J. Med. Entomol. Vol. 24, no. 1: 40–45 January 1987

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ISOZYME EVIDENCE OF LACK OF SPECIATION BETWEEN WILD AND DOMESTIC *TRIATOMA INFESTANS* (HETEROPTERA: REDUVIIDAE) IN BOLIVIA[1]

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Abstract. Wild and domestic *Triatoma infestans* from the Cochabamba region of Bolivia were virtually identical at 19 gene loci coding for enzymes. No allele was distinctive of either the wild or the domestic populations. Hence, there is no evidence that the 2 populations are different species. Domestic populations separated by 20 km showed statistically significant differences in allelic frequencies; this is compatible with the hypothesis of other authors that migrations of *T. infestans* are limited when feeding conditions are satisfactory. Fifteen trypanosomatid stocks isolated from wild *T. infestans* were shown by isozyme analysis to be *Trypanosoma cruzi*. This provides evidence that wild *T. infestans* are involved in the Chagas' disease cycle. The *T. cruzi* isozymic strains from wild *T. infestans* were genetically similar to those isolated from domestic *T. infestans* in the same region. This supports the hypothesis that there is no speciation between wild and domestic *T. infestans*, and that wild and domestic *T. cruzi* cycles may overlap in this region.

Among *T. cruzi* vectors, *Triatoma infestans* (Klug) is the best adapted to human habitats (Lent & Wygodzinsky 1979). Its wide distribution in South America may be due to the recent spread of human populations on that continent. Before it adapted to human habitats, *T. infestans* probably fed on wild animals (Usinger et al. 1966) and was less numerous and less widespread than at present.

Wild *T. infestans* populations occur in the Cochabamba Valley of Bolivia, where they live in rocky habitats associated with wild guinea pigs (Fig. 1). Wild *T. infestans* are morphologically indistinguishable from domestic specimens.

To clarify the taxonomic and evolutionary relationships between wild and domestic *T. infestans*, we compared them genetically by means of isozyme analysis and similarly compared the *Trypanosoma cruzi* isozymic strains isolated from them. The results bear on the important question of whether wild *T. infestans* might be involved in the domestic cycle of Chagas' disease.

MATERIALS AND METHODS

We studied 32 *T. infestans* from a wild population and 86 from 3 domestic populations in the Cochabamba region, Department of Cochabamba, Bolivia, where the wild populations occur (Table 1, Fig. 2). Some 3rd-, 4th-, and 5th-instar nymphs were included in the present study, as were male and female adults. Indeed, the enzyme patterns studied here showed neither ontogenic nor sex specificity (Dujardin, unpubl.). Domestic *T. infestans* populations sampled from or near human habitats were designated "1," "2," and "3." The wild population, collected from wild guinea pig (*Cavia* sp.) habitats about 15 km south of the town of Cochabamba, was designated population "S." Domestic population 3 and the wild population S are separated by ca. 1 km; population 2 is the farthest from them, ca. 20 km away.

Genetic analysis was performed by electrophoretic separation of isozymes on cellulose acetate plates (Helena Laboratories, Beaumont, TX). The following enzyme systems were assayed: aconitase (aconitate hydratase, EC 4.2.1.3, ACON); glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PD); glucose-6-phosphate

1. This study was carried out with the support of the French Technical Cooperation Program, of the Ministère de l'Industrie et de la Recherche (extension of grant no. PVD/81/L-1423), and of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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Fig. 1. Rocky habitat of wild *Triatoma infestans*. Feces from guinea pig hosts can be seen on stones at bottom. Area covered by photograph is ca. 1 m².

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isomerase (EC 5.3.1.9, GPI); αglycerophosphate dehydrogenase (EC 1.1.1.8, αGPD); isocitrate dehydrogenase (EC 1.1.1.42, IDH); leucine aminopeptidase (cytosol aminopeptidase, EC 3.4.11.1, LAP); malate dehydrogenase (EC 1.1.1.37, MDH); malate dehydrogenase (oxaloacetate decarboxylating) (NADP+) or malic enzyme (EC 1.1.1.40, ME); peptidase 1 (ficin, EC 3.4.22.3, formerly EC 3.4.4.12, substrate: leucyl-leucyl-leucine); phosphoglucomutase (EC 5.4.2.2, formerly EC 2.7.5.1, PGM); and 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6PGD). Conditions for sample preparation, electrophoresis, and enzyme staining have been previously described (Dujardin & Tibayrenc 1985).

