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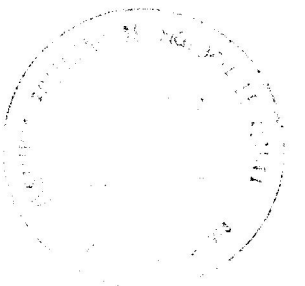

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ACTROP 00536

# Genetic analysis of *Triatoma infestans* following insecticidal control interventions in central Bolivia

JP. Dujardin [a,*], L. Cardozo [b], C. Schofield [c]

[a] *UMR CNRS-ORSTOM 9926, 'Génétique Moléculaire des Parasites et des Vecteurs', ORSTOM, BP 5045, 911 avenue Agropolis, 34032, Montpellier Cedex 01, France*
[b] *CENETROP, Casilla 7924, Santa Cruz de la Sierra, Bolivia*
[c] *Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London WC1 E7HT, UK*

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Key words: *Triatoma infestans*; Vector control; Entomological surveillance; Isoenzymes

Control of Chagas disease vectors relies primarily on spraying infested dwellings with pyrethroid insecticides. After the initial intervention, however, it is important to continue entomological surveillance so that any new infestations can be selectively retreated. The reappearance of domestic vectors may be due to immigrants or to small populations that survive the initial treatment. In this paper we show how a genetic analysis, based on isoenzymes, can be used to infer the source of new infestations of *Triatoma infestans* (Klug), the main vector of Chagas disease in Bolivia and neighbouring southern cone countries.

Our study site was the region of Vallegrande in central Bolivia, some 250 km SE of Santa Cruz de la Sierra (Fig. 1). Villages in this region tend to be heavily infested with *T. infestans*, and the local population shows a high prevalence of Chagas disease (WHO, 1986). Villagers of Moro Moro, for example, showed 70.4% seroprevalence of infection with *Trypanosoma cruzi* during a 1983 survey (WHO, 1986).

During 1986, the 186 houses in the village of Moro Moro were sprayed with deltamethrin (K-othrin 2.5 SC @ 25 mg a.i. per sq.m.) as part of a village field trial carried out by the CENETROP authorities. Three neighbouring villages (Candelaria, El Bello and San Geronimo) were left untreated. In Moro Moro, the number of houses apparently infested with *T. infestans* fell to zero after treatment, but 3 houses were found to be 'reinfested' 6 months later.

From standard isoenzyme studies on cellulose acetate (Dujardin and Tibayrenc, 1985), it appeared that gene frequencies of reinfestant specimens were nearly identical to those collected from the village (Moro Moro) before insecticide treatment (Table 1), suggesting that the reinfestations could be due to bugs surviving the initial treatment. However, gene frequencies in those specimens were also similar to those of bugs collected from nearby localities that had not been sprayed, so the idea that

* *Corresponding author. c/o Embajada de Francia, CP 717, La Paz, Bolivia. Fax: (+591) 2 39 14 16.*

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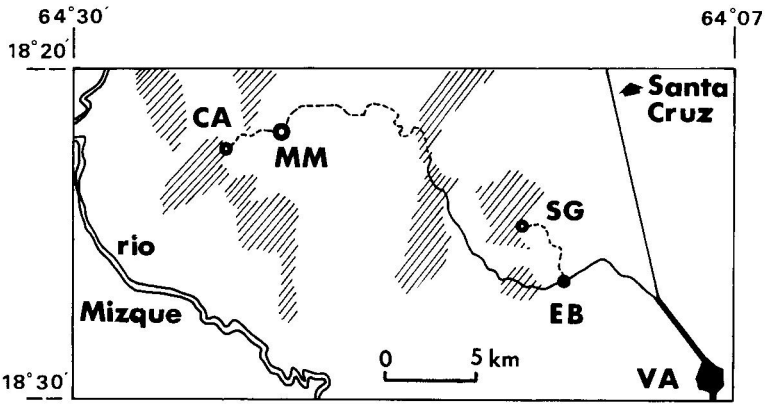


Fig. 1. Sketch map of Moro Moro. CA = Candelaria, EB = El Bello, MM = Moro Moro, SG = San Geronimo and VA = Vallegrande. This area is at an altitude of about 2500 metres. Dashed zones represent hish ground.

Table 1
Gene frequencies in Vallegrande

| Loc | Year | 6*pgdh*-1 | *n* | *Pgm*-2 | *n* |
|---|---|---|---|---|---|
| MM | 1986 | 0.253 | 75 | 0.873 | 75 |
| EB | 1986 | 0.241 | 29 | 0.917 | 30 |
| SG | 1986 | 0.324 | 34 | 0.925 | 40 |
| CA | 1986 | 0.321 | 39 | 0.974 | 38 |
| MM | 1987 | 0.257 | 70 | 0.864 | 66 |
| EB | 1987 | 0.313 | 24 | 0.952 | 21 |

6*pgdh*-1 is the fastest migrating allele at the 6*pgdh*-1 locus, *Pgm*-2 is the most frequent allele in Bolivia at the *Pgm* locus, having an intermediate rate of migration between those of *Pgm*-1 and *Pgm*-3. Loc = locality; $n$ = number of specimens; MM = Moro Moro; EB = El Bello; SG = San Geronimo; CA = Candelaria. MM (1987) represents the reinfestant population.

the reinfestations were due to immigrant bugs could not be immediately rejected. We therefore wished to see if it were possible to confirm or reject the untreated neighbouring localities as sources of the reinfestation.

