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

M. TIBAYRENC[1], L. ECHALAR[2], J. P. DUJARDIN[3], O. POCH[4] AND P. DESJEUX[5]

[1]*ORSTOM-IBBA, Embajada de Francia, Casilla 824, La Paz, Bolivia;* [2]*Universidad Major de San Andrès, and IBBA;* [3]*Institut de Médecine tropicale Prince Leopold (Antwerp), and IBBA;* [4]*Université Louis Pasteur (Strasbourg), & IBBA;* [5]*Institut Pasteur de Paris, and IBBA*

Summary

132 *Trypanosoma cruzi* stocks were collected in southern Bolivia (99 stocks in Tupiza, 33 in Tarija), and were characterized using five enzymes (six loci). From these 132 stocks, a sample of 21 was studied using 10 enzymes (12 loci) to establish the genetic distances between them. Only five different isoenzymic strains were registered among the 132 stocks: the taxonomic status of these strains is discussed. The distribution of the strains indicated that a Founder effect was not a constant fact at the level of the house and of the suburb, but that a Founder effect was more apparent for greater geographical distances. All strains were transmitted sympatrically by the same vector *Triatoma infestans*. Genotype frequencies demonstrated the lack of Mendelian sexuality among stocks of *T. cruzi* from southern Bolivia, confirming our previous results.

Introduction

The presence of different isoenzymic strains within the taxon *Trypanosoma cruzi* was first demonstrated by MILES *et al.* (1977) using an intuitive interpretation of zymograms. READY & MILES (1980) distinguished, by numerical taxonomy, three main strains ("zymodemes") in Brazil.

The genetic interpretation of zymograms led us to propose that *T. cruzi* was diploid (TIBAYRENC *et al.*, 1981a) and lacked present sexuality (TIBAYRENC *et al.*, 1981b). These theoretical assumptions allowed the use of genetic distances (NEI, 1972; TIBAYRENC, 1980) to classify isoenzymic strains of *T. cruzi*. The geographical frequencies of the principal isoenzymic strains in Bolivia were described in a previous study (TIBAYRENC & DESJEUX, 1983).

In this paper we present a microgeographical study of the Bolivian towns of Tupiza and Tarija, which clarifies the distribution of the isoenzymic strains at the level of the suburb and the house. The taxonomic status of *T. cruzi* isoenzymic strains is again discussed and the hypothesis of lack of mating in *T. cruzi* is once more considered.

Material and Methods

All stocks were isolated from *Triatoma infestans* (Hemiptera, Reduviidae) using a method described elsewhere (TIBAYRENC *et al.*, 1982). Triatomine bugs were collected in Tupiza and Tarija in December 1981. 99 stocks of *T. cruzi* from Tupiza were collected from 42 different houses and 16 different suburbs or near villages. 33 stocks of *T. cruzi* were collected from 12 different houses and four different suburbs or near villages (Table I; Figs. 1, 2 and 3).

All stocks were grown in GLSH monophasic medium. Stocks were harvested by centrifugation and pellets were stored, without washing, at −70°C. Pellets were lysed just before electrophoresis in an equal volume of hypotonic enzyme stabilizers (dithiothreitol, e-aminocaproic acid, EDTA, each at 2 mM) by standing for 20 min on ice, followed by freeze-thawing. Single culture tubes were sufficient to study the range of enzymes used.

