic differences between localities increased with their geographical separation, indicating "isolation by distance" as the most convenient model for describing *T. infestans* population structure (Dujardin et al. 1994, 1998). Spatial differentiation in gene frequencies allowed an indirect estimate of field dispersal by *T. infestans* (Dujardin et al. 1988), and was used recently to identify the origin of reinfesting individuals appearing several months after insecticide application (Dujardin et al. 1996).

Previous studies assumed free gene flow within compared localities (Dujardin et al. 1998). To further explore this hypothesis, the current study used large sample sizes collected in new areas of Bolivia. Explanations as to why a large proportion of the localities showed departures from Hardy–Weinberg equilibrium are offered.

Materials and Methods

Study Area. The Yungas area (department of La Paz), in the provinces of North Yungas (16° 15′ S, 67° 40′ W) and Caranavi (15° 45′ S, 67° 32′ W), was investigated. The Yungas are steep-sided valleys located in the Eastern Andean Cordillera that were covered with forest but today largely are replaced by coffee, cocoa, banana, and particularly coca plantations. The climate is subtropical. Although few data are available, Chagas disease in the Yungas presently is considered to be nonendemic by the National Con-

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trol Program. However, a recent serological survey detected 12.7% seroprevalence for *T. cruzi* infection in the North Yungas and 2.2% in Caranavi (unpublished data). In both provinces, *T. infestans* was found at almost all of the sites inspected. The *T. cruzi* infection rate of *T. infestans* was 10.7% in North Yungas and 6.6% in Caranavi.

In the department of Cochabamba where the climate is temperate, allowing the cultivation of corn, grapes, and vegetables, 2 provinces were investigated: Capinota (17° 44′ S, 66° 16′ W) and Campero (18° 14′ S, 65° 14′ W). Both provinces were known to be highly endemic for Chagas disease (Pless et al. 1992).

Collections. From 1992 to 1993, a total of 1,286 adults and nymphs was collected in the La Paz and Cochabamba departments. Collections were performed at North Yungas (La Paz) between February and June 1992 (8 localities, 631 specimens) and in 1993 (3 localities among the 8 sampled previously, 163 specimens), at Caranavi (La Paz) in February 1992 (11 localities, 126 specimens), at Capinota (Cochabamba) in July 1992 (3 localities, 207 specimens), and at Campero (Cochabamba) in June 1993 (2 localities, 156 specimens).

In the North Yungas province, the altitude of the collection sites ranged from 1,200 to 1,800 m, whereas, in Caranavi province, sites ranged from 750 to 1,500 m. Altitudes in the Capinota and Campero provinces (Cochabamba) were higher, ranging from 2,000 to 2,400 m.

Geographical distances between localities were similar in both provinces of the department of La Paz, ranging from 2.5 to 35.2 km. The Capinota localities, located along the Arque River, also had short distances between them, ranging from 5.0 to 17.5 km. The 2 Campero communities were 50 km distant from each other.

Triatomines were collected in sleeping rooms of houses (wall and bed) and in peridomestic structures (shelters) located 5–10 m from houses. Insects were stored in plastic bottles containing filter paper, and transported live to the laboratory. All study areas were free from insecticide treatment.

Sampling. Genotype frequencies were studied at several levels of increasing collection area, starting from the smallest subpopulation unit (single capture sites such as houses or chicken coops) and expanding to localities (or villages), provinces, and departments. Three of 11 Caranavi localities yielded an adequate sample size for genetic analysis; the remaining 8 were grouped together.

Electrophoresis. Electrophoretic analyses were conducted using the head and thorax from each specimen preserved at −70°C according to techniques slightly modified from Dujardin and Tibayrenc (1985a). The following 11 enzyme systems were analyzed: alpha glycerophosphate dehydrogenase (EC 1.1.1.8, αGPD), glucose phosphate isomerase (EC 5.3.1.9, GPI), glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PD), isocitrate dehydrogenase (EC 1.1.1.42, IDH), leucine aminopeptidase (EC 3.4.11.1, LAP), malate dehydrogenase (EC 1.1.1.37, MDH), malic enzyme (EC 1.1.1.40, ME), peptidases, substrate: L-leucyl-leucyl-leucine (EC 3.4.11.11, PEP1), and Leucyl-L-alanine (EC 3.4.11.13, PEP2), phosphoglucomutase (EC 2.7.5.1, PGM), and 6 phosphogluconate dehydrogenase (EC 1.1.1.44, 6PGDH). The 3 previously described variable enzyme systems were resolved from each individual (PGM, 6PGDH and αGPD). Other systems were tested on a subset of bugs from each sample (24 individuals from North Yungas, 24 from Capinota, and 24 from Campero provinces).