If bugs were randomly migrating to Moro Moro from neighbouring localities, they would tend to be few in number (Schofield, 1985) and probably unrepresentative of the full set of gene frequencies of their source populations. For the single locus statistical comparisons, we thus gave the gene frequencies of reinfestant specimens $[p(r)]$ the variance of the putative source populations $[v(s)]$ (Table 2). The $P$-value for each locus was read from normal tables after computing the standard normal deviate $z$, where:

$$z = [p(r) - p(s)] / \sqrt{[v(s)]}$$

where $p(r)$ is the gene frequency in the reinfestant population, and for each putative source population $p(s)$ and $v(s)$ are the gene frequency and variance $(p(s) \cdot [1 - p(s)] \cdot 1/n)$, where $n$ is the total number of individual genes (i.e., twice the number of insects examined, since these insects are diploid).

Table 2
Single locus statistics

| Loc | Year | n | x | p(s) | sqrt[v(s)] | z | P |
|---|---|---|---|---|---|---|---|
| *6Pgdh* locus | | | | | | | |
| MM | 1986 | 150 | 38 | 0.253 | 0.035 | 0.11 | >O.91 |
| EB | 1987 | 48 | 15 | 0.313 | 0.067 | 0.83 | >O.40 |
| SG | 1986 | 68 | 22 | 0.324 | 0.057 | 1.17 | >0.24 |
| CA | 1986 | 78 | 25 | 0.321 | 0.053 | 1.19 | >0.23 |
| *Pgm* locus | | | | | | | |
| MM | 1986 | 150 | 131 | 0.873 | 0.027 | 0.64 | >0.52 |
| EB | 1987 | 42 | 40 | 0.952 | 0.033 | 2.93 | <0.01 |
| SG | 1986 | 80 | 74 | 0.925 | 0.029 | 2.34 | <0.02 |
| CA | 1986 | 76 | 74 | 0.974 | 0.018 | 6.41 | <0.00 |

Computed risks of error ($P$) in rejecting different localities as the source of reinfestant bugs. The gene frequency in the reinfestant population is not shown: it was 0.257 for the *Pgm* locus ($n=150$) and 0.860 for the *6Pgdh* locus ($n=150$). Loc = putative source locality; $n$ = number of genes (twice the number of individuals); $x$ = the number of alleles found (*Pgm*-2 or *6Pgdh*-1); $p(s)=x/n$ and $\sqrt{v(s)}=\sqrt{[p(s)(1-p(s)]}$ $n$ are the gene frequency and its standard error for each putative source population; $z$ = standard normal deviate; MM = Moro Moro; EB = El Bello; SG = San Geronimo; CA = Candelaria.

For this study we used data from two gene loci, *6Pgdh* and *Pgm*, which represent two of the most frequently polymorphic enzymes found in *T. infestans* (Dujardin and Tibayrenc, 1985b; Dujardin, 1990). The $P$-value on the null hypothesis of each locality as the source of the reinfestant bugs was much lower for the neighbouring localities than for Moro Moro (Table 2). These $P$-values can be envisaged as representing the proportionate risk of error in rejecting each locality as the source of reinfestant bugs, and this risk was greater for Moro Moro than for the remaining localities. Moreover, because *6Pgdh* and *Pgm* can be considered as independent loci (Dujardin, 1990), the $P$-values from the significance tests on each locus ($i$) can be combined to produce a chi-squared value as recommended by Fisher (1950), where $X^2=-2\sum\ln(P_i)$, with 4 degrees of freedom (i.e., twice the number of independent tests) (Table 3). This analysis confirmed a significant difference between the reinfestant bugs and the untreated localities ($P$ ranging between $<0.001$ and $<0.01$), while no significant difference was detected with Moro Moro ($P<0.50$). Besides these tests, the gene frequencies of reinfestant specimens and putative source populations were also compared according to the standard test for the significance of the

Table 3
Combined statistics

| Locality | Year | $X^2$ | $n$ | Significance |
|---|---|---|---|---|
| MM | (86) | 3.025 | 4 | <0.50 |
| EB | (87) | 15.93 | 4 | <0.01 |
| SG | (86) | 13.51 | 4 | <0.02 |
| CA | (86) | 36.53 | 4 | <0.001 |

Chi-squared ($X^2$) for combining $P$-values from the tests in Table 2 for the two loci *6Pgdh* and *Pgm*. $X^2=-2\sum\ln(P_i)$; $n$ = degrees of freedom; MM = Moro Moro; EB = El Bello; SG = San Geronimo; CA = Candelaria. MM (1986), EB, SG and CA are the putative source populations.

difference between two proportions based on measurements of samples of size $n_1$ and $n_2$, leading to similar conclusions (not shown).

Our analysis clearly supports the idea that, in this case, the reinfestant specimens did not come from the untreated localities, but instead represented survivors from the original bug population in the treated locality. This type of analysis may prove to be useful in entomological surveillance of Chagas vector control programmes, since it provides a way of distinguishing between true recolonisation and apparent treatment failure.

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