Fifteen trypanosomatid stocks were isolated from wild *T. infestans* using the method described by Tibayrenc et al. (1982). These were also studied by enzyme electrophoresis on cellulose acetate plates. The 12 enzyme systems assayed were glucose-6-phosphate dehydrogenase; glucose-6-phosphate isomerase; glutamate dehydrogenase NAD+ (EC 1.4.1.2, GDH

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TABLE 1. Genotype numbers and allele frequencies at 2 loci in 4 populations of *Triatoma infestans.*

Genotypes and alleles	Populations (sample date and size) 1: Cerro San Miguel (Dec. 1982; $n = 12$)	2: Pueblo Maica (Feb. 1984; $n = 44$*)	3: Laguna Angostura (Mar. 1984; $n = 30$*)	S: Laguna Angostura strands (Mar. 1984; $n = 32$)
Genotype numbers				
Pgm 1/1	1	0	4	3
Pgm 1/2	2	6	9	8
Pgm 2/2	9	38	17	21
χ^2**	0.01***	0.01	0.33	0.29
P	0.9	0.9	0.5	0.5
6Pgd 1/1	0	0	4	2
6Pgd 1/2	8	17	13	16
6Pgd 2/2	4	23	9	14
χ^2	0.6	0.33	0.03	0.15
P	0.3	0.5	0.5	0.5
Allele frequencies				
Pgm 1	0.167	0.068	0.283	0.219
Pgm 2	0.833	0.932	0.717	0.781
6Pgd 1	0.333	0.213	0.404	0.313
6Pgd 2	0.667	0.787	0.596	0.687

* At the *6Pgd* locus, only 40 individuals were analyzed in population 2 and 26 in population 3.

** Because numbers were low, the 2 less numerous genotypes were combined in the calculations of all χ^2's.

*** Yates' corrected χ^2.

NAD+); glutamate dehydrogenase NADP+ (EC 1.4.1.4, GDH NADP+); isocitrate dehydrogenase; leucine aminopeptidase; malate dehydrogenase; malic enzyme; peptidase 1 (substrate: leucyl-leucyl-leucine); peptidase 2 (bromelain, EC 3.4.22.4, formerly EC 3.4.4.24, substrate: leucyl-L-alanine); phosphoglucomutase; and 6-phosphogluconate dehydrogenase. The technical conditions were as described previously by Tibayrenc & Le Ray (1984). Two reference strains were added on each electrophoresis plate: Tehuantepec (isozyme strain 12) and Tulahuen (isozyme strain 43); the classification of *T. cruzi* isozyme strains is according to Tibayrenc et al. (1986b).

RESULTS

Wild and domestic Triatoma infestans

No diagnostic loci between the wild and domestic populations were found among the 19 gene loci (11 enzyme systems) studied. The nearest domestic population was virtually identical to the wild population genetically, and the other 2 domestic populations were only slightly different from them and about equally different from both.

Sixteen gene loci (8 enzyme systems: ACON, G6PD, GPI, IDH, LAP, MDH, ME, and PEP 1) have been reported as monomorphic in *T. infestans* (Dujardin & Tibayrenc 1985). The present study confirms this and reveals exactly the same enzyme patterns in both the wild and domestic specimens. In other domestic populations from Bolivia, αGPD exhibits variability (Dujardin, unpubl. data), but it is monomorphic in the present samples, which show the same phenotype in wild and domestic bugs. This low level of polymorphism cannot be due to our electrophoretic procedures, because similar methods have been used to study the *Trypanosoma cruzi* stocks, which exhibit high allozyme variability.

Table 1 gives results for the 2 polymorphic loci, *Pgm* and *6Pgd.* For each locality we list the number of individuals exhibiting each genotype and the allele frequencies. These 2 loci are polymorphic in other domestic populations (Tibayrenc et al. 1981; Dujardin & Tibayrenc 1985), and only 2 alleles that are the same have been observed in the 4 populations now surveyed. The heterozygous patterns observed in these populations are consistent with the quaternary structures previously inferred for domestic samples: PGM is monomeric (2-banded pattern in the heterozygotes) and 6PGD is dimeric (3-banded pattern in the heterozygotes) (Tibayrenc et al. 1981; Dujardin & Tibayrenc 1985).