Most of the 132 stocks were characterized using five enzymes, namely, phosphoglucomutase (E.C.2.7.5.1., PGM), glucosephosphate isomerase (E.C.5.3.1.9., GPI), malate dehydrogenase (oxaloacetate decarboxylating (NADP+) (E.C.1.1.1.40., ME), 6-phosphogluconate dehydrogenase (E.C.1.1.1.44., 6PGD) and isocitrate dehydrogenase (E.C.1.1.1.42., ICD). These enzymes were sufficient to determine the isoenzymic strain and to test the hypothesis of lack of mating. A sample of 21 stocks (15 from Tupiza, six from Tarija) was studied using 10 enzymes to examine phylogenetic relationships between the isoenzymic strains. We used as reference stocks C8 clone 1 (Bolivian zymodeme 1), SC43 clone 2 (Bolivian zymodeme 2), the Tulahuen strain and Esmeraldo clone 3 (Brazilian zymodeme 2) (TIBAYRENC & MILES, 1983). The additional five enzymes used were: malate dehydrogenase (E.C.1.1.1.37., MDH), glucose-6-phosphate dehydrogenase (E.C.1.1.1.49., G6PD), glutamate dehydrogenase (NADP+) (E.C.1.4.1.2., GD), aminopeptidase (cytosol) (E.C.3.4.11.1., PEP; substrate: leucyl-leucyl-leucine), and leucine aminopeptidase (E.C.3.4.11., LAP; substrate: L-leucine β naphthyl amide). All electrophoresis was carried out on cellulose acetate plates (Helena[r] laboratories). The recipes used were adapted from LANHAM *et al.* (1981). Electrophoresis was performed at 200 volts for 30 min throughout and all tank buffers contained 20% sucrose. Tank buffer No. III of SHAW & PRASAD (1970) was used for the enzymes GPI and 6PGD and the same buffer diluted (1:10) for soaking plates. Buffer Helena[r] HR made up to 750 ml, plus 1·0 mM $MgCl_2$, was used as tank buffer for G6PD and GD. For GD, the developer solution consisted of: Tris 0·05 M, L-glutamic acid 0·1 M, adjusted to PH 7·0; with the quantities of NADP, NBT and PMS given by LANHAM *et al.* (1981). For LAP, the buffer was No. II of SHAW & PRASAD (1970) and the developer solution was as they described (SHAW & PRASAD, 1970).

Genetic interpretation was based on previous assumptions (TIBAYRENC *et al.*, 1981a, b). Genetic distance calculations used Nei's standard distance (NEI, 1972) with the modifications proposed for asexual, diploid flagellates (TIBAYRENC, 1980; TIBAYRENC *et al.*, 1981a) (Tables II and III). All calculations were performed with a microcomputer Casio[r] FX-702P. Computer programmes were composed for genetic distance calculations, and for all statistical tests used in this work.

Results and Discussion

Only five different isoenzymic strains were encountered among the 132 *T. cruzi* stocks examined. Two of

Table I—Origin of the stocks examined. (Number of stocks collected in 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)

Isoenzymic strain: / *Place:*	1	2	2a	2b	2c
TUPIZA					
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 Suycuchacra					
House 1	2	—	—	—	—
2	1	1	2	—	—
3	1	—	—	—	—
4	6	—	—	—	—
5	4	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					
House 1	—	3	—	—	—
2	—	1	—	—	—
3	—	6	—	—	—
Suburb San Martin					
House 1	—	2	4	—	—
TOTAL TARIJA:	8	20	4	1	0
Percentage:	24	60	12	0·03	0

Fig. 1. General map of Bolivia, with the situations of Tupiza and Tarija.

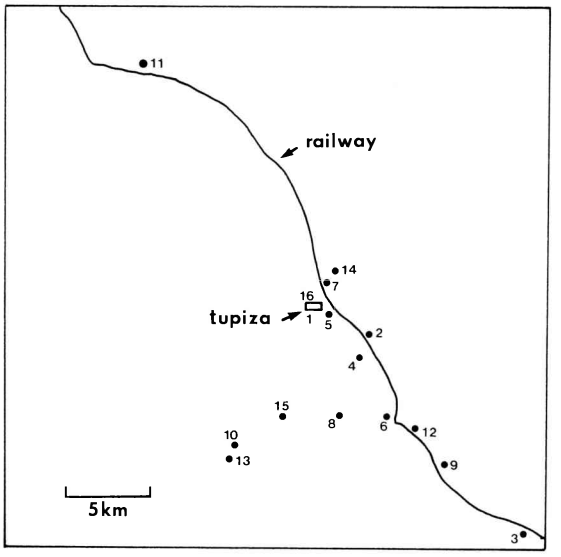

Fig. 2. Outskirts of Tupiza, showing the suburbs and villages studied (up to 20 km from Tupiza). See also Table I. Numbers refer to suburbs and villages: 1 = Rencillas; 2 = Santa Rosa; 3 = Suypacha; 4 = Zona Bolivar; 5 = Deseada; 6 = Angostora; 7 = Villa Remedios; 8 = Quebrada Seca; 9 = Suycuchacra; 10 = Pueblo Nuevo; 11 = Oploca; 12 = Tocloca; 13 = Chacopampa; 14 = Villa Betania; 15 = Palquiza; 16 = Villa Fatima.

them have already been recorded in Bolivia (TIBAYRENC & DESJEUX 1983). The three others were observed in this country for the first time: 2a was found in both Tupiza and Tarija; 2b (a single stock) was found only in Tarija, and 2c only in Tupiza.

