

The microdistribution of isoenzymic strains of *Trypanosoma cruzi* in southern Bolivia; new isoenzyme profiles and further arguments against Mendelian sexuality

M. <sup>Michal</sup> TIBAYRENC<sup>1</sup>, L. ECHALAR<sup>2</sup>, J. P. DUJARDIN<sup>3</sup>, O. POCH<sup>4</sup> AND P. DESJEUX<sup>5</sup>  
<sup>1</sup>ORSTOM-IBBA, Embajada de Francia, Casilla 824, La Paz, Bolivia; <sup>2</sup>Universidad Major de San Andrés,

Table I—Origin of the stocks examined. (Number of stocks referred to each house referred to each isoenzymic strain. Groups A and B of suburbs and villages are presented separately (see text). Suburb Oploca was not included in groups A and B)

<b>TUPIZA</b>					
Group A of suburbs:					
Suburb Rencillas					
House 1	—	2	—	—	—
Suburb Santa Rosa					
House 1	1	—	—	—	1
Suburb Zona Bolivar					
House 1	—	4	—	—	—
2	—	2	—	—	—
3	—	5	1	—	—
Suburb Deseada					
House 1	—	2	2	—	—
Suburb Villa Remedios					
House 1	2	3	—	—	—
2	1	—	—	—	—
3	—	—	1	—	—
4	1	—	—	—	—
5	—	—	1	—	—
Suburb Villa Betania					
House 1	2	1	2	—	1
Suburb Villa Fatima					
House 1	—	2	—	—	—
Total group A:					
	7	21	7	0	2
Group B of suburbs:					
Suburb Suypacha					
House 1	1	—	—	—	—
2	1	—	—	—	—
3	—	—	—	—	2
4	—	—	—	—	1
Suburb Angostora					
House 1	1	—	—	—	—
2	—	—	1	—	—
Suburb Quebrada Seca					
House 1	—	—	—	—	1
2	1	—	—	—	—
Suburb Suycuchaca					
House 1	2	—	—	—	—
2	1	1	2	—	—
3	1	—	—	—	—
4	6	—	—	—	—
5	4	1	—	—	1
6	—	—	—	—	1
Suburb Pueblo Nuevo					
House 1	—	—	—	—	1
Suburb Tocloca					
House 1	2	—	—	—	—
2	—	1	1	—	—
3	—	1	—	—	—
4	3	—	—	—	—
5	2	—	—	—	—
6	1	—	—	—	—
Suburb Chacopampa					
House 1	2	1	1	—	—
2	1	2	—	—	—
3	1	—	—	—	—
4	—	—	1	—	—
5	1	—	—	—	—
6	1	—	—	—	1
Suburb Palquiza					
House 1	3	—	1	—	—
2	1	1	—	—	—
3	—	1	—	—	—
Total group B:					
	36	9	7	0	7
Suburb Oploca					
House 1	2	1	—	—	—
TOTAL TUPIZA: (and percentage)					
	45	31	14	0	9

**TARIJA:**

Suburb Defensor del Chaco					
House 1	5	—	—	—	—
2	—	1	—	1	—
3	—	1	—	—	—
4	1	—	—	—	—
Suburb Tomatitas					
House 1	—	3	—	—	—
2	1	—	—	—	—
3	—	1	—	—	—
4	1	2	—	—	—
Suburb Villa Pisaro					

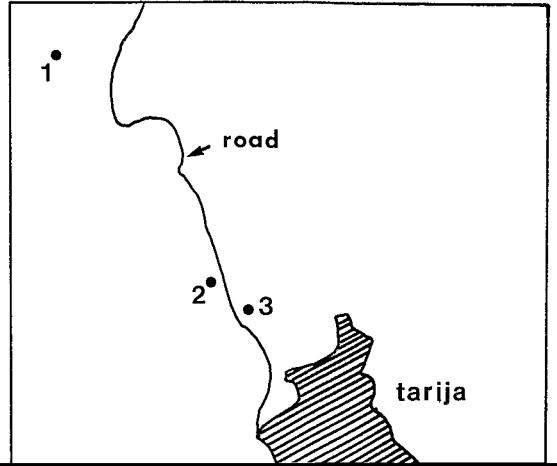


Table II—Genetic interpretation of the zymograms: allele frequencies. (Z2 = Brazilian zymodeme 2)