Chi-square tests showed no significant departures from the expectations of Hardy-Weinberg equilibrium for any of the 4 populations (see Table 1). If the data for the 4 populations are pooled, the combined genotype numbers are also consistent with a Hardy-Weinberg equilibrium ($\chi^2 = 0.54$ and 3.14, $P > 0.3$ and > 0.2, for *Pgm* and *6Pgd*, respectively). For *Pgm* we had to pool the 2 less numerous genotypes to obtain sufficiently large expected numbers, and so there is only 1 degree of freedom; for *6Pgd* there are 2. In Table 2 standard deviate statistics are presented for comparing the allelic frequencies in the various populations: $\epsilon = (p_A - p_B)/(pq/N_A + pq/N_B)^{1/2}$, where p_A and p_B are the frequencies of a given allele in populations A and B, p is the frequency of this allele in the 2 populations con-

TABLE 2. Standard deviate statistics for pairwise comparison between allelic frequencies using individual or grouped populations. Levels of significance (*P*) are in parentheses. The results are for *Pgm* (above) and *6Pgd* (below).

Populations	1	2	3	S
2	*			
	1.21 (NS)**			
3	1.11 (NS)	3.55 (0.001)		
	0.59 (NS)	2.37 (0.02)		
S	*	2.72 (0.01)	0.82 (NS)	
	0.18 (NS)	1.36 (NS)	1.02 (NS)	
1 + 3	—	3.28 (0.001)	—	0.44 (NS)
	—	2.31 (0.03)	—	0.85 (NS)
1 + 3 + S	—	3.29 (0.001)	—	—
	—	2.13 (0.04)	—	—

* Numbers are too small to make calculation meaningful.

** NS = not significant, i.e., $P > 0.05$.

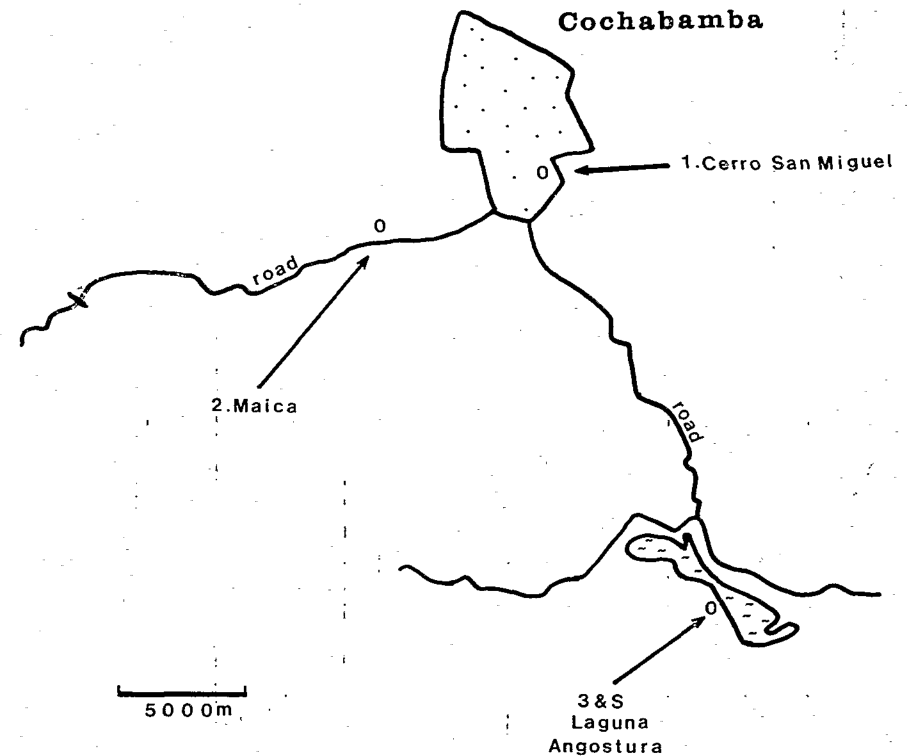

FIG. 2. Vicinity of Cochabamba, Bolivia, showing the collection sites.

sidered as one, $q = 1 - p$, and N_A and N_B are the numbers of alleles sampled in each population (see Schwartz 1963, p. 58). Populations 2 and 3, which are farthest apart geographically, have significantly different allele frequencies at both loci *Pgm* and *6Pgd*. Population 2 is significantly different from the wild population at the *Pgm* locus but not at *6Pgd*. Population 1 is not significantly different from any of the other 3 populations at either locus. Population 3, which is geographically closest to the wild population (ca. 1 km), does not differ from it at either locus.