Data Analysis. Temporal and spatial structuring were investigated. Genetic interpretation was based on previous descriptions and experimental crosses showing Mendelian segregation for *Pgm* and *6Pgdh* (Dujardin and Tibayrenc 1985a, b). For the αGPD system, various phenotypes were observed previously (αGPD-1 and αGPD-a), of which allelic interpretation was not resolved (Dujardin et al. 1998, Pereira et al. 1996), but equated to distinct genotypes. Research on the panmictic unit was based on frequencies of *Pgm* and *6Pgdh* (alleles). Fixation indexes and *F* statistics were computed according to Nei (1987) for expanding geographical units. To explore spatial or temporal differentiation, in addition to the *F* statistics, we used both gene and genotype frequency analysis. The Workman and Niswander test compared gene frequencies between groups, and the contingency chi-square test was applied to analyze the distribution of αGPD genotype frequencies.

The fixation index (F) was $F = (4n_{11}n_{22} - n_{12}^2)/(2n_{11} + n_{12})(2n_{22} + n_{12})$, with n_{11} and n_{22} representing the number of homozygotes, and n_{12} representing the number of heterozygotes. Statistical significance was tested by chi-square, with Yates correction applied when expected values were <5. For limiting the overall experimental error rate, the Bonferroni method was applied according to the Dunn–Sidák calculation (Sokal and Rohlf 1995). Among a sample of k tests, the smallest probability P_1 was compared with $1 - (1 - \alpha)^{1/k}$ with $\alpha = 0.05$. If P_1 was greater than the critical value, then all tests were not significant. If P_1 was less than or equal to the critical value, this contrast was significant and the 2nd smallest probability, P_2, compared with $1 - (1 - \alpha)^{1/(k-1)}$. Testing successively larger probabilities was continued in the same manner. The significance of the fixation index (F) was examined separately for *Pgm* and *6 Pgd* loci according to the Bonferroni method.

In each combination of geographical groups, the *F* statistics provided information about the respective contributions of genetic disequilibrium and spatial differentiation to the total genetic heterogeneity. F_{ST} measured the amount of differentiation among populations, whereas F_{IS} and F_{IT} measured deviation from panmixis in subpopulations on average and in the total population, respectively. The significance of F_{ST} was elucidated using the chi-square test, $\chi^2 = 2NF_{ST}(k\text{-}1)$ with $(k\text{-}1)(s\text{-}1)$ df. In this formula, N is the total sample size, k is the number of alleles per locus, and s is the number of subpopulations (Bilton 1992). The significance of the deviations of F_{IS} or F_{IT} from zero was tested using the formula, $n(F_{IS})^2$ where n is the total

number of individuals in a chi-square distribution with 1 df (Bilton 1992).

The statistics of Workman and Niswander (1970) analyze homogeneity in allelic frequencies (*Pgm* and *6Pgdh* loci) between 2 populations. Briefly, if there are k alleles at a locus, then the chi-square statistic is given by

$$2N\left(\sum_{j=1}^{k} v_{Pj}^2 / P_j\right)$$ with v_{Pj} and P_j the weighted mean and variance

of the frequencies of the jth allele at the locus. For each allele, $P = \Sigma(N_i/N)p_i$, p_i is the frequency of the allele in the ith population, N_i sample size and N the size of all populations; $v^2{}_P = \Sigma\ (N_i/N)p_i{}^2 - P^2$. The degrees of freedom are given by $(k\text{-}1)(r\text{-}1)$, where r is the number of populations examined.

In addition, differences among populations in the frequency of the 2 αGPD genotype (αGPD-1 and αGPD-a, see below) were evaluated by an $m \times n$ heterogeneity chi-square test, with Yates correction applied when expected values were <5.