Isoenzymic strain:		1	2	2a	2b	2c	Z2
Locus PGM	Allele no.						
	1	1	0	0	0	0	0
	2	0	0.5	0.5	0	0	0
	3	0	0.5	0.5	1	1	1
GPI	1	0	0	0	0	0	0.5
	2	0	0.5	0	0.5	0	0
	3	0	0	0.5	0	1	0.5
	4	0	0.5	0.5	0.5	0	0
	5	1	0	0	0	0	0
6PGD	1	0	0	0	0	1	0
	2	0	0.5	0.5	0.5	0	0
	3	0	0	0	0	0	1
	4	0	0.5	0.5	0.5	0	0
	5	1	0	0	0	0	0
PEP	1	1	0	0	0	0	0
	2	0	0	0	0	0	1
	3	0	1	1	1	0	0
	4	0	0	0	0	1	0
G6PD	1	0	0	0	0	0	1
	2	0	0	0	0	1	0
	3	0	1	1	1	0	0
	4	1	0	0	0	0	0
ICD	1	0.5	0	0	0	0	0
	2	0.5	0	0	0	0	0
	3	0	1	1	1	1	1
ME1	1	0	1	1	1	1	1
	2	1	0	0	0	0	0
ME2	1	1	0	0	0	0	0
	2	0	1	1	1	1	1
MDH1	1	0	1	1	1	1	1
	2	1	0	0	0	0	0
MDH2	1	1	1	1	1	1	1
LAP	1	1	0	0	0	0	0
	2	0	1	1	1	1	1
GD	1	1	1	1	1	1	1

Table III—Matrix of genetic distances

	1	2	2a	2b	2c
1	0				
2	1.71	0			
2a	1.71	0.03	0		
2b	1.73	0.03	0.05	0	
2c	1.77	0.41	0.34	0.36	0
Z2	1.75	0.39	0.35	0.34	0.32

14% (18 stocks out of 132). Although this strain is closely related to IS 2 (genetic distance 0.03), it may be epidemiologically significant because it has also been found in French Guyana (Tibayrenc, in preparation), in Chile (the laboratory reference strain "Tulahuén") and in the USA (Tibayrenc & Le Ray, in preparation). In Bolivia, IS 2c was only observed in Tupiza. This town is a centre of migration between Bolivia and Argentina. The relationships between IS 2c and Brazilian zymodeme 2 (genetic distance 0.34) has to be studied more closely. Brazilian zymodeme 2 was recorded by MILES *et al.* (1981a) in central and eastern Brazil. IS 2b, closely related to IS 2 (genetic distance 0.03), was observed in Tarija, as a single stock. IS 2 and IS 1 were observed both in Tupiza and

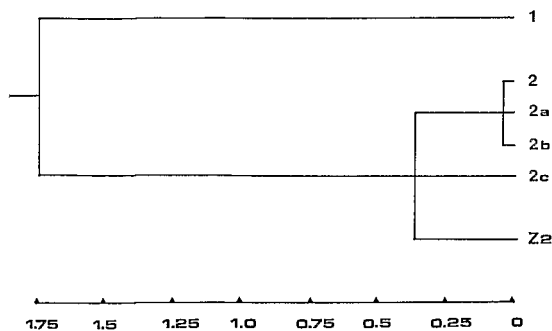


Fig. 4. Dendrogram of genetic distances among the isoenzymic strains, established using UPGMA cluster method.

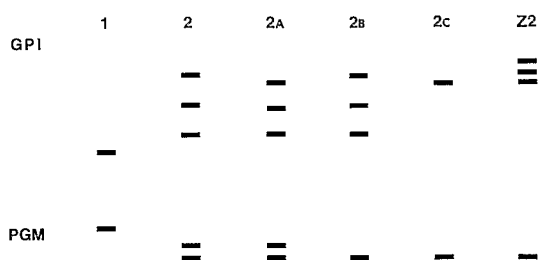


Fig. 5. Zymograms of the isoenzymic strains for the enzymes GPI and PGM (see also Table II: genetic interpretation of the zymograms, and Fig. 6).

Tarija, and also in all the Bolivian towns we have studied (TIBAYRENC & DESJEUX, 1983). It is interesting to notice that the frequency of IS 2 is higher (60%) in Tarija (altitude 1600 m) than in Tupiza (31%, altitude 2600 m). We have already pointed out (TIBAYRENC & DESJEUX, 1983) that IS 1 seems to be more frequent at high altitude, and that IS 2 seems to be more frequent at lower altitude. Further studies are in hand to verify the correlation between altitude and frequency of the isoenzymic strains.

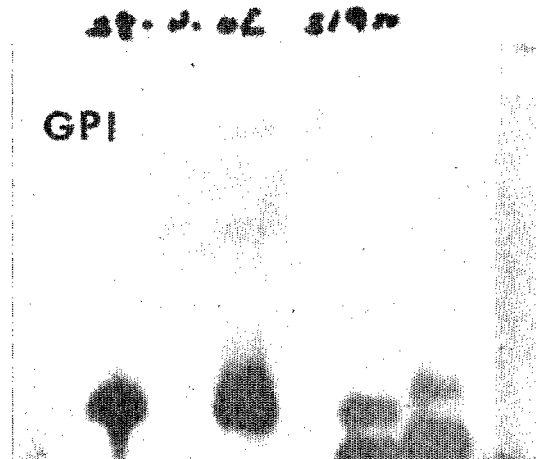
#### Microdistribution

It is interesting to notice that it is easy to collect different isoenzymic strains in the same suburb and even in the same house, as seen previously (TIBAYRENC & DESJEUX, 1983). For example in

groups are separated by 5 to 10 km, and a natural obstacle (a small mountain). We used a  $\chi^2$  test, grouping the data for IS 2a and IS 2c in order to obtain sufficient expected values. The differences are highly significant ( $\chi^2 = 21.53$  with degree of freedom = 2). This indicates clearly that the homogenization of the strain frequencies between the two groups is not effective, unless the human communications are important, and the ecological conditions are similar. LEHANE & SCHOFIELD (1981) indicated that flights of several kilometers were possible for triatomine bugs. Nevertheless, our data seem to indicate that the homogenization of strain frequencies is not effective for relatively small distances, and that at this level, a Founder effect can be supposed.