Phylogenetic relationships

Both intuitive interpretation and genetic distances (Tables II and III, Fig. 5) show that the three new isoenzymic strains are more closely related to Bolivian

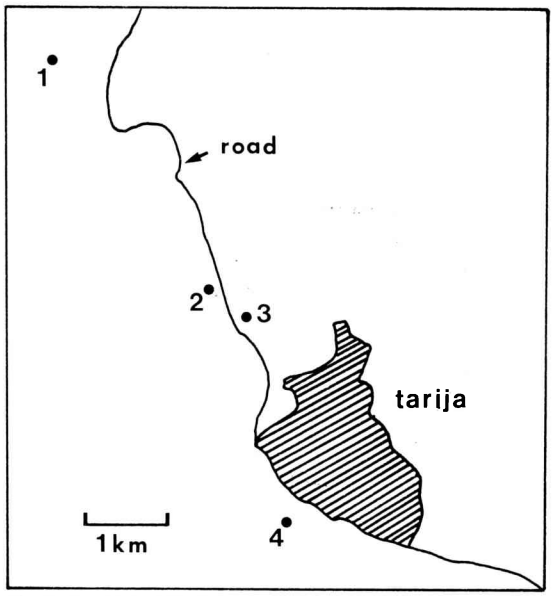

Fig. 3. Outskirts of Tarija, showing the suburbs and villages studies (up to 8 km from Tarija). Numbers refer to suburbs and villages: 1 = Tomatitas; 2 = Villa Pisaro; 3 = Defensor de Chaco; 4 = Sant Martin.

zymodeme 2 than to Bolivian zymodeme 1(TIBAYRENC & MILES, 1983). Strains 2a and 2b differ from Bolivian zymodeme 2 than to Bolivian zymodeme 2 by one allele only. Strain 2c can be related intuitively to Brazilian zymodeme 2 because they have several alleles in common. Nevertheless the genetic distance between 2c and Brazilian zymodeme 2 is not negligible (0·34) and is comparable to the genetic distance between 2c and Bolivian zymodeme 2 (0·41). In another paper (TIBAYRENC *et al.*, 1983) we discuss some taxonomic and terminological problems concerning isoenzymic strains of *T. cruzi*. Several genetic arguments (principally: lack of continuum, lack of high heterozygosity, frequent passage from a homozygote state to another) suggest that "principal" isoenzymic strains can be the result of real speciation (we refer to biological concept of species), which would have occurred before the loss of hypothetical sexuality of *T. cruzi*. Further investigations are needed to confirm the distinction between "principal" and "lesser" isoenzymic strains and intermediate cases are apparent (TIBAYRENC & MILES, 1983). In previous papers (TIBAYRENC & DESJEUX 1983; TIBAYRENC & MILES, 1983), we used the word "zymodeme" for "principal" isoenzymic strains, as done by MILES *et al.* (1980) and READY & MILES (1980). But as the distinction between "principal" and "lesser" strains becomes less clear, we prefer for the moment not to use this word: each strain defined by isoenzymes ("principal" or "lesser") is called "isoenzymic strain" (IS) without consideration of its taxonomic or medical importance. Only an extensive survey of the total variability of *T. cruzi* throughout its geographical range will allow the definition of a more precise and rigorous terminology.

Geographical distribution

In Bolivia, IS 2a was only observed in the southern part of the country (Tupiza, Tarija), at a frequency of

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

Isoenzymic strain:		1	2	2a	2b	2c	Z2
Locus	Allele no.						
PGM	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 "Tulahuen") 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

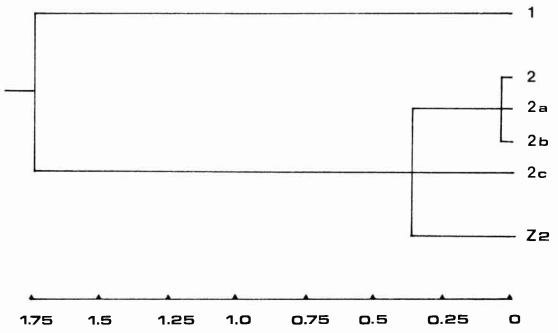

Fig. 4. Dendrogram of genetic distances among the isoenzymic strains, established using UPGMA cluster method.