To explore the population model, non-euclidean Nei's (Nei 1972) and euclidean Roger's (Roger 1972) genetic distances were plotted against geographical distances between localities compatible with Hardy–Weinberg equilibrium (21 pairwise comparisons) and collection sites (78 pairwise comparisons) studied during 1992. The significance of correlation between genetic and geographical distances was assessed by the Mantel test (Mantel 1967) after 10,000 random matchings (Chessel and Dolédec 1992).

Results

Isoenzymatic Variability. Of the 11 enzyme systems tested, 4 (*Me, Mdh, Lap,* and *Pep1*) exhibited 2 zones of activity interpreted as 2 separate loci. Therefore, 15 loci were analyzed, of which 3 exhibited variability (Table 1). Two allelic positions were scored for the PGM and the 6PGDH systems. For αGPD, 2 different apparently non-allelic patterns, named αGPD-1 and αGPD-a by Dujardin and Tibayrenc (1985a), were observed and equated to distinct genotypes. The monomorphic patterns of the other 8 enzyme systems were confirmed on a subsample of 72 individuals (see *Materials and Methods*). Overall, the polymorphism rate was (3/15) 0.20 and the average number of alleles per locus was 1.14.

Fixation Index. Fixation index (F) was calculated in different subpopulations progressing from the small to large units (i.e., a single capture site [house and chicken-coop]), a single locality, a single area, and the entire populations sampled in 1992 and 1993. Values of the F-index at each population level are presented in Table 1. Table 1 identifies groups responsible for the significant F_{IS} values given in Table 2.

In agreement with F_{IT} being generally significant (see below), observed genotype frequencies at *Pgm* and *6Pgdh* loci for the whole population were significantly different from Hardy–Weinberg expectations. When single localities (19), sampled in either 1992 or 1993, were examined, genotype frequencies were consistent with random mating expectations in 14 of 19 cases (73.7%) according to the Bonferroni method. However, when populations from smaller subunits such as houses or chicken-coops were tested (16), genotype frequencies were consistent with random mating expectations in 14 of 16 cases (87.5%). In all cases, significant F values were positive, indicating the deficiency of heterozygotes.

***F* Statistics.** In most cases, significant F_{IS} and F_{IT} as well as significant F_{ST} estimates were found, even among neighboring localities (Table 2). In all cases, significant F_{IT} and F_{IS} values were positive, indicating deficiencies of heterozygotes.

Spatial Structuring. In agreement with the significant overall F_{ST}, pairwise comparisons of adjacent localities (e.g., Auquisamaña and Pararani, 2.5 km) for *Pgm* or *6Pgdh* gene frequencies were also significant (Workman and Niswander tests, detailed results not shown). In addition, genotype frequencies of αGPD-1 and αGPD-a generally showed significant differences, regardless of the distances between localities (Table 2).

Temporal Structuring. Significant differences in allele frequencies occurred between 1992 and 1993 for at least 1 of the variable loci of the 3 populations tested in the La Paz department (North Yungas province). Heterogeneity in *αGpd* genotypes was also significant in 2 of 3 cases (Table 3).

Population Model. The relationships between geographical and genetic distances between the capture sites sampled in 1992 generally were in agreement with panmixis (Fig. 1a). Geographical distances ranged from 0.5 to 58.6 km. Correlations of these distances with either Nei's and Roger's genetic distances were found to be positive and significant (r = 0.29 and r = 0.32; Mantel test, $P < 0.0019$ and $P < 0.0001$, respectively). This analysis also was performed among localities sampled in 1992 that showed Hardy–Weinberg equilibrium and presented an apparent genotypic equilibrium (7 populations, Fig. 1b). Correlation with Nei's or Roger's distances was positive and significant (r = 0.32 and r = 0.40; Mantel tests, P = 0.0002 and $P < 0.0001$, respectively).

Discussion

Isoenzymatic Variability. The 3 variable enzyme systems, PGM, 6PGDH, and αGPD, as well as the general patterns of variation (polymorphism rate, mean allelic number) in the current study were similar to Dujardin and Tibayrenc (1985a, b). However, the peptidase systems showed 2 instead of 4 bands (Dujardin and Tibayrenc 1985a). Differences between genetic variability estimates derived from our techniques and those used by Garcia et al. (1995) were discussed previously in Dujardin et al. (1998) and attributed to different electrophoresis systems, and the use of laboratory reared insects.