#### Vector specificity

MILES *et al.* (1981b) have suggested that different *T. cruzi* zymodemes may be adapted to particular vector species. In Bolivia, that does not seem to be the case, at least for the isoenzymic strains considered and in domestic transmission cycles: different strains are all transmitted by *Triatoma infestans*, including the "principal" strains. This is obvious in Tupiza, where we observed totally sympatric transmission by the same insect vector and twice recorded an apparently



mixed infection with IS 1 and IS 2 in the same triatomine bug. This lack of vector specificity among the different *T. cruzi* strains in Bolivia is important epidemiologically.

#### Lack of mating

Genetic arguments for this hypothesis were given previously (TIBAYRENC *et al.*, 1981b). Nevertheless, we had not a sufficient number of stocks to test a Hardy-Weinberg equilibrium. In the present work, we calculated Hardy-Weinberg genotype frequencies based on the hypothesis of diploidy in *T. cruzi* (see TIBAYRENC *et al.* 1981a; LANAR *et al.* 1981). We considered the following enzyme loci: PGM and GPI (each locus being considered independently). Table IV gives the observed and expected genotype numbers in Tupiza. The  $\chi^2$  test shows clearly that the results do not accord with genetic exchange between the strains by means of Mendelian sexuality (for example, locus GPI:  $\chi^2 = 198$ , with degree of freedom = 9). It is also interesting to notice the frequent presence of different alleles between IS 1 and IS 2 (for example in the cases of PGM and GPI) without corresponding heterozygous patterns for these different alleles: the genotypes PGM 1/2 and GPI 4/5 were not encountered. This is a common argument to prove lack of genetic exchange between sympatric taxa. The observed and expected genotype numbers in Tupiza may be calculated if we assume that genetic exchange occurs only between IS2, 2a and 2c, which are closely related. The results obtained (Table V) also argue against this hypothesis (GPI:  $\chi^2 = 66$ , with degree of freedom = 5). On the other hand, one can see that IS 2 and 2a are constantly heterozygous for PGM, GPI and 6 PGD (this was previously noticed for IS 2: TIBAYRENC *et al.*, 1981b; TIBAYRENC & DESJEUX, 1983). This "fixed heterozygosity" further suggests that there is no genetic exchange within these strains. Finally, the patterns for the six loci examined are constant within each strain, with no recombination among the different strains (that is GPI, PGM, ME1, ME2, ICD and 6PGD patterns are specific for each strain). This lack of recombination is also observed with the sample of 21 stocks characterized using 10 enzymes (12 loci). The only exception is the case of IS 2b in Tarija (a single stock), which has all enzymic patterns of IS 2, but with a PGM pattern of IS 2c. The unlikely phenomenon of parasexuality in *T. cruzi* (TIBAYRENC *et al.*, 1983) (see the common PGM pattern IS 2b and IS 2c), with genetic exchange involving a small part of the genome, might be considered. Mendelian sexuality (with recombination of the whole genome) seems to be exceptional or absent. This confirms our previous data (TIBAYRENC *et al.*, 1981b) and shows a situation that is quite different to the one observed in *Trypanosoma brucei* (TAIT, 1980).

The present work makes clearer some points of the distribution of *Trypanosoma cruzi* isoenzymic strains and allowed us to verify the hypothesis of lack of mating at present. In the field of taxonomy, for the moment, more data are required to estimate the real genetic variability of *T. cruzi* and to establish reliable correlations between biochemical taxonomy and medical data (TIBAYRENC *et al.*, 1983, BARNABE *et al.*, 1984.

**Table IV—Observed and expected numbers of genotypes among the isoenzymic strains 1, 2, 2a and 2c in Tupiza**

Enzyme GPI Genotypes:	Observed	Expected
3/3	9	2.5
5/5	45	21
2/4	31	6.8
3/4	14	7.2
2/2	0	2.3
4/4	0	5.3
2/3	0	4.8
2/5	0	13.6
3/5	0	14.6
4/5	0	20.9
Enzyme PGM Genotypes:		
1/1	45	20.9
3/3	9	10.1
2/3	45	13.9
2/2	0	4.9
1/2	0	20.1
1/3	0	29.1

**Table V—Observed and expected numbers of genotypes among the isoenzymic strains 2, 2a and 2c in Tupiza**

Enzyme GPI Genotypes:	Observed	Expected
3/3	9	4.9
2/4	31	12.8
3/4	14	13.2
2/2	0	4.6
4/4	0	9.1
2/3	0	23.1
Enzyme PGM Genotypes:		
3/3	9	18.2
2/3	45	26.3
2/2	0	9.5

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