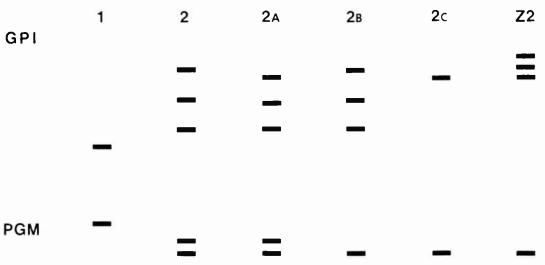

Fig. 5. Zymograms of the isoenzymic strains for the enzymes GPI and PGM (see also Table II: genetic interpretation of the zymograms, and Fig. 6).

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 house No. 1 of Villa Betania (Table I), we collected four different strains out of six stocks. On the contrary, in house No. 4 of Suycuchacra, we collected only one strain out of six stocks. These data indicate that a Founder effect is not a constant feature of the dispersal of the strains at the level of the suburb and of the house. In Tupiza, of 44 houses, nine had two different isoenzymic strains, two had three different strains, and one had four different strains (the average number of stocks collected per house was 2.25). This may in fact suggest that the migration of triatomine bugs from house to house is important as pointed out by LEHANE & SCHOFIELD (1981). On the other hand, we compared the strain repartition between two groups of suburbs and villages. Group A: suburbs 1, 2, 4, 5, 7, 14, 16: 13 different houses, 37 stocks; Group B; suburbs 3, 6, 8, 9, 10, 12, 13, 15: 30 different houses, 59 stocks (see Fig. 2). These two groups are separated by 5 to 10 km, and a natural obstacle (a small mountain). We used a χ^2 test, grouping the data for IS 2a and IS 2c in order to obtain sufficient expected values. The differences are highly significant ($\chi^2 = 21{\cdot}53$ 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

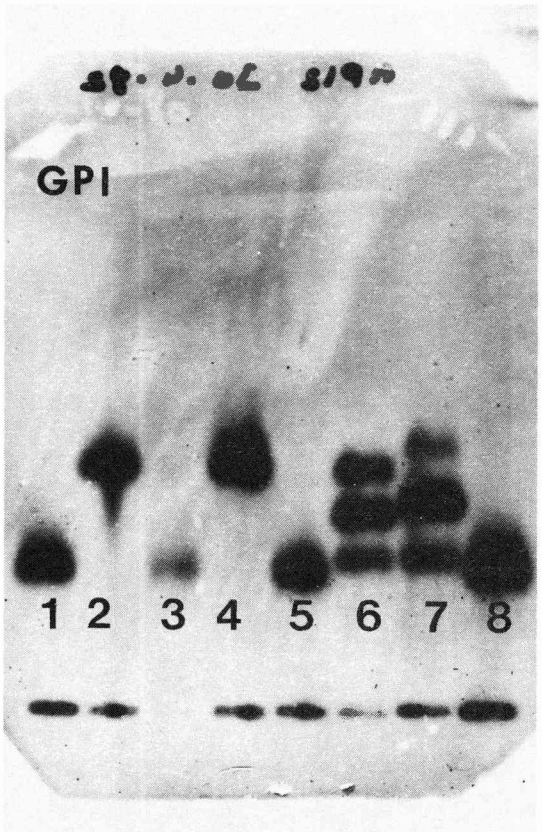

Fig. 6. Zymogram of the enzyme GPI for different isoenzymic strains. Samples 1, 2, 5 & 8: Isoenzymic strain 1 (genotype 5/5); samples 2 & 4: isoenzymic strain 2c (genotype 3/3); sample 6: isoenzymic strain 2a (genotype 3/4); sample 7: isoenzymic strain 2 (genotype 2/4). See also Fig. 5 and Table II.

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 χ^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

Acknowledgements

We are indebted to Dr. M. A. Miles (London School of Tropical Medicine and Hygiene), who cloned C8 and SC43 stocks, and kindly provided Brazilian zymodeme 2, and to Dr. D. Le Ray (Institute of Tropical Medicine, Antwerp), who provided kindly the strain Tulahuen.