Panmictic Unit. Dujardin et al. (1988, 1998) suggested that the panmictic unit for *T. infestans* was a group of villages separated by 50 km in Vallegrande

Table 1. Genotype frequencies of *T. infestans* populations and subpopulations and *F* values

Populations		Locus									
		Pgm				*6Pgdh*				*α Gpd*	
Department (Province)	Locality Site	Genotypes 1/1	1/2	2/2	*F* value	Genotypes 1/1	1/2	2/2	*F* value	Genotypes 1	a
La Paz 92	Auquisamaña	1	5	71	0.25	0	15	64	−0.1	41	38
(North Yungas)	Chicken-coop 1	0	4	30	−0.06	0	12	24	−0.2	18	18
	Coscoma	43	6	102	0.90*	38	61	50	0.17	112	39
	Chicken-coop 1	15	2	66	0.92*	16	37	31	0.09	65	19
	Chicken-coop 2	23	2	4	0.76*	8	12	8	0.14	15	12
	House 1	0	0	21	Mono	2	7	11	0.12	20	0
	Marquirivi	11	45	61	0.06	6	25	102	0.21	70	26
	Chicken-coop 1	1	11	18	−0.08	1	3	26	0.35	19	4
	Chicken-coop 2	5	17	23	0.1	1	12	32	−0.02	32	8
	House 1	3	10	5	−0.12	2	5	14	0.3	7	0
	Pararani	8	27	42	0.13	3	31	33	−0.16	58	18
	Chicken-coop 1	4	8	17	0.31	3	15	9	−0.17	25	4
	House 1	0	3	11	−0.12	0	6	3	−0.5	5	5
	San Juan	4	14	25	0.14	8	17	12	0.07	20	21
	House 1	0	6	11	−0.21	5	8	1	−0.24	7	9
	Siete Lomas	20	24	36	0.37*	6	20	54	0.22	28	48
	Chicken-coop 1	4	19	27	0.04	4	9	35	0.36	15	34
	Trinidad Pampa	4	3	1	0.13	1	7	6	−0.15	5	13
	Yolosa	40	28	10	0.16	0	0	94	Mono	32	26
	Chicken-coop 1	9	4	0	−0.18	0	0	21	Mono	7	14
	House 1	7	9	2	−0.08	0	0	20	Mono	14	1
	Total	131	152	348	0.45***	62	176	455	0.25***	366	229
La Paz 92	Bartos	1	7	8	−0.08	0	0	16	Mono	11	3
(Caranavi)	San Lorenzo	5	6	10	0.39	0	0	18	Mono	8	7
	Villa Oriente	0	5	14	−0.15	0	0	18	Mono	9	7
	Others	19	24	17	0.2	1	2	56	0.48*	23	32
	Total	25	42	49	0.24**	1	2	108	0.49**	51	49
Cochabamba 92	Capinota	2	12	48	0.14	8	14	40	0.38	57	5
(Capinota)	Sarcobamba	10	21	60	0.34*	3	31	55	−0.06	85	3
	Sicaya	0	14	40	−0.15	11	14	29	0.42*	53	1
	Total	12	47	148	0.20**	22	59	124	0.23***	195	9
La Paz 93	Coscoma	1	1	57	0.66	22	22	15	0.24	48	11
(North Yungas)	Pararani	0	1	27	−0.02	0	7	21	−0.14	11	16
	Trinidad Pampa	0	0	76	Mono	8	26	42	0.14	73	3
	House 1	0	0	10	Mono	1	5	4	−0.1	10	0
	Chicken-coop 1	0	0	8	Mono	0	4	4	−0.33	8	0
	Chicken-coop 2	0	0	12	Mono	1	2	9	0.4	12	0
	Total	1	2	160	0.49**	30	55	78	0.26***	132	30
Cochabamba 93	Ailquile	2	12	48	0.14	8	14	40	0.38	57	5
(Campero)	Mizque	80	10	4	0.39*	21	40	31	0.12	63	30
	Total	82	22	52	0.71***	29	54	71	0.24**	120	35
La Paz and	1992	168	241	541	0.40***	85	237	647	0.26***	612	287
Cochabamba	1993	83	24	212	0.82***	59	109	149	0.25***	252	65
	1992 + 1993	251	265	753	0.50***	144	346	796	0.27***	864	352

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Mono, monomorphic. The significance of *F* values for localities and sites was according to the Bonferroni method.