When the populations were grouped the results were as follows: Population 2, geographically most distant from the others, was significantly different from the other 3 populations combined and also from the pooled data for populations 1 and 3, but these 2 combined were not significantly different from the wild population.

We calculated the genetic distance, D (which estimates the average number of codon differences per locus; Nei 1972), between the various populations. For the comparison between the wild population and population 3, which is geographically closest, $D = 0.001$; for the comparisons between the wild population and populations 1 and 2, $D = 0.001$ and 0.002, respectively. For the comparisons between population 1 and either 2 or 3, $D = 0.001$; and for the comparison between populations 2 and 3, the 2 domestic populations farthest apart, $D = 0.004$. These results confirm the extreme genetic similarity among the 4 populations.

Analysis of the trypanosomatid stocks isolated from wild T. infestans

The 15 trypanosomatid stocks isolated from wild *T. infestans* were assayed for 13 genetic loci (ME exhibiting the activity of 2 loci). According to their isozyme patterns, all 15 stocks were determined as *Trypanosoma cruzi*. This is evidence that wild *T. infestans* are indeed vectors in the Chagas' disease cycle.

Using the classification presented by Tibayrenc et al. (1986b), the 15 stocks are as follows: 11 are identical to isozyme strain 20 (IS 20), 3 are identical to IS 39, and 1 is a mixed stock of these 2 strains. We have isolated from domestic *T. infestans* in the same region 14 *T. cruzi* stocks (Tibayrenc et al. 1986a). Eight belong to IS 20, 5 are IS 39, and 1 is IS 32 (closely related to IS 39). Hence, the 2 isozyme strains isolated from wild *T. infestans* are strains already recorded in domestic bugs and the numbers of stocks of the 2 isozyme strains of wild and domestic origin are not significantly different ($\chi^2 = 0.61$, $P = 0.3$).

DISCUSSION

The comparison between wild and domestic *Triatoma infestans* suggests that they are members of the same species. No distinctive allele exists in either group: all 17 monomorphic loci are identical and the 2 polymorphic loci exhibit the same alleles with similar frequencies. The genetic distances between the wild population and the domestic ones are extremely small. The lack of speciation between wild and domestic *T. infestans* is corroborated by the fact that the 2 *Trypanosoma cruzi* isozyme strains found in wild *T. infestans* are the same ones found in domestic *T. infestans* from the same region, in comparable frequencies.

Wild and domestic *T. infestans* might differ at some loci not surveyed in the present study, but the similarity of allelic and genotypic frequencies at the loci sampled make it highly unlikely that these populations have been breeding independently for very long. Indeed, the genetic distances observed are very small, even for local populations of the same species (Ayala 1982). We then accept as a working hypothesis that the wild and domestic *T. infestans* are members of the same species.

There are 2 obvious alternatives as to the origin of the wild population. First, it might be a relic of the ancestral population from which the domestic *T. infestans* derived. The great genetic similarity between the wild and domestic populations could be a consequence of occasional gene migration (interbreeding) between them. The 2nd alternative is that the wild population has derived from domestic *T. infestans* that would have relatively recently colonized wild guinea pig habitats. Only the 1st possibility has been previously considered by other authors (Usinger et al. 1966); yet the isozyme data are equally consistent with both alternatives.

An important consideration epidemiologically is that the wild *T. infestans* is infected with *Trypanosoma cruzi* and does not appear to be reproductively isolated from the domestic *T. infestans*. No data are available on the possible infection of wild guinea pigs by *T. cruzi*, but this seems most probable, because the triatomine bugs that we have found in these wild guinea pig habitats were naturally infected. In addition, we found eggs, exuviae, and larvae of *Triatoma infestans* in wild guinea pig nests, indicating that the triatomine bugs breed in these nests. Given that no other warm-blooded animals are available, the bugs probably also feed on wild guinea pigs. In this area, therefore, the wild population of *T. infestans* could act as a reservoir to recolonize houses following attempts to control domestic populations of this species.

We have found significant differences in allelic frequencies between domestic populations of *T. infestans* separated by relatively short distances (20 km). This is consistent with the hypothesis of Schofield (1985) that *T. infestans* has little tendency to migrate when it enjoys satisfactory feeding and habitat conditions. Nevertheless, we were unable to find any deviation from Hardy-Weinberg equilibria, either within populations or between them. This suggests that the populations, at the level studied, tend towards panmixia.

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