(department of Santa-Cruz, Bolivia) and only few kilometers in the Yungas valleys. However, the current study showed strong departures from Hardy–Weinberg equilibrium within localities. Out of 19 localities, 5 (26.3%) showed significant Hardy–Weinberg disequilibrium for *Pgm*, *6Pgdh*, or both loci in both departments, and this was always due to heterozygote deficiency. This high percentage is probably an overestimate because several samples did not provide ideal data for chi-square analysis (i.e., expected frequencies <5). The existence of undetected null allele may explain lack of heterozygotes (Pasteur et al. 1987). This explanation was discarded because we never observed the absence of enzymatic activity (homozygotes for the null allele) in any individual.

Two other explanations included unknown selective factors acting locally and biological factors impeding random mating (cryptic speciation). Selection against heterozygotes is uncommon, especially for enzyme loci. Dujardin et al. (1998) showed that infected and uninfected adults bugs exhibited strong genetic differences at 1 locus, even in sympatric conditions which may account for local disequilibria where infected bugs are numerous. However, the similar finding of local disequilibria in areas with low endemicity does not support this hypothesis. Cryptic speciation also could produce local departures from panmixis. However, in the absence of fixed diagnostic alleles allowing the recognition of 2 sets of different genotypes, this hypothesis is difficult to refute. Genetic similarity among distant localities does not favor this hypothesis.

The subdivision of one locality (e.g., Coscoma) into smaller panmictic subunits (i.e., the Walhund effect)

Table 2. Heterogeneity in genotype frequencies among *T. infestans* populations according to level of geographical association

Geographic origin of compared populations	N	Maximal distance, km	Contingency chi-square test α *Gpd* χ^2 value	*F* statistic *Pgm* Fis	*Pgm* Fit	*Pgm* Fst	*6Pgd* Fis	*6Pgd* Fit	*6Pgd* Fst
Among departments									
All over localities 1992 + 1993	19	790	213.94***	0.23***	0.46***	0.29***	0.14***	0.28***	0.16***
All over localities 1992	14	634	152.2***	0.23***	0.36***	0.17***	0.12***	0.26***	0.16***
All over localities 1993	5	690	51.55***	0.27***	0.79***	0.71***	0.18***	0.27***	0.11***
Within departments									
All over localities La Paz 1992 + 1993	14	634	112.11***	0.25***	0.43***	0.24***	0.08**	0.27***	0.21***
All over localities La Paz 1992	11	172	59.9***	0.25***	0.38***	0.17***	0.05	0.25***	0.20***
Within provinces									
Caranavi province	3	30	2.34	0.13	0.19	0.06*	Mono	Mono	Mono
North Yungas province 1992	8	35	57.60***	0.28***	0.42***	0.18***	0.05	0.17***	0.13***
North Yungas province 1993	3	14	40.39***	0.38***	0.39***	0.01	0.14	0.27***	0.15***
Campero province	2	50	12.45***	–	–	–	–	–	–
Capinota province	3	17.5	3.26	0.15*	0.17**	0.02*	0.27***	0.29***	0.02*
Among close localities									
San Juan/Marquirivi/Siete Lomas	3	8.5	23.24***	0.21**	0.23***	0.02**	0.15**	0.23***	0.09***
Coscoma/Auquisamaña/Pararani	3	5.2	14.57***	0.50***	0.54***	0.08***	0.01	0.12*	0.11***
Within a locality									
Capture sites from Coscoma	3	0.5	12.96**	0.84***	0.93***	0.55***	0.13	0.17*	0.04**
Capture sites from Marquirivi	3	0.5	2.7	−0.02	0.03	0.05	0.21*	0.23*	0.03
Capture sites from Trinidad Pampa 1993	3	0.5	Mono	Mono	Mono	Mono	0.001	0.06	0.06

For the geographic origin of populations see Table 1. Mono, monomorphic system; *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$.

appeared to be the most likely explanation for the overall results. If the subunits differed in allele frequency at a locus with 2 alleles, and a sample set is collected that includes some members from each unit, then a deficiency in the number of heterozygotes will be observed relative to that predicted under Hardy–Weinberg equilibrium conditions (Wahlund 1928). Indeed, when populations composed of insects from the same capture site (house or chicken-coop) were analyzed instead of all the insects from a common locality, nonsignificant F-fixation indexes were observed in 12.5% of the sites. The reasons why these subunits differed in gene frequencies could be the lack or low level of genetic exchange, allowing genetic drift, and the probable founding of the population by very few females. In low or recent house infestation by *T. infestans* (low endemicity), it is possible that a population at a single site may arise from one female.

Spatial and Temporal Structuring. Significant F_{ST} values, even among nearby localities, were confirmed by pairwise comparisons of either gene or genotype frequencies between localities. Allelic differences between nearby localities may be attributed to the poor dispersal ability of *T. infestans* as previously reported by Lehane and Schofield (1981). Other factors, especially the topography of the sub-Andean region, also could play a significant role (Dujardin et al. 1998). Dujardin et al. (1997) showed stable gene frequencies across years in 4 communities in Vallegrande region (department of Santa Cruz), but significant changes at one locality in North Yungas province (department of La Paz). We showed similar changes in the same department, which were apparently not seasonal because the localities were sampled during the same month in 2 different years. The most likely explanation could be the substructuring of localities into smaller subunits, sampling different ones in different years.

Table 3. Temporal heterogeneity in genotypes frequencies between *Triatoma infestans* populations

Populations	N	Workman and Niswander test *Pgm* χ^2	Workman and Niswander test *6Pgdh* χ^2	Contingency χ^2 test α *Gpd* χ^2
Coscoma 1992/1993	2	37.79***	3.35	1.2
Pararani 1992/1993	2	16.93***	5.07*	11.40**
Trinidad Pampa 1992/1993	2	111.82***	0.24	46.18***

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Population Model. The positive and significant correlation between genetic and geographical distances, between capture sites and localities in Hardy–Weinberg equilibrium, was in agreement with the hypothesis of isolation by distance. This model became more apparent as more distant populations were added (Dujardin et al. 1998).

Control Implications. Our data indicate that the panmictic unit of *T. infestans* may be no larger than a single locality, and they confirm a strong spatial structuring of these populations. These biological features helped to interpret the results of recent studies trying to identify, with the use of genetic markers, the causes of reinfestation after insecticide treatment. Recrudescence from a residual population rather than reinva-

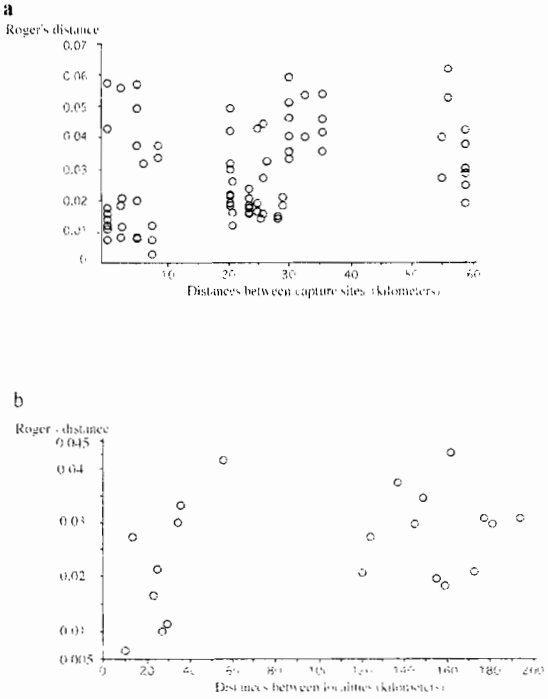

Fig. 1. Plots of Roger's distances against (a) geographical distances for the capture sites of *Triatoma infestans* sampled in 1992 (b) and *T. infestans* populations sampled in 1992 from localities exibiting Hardy-Weinberg equilibrium.

sion from surrounding localities, seems to provide the best explanation (Dujardin et al. 1994, 1996, 1998). Indeed, in the case of structured populations among localities, the probability of reinvasion from nearby, untreated localities would be low (Dujardin et al. 1997), but it would be even lower, if structuring occurred within localities.

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