This study was carried out with the aid of the French Technical Cooperation and the aid of the Délégation générale à la Recherche Scientifique Scientifique et Technique, n° of aid: PVD/81/L-1423.

References

Barnabé, C., Tibayrenc, M. & Dujardin, J. P. (1983). *Trypanosoma cruzi*: a pharmacological comparison of some Bolivian isoenzymic strains. *Annales de la Société belge de Médecine tropicale*, **63**, 319-324.

Lanar, D. E., Levy, L. S. & Manning, J. E. (1981). The complexity and content of the DNA and RNA in *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology*, **3**, 327-341.

Lanham, S. M., Grendon, J. M., Miles, M. A., Povoa, M. & Souza, A. A. de (1981). A comparison of electrophoretic methods for isoenzyme characterization of Trypanosomatids. I: Standard stocks of *Trypanosoma cruzi* zymodemes from northeast Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **75**, 742-750.

Lehane, M. J. & Schofield, C. S. (1981). Field experiments of dispersive flight by *Triatoma infestans*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **75**, 399-400.

Miles, M. A., Toyé, P. J., Oswald, S. C. & Godfrey, D. G. (1977). The identification by isoenzyme patterns of two distinct strain-groups of *Trypanosoma cruzi*, circulating independently in a rural area of Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 217-22.

Miles, M. A., Lanham, S. M. Souza, A. A de & Povoa, M. (1980). Further enzymic characters of *Trypanosoma cruzi* and their evaluation for strain identification. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 221-237.

Miles, M. A., Cedillos, R. A., Povoa, M., Souza, A. A. de, Prata, A. & Macedo, V. (1981a). Do radically dissimilar *Trypanosoma cruzi* strains (zymodemes) cause Venezuelan and Brazilian forms of Chagas' disease? *Lancet*, i, 1338-1340.

Miles, M. A., Povoa, M., Souza, A. A. de, Lainson, R., Shaw, J. J. & Ketteridge, D. S. (1981b). Chagas' disease in the Amazonian basin. II: The distribution of *Trypanosoma cruzi* zymodemes 1 and 3 in Para State, North Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **75**, 667-674.

Nei, M. (1972). Genetic distances between populations. *American Naturalist*, **106**, 283-292.

Ready, P. D. & Miles, M. A. (1980). Delimitation of *Trypanosoma cruzi* zymodemes by numerical taxonomy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 238-242.

Shaw, C. R. & Prasad, R. (1970). Starch-gel electrophoresis of enzymes: a compilation of recipes. *Biochemical Genetics*, **4**, 297-320.

Tait, A. (1980). Evidence for diploidy in trypanosomes. *Nature*, **287**, 536-537.

Tibayrenc, M. (1980). Application of the calculations of genetic distance for flagellate systematics. *Cahiers ORSTOM série Entomologie médicale et Parasitologie*, **18**, 301-302.

Tibayrenc, M. & Desjeux, P. (1983). The presence in Bolivia of two distinct zymodemes of *Trypanosoma cruzi*, circulating sympatrically in a domestic transmission cycle. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **77**, 73-75.

Tibayrenc, M. & Miles, M. A. (1983). A genetic comparison between Brazilian and Bolivian zymodemes of *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **77**, 76-83.

Tibayrenc, M., Cariou, M. L. & Solignac, M. (1981a). Interprétation génétique des zymogrammes de flagellés des genres *Trypanosoma* et *Leishmania*. *Comptes-rendus de l'Académie des Sciences, Paris*, **292**, 623-625.

Tibayrenc, M., Cariou, M. L., Solignac, M. & Carlier, Y. (1981b). Arguments génétiques contre l'existence d'une sexualité actuelle chez *Trypanosoma cruzi*. Implications taxonomiques. *Comptes-rendus de l'Académie des Sciences, Paris*, **293**, 207-209.

Tibayrenc, M., Echalar, L. & Desjeux, P. (1982). Une méthode simple pour obtenir directement des isolats de *Trypanosoma cruzi* à partir du tube digestif du triatome vecteur. *Cahiers ORSTOM série Entomologie médicale et Parasitologie*, **20**, 187-188.

Tibayrenc, M., Echalar, L., Brenière, F., Lemesre, J. L., Barnabé, C. & Desjeux, P. (1983). Sur le statut taxonomique et médical des souches isoenzymatiques de *Trypanosoma cruzi*. Considérations sur la valeur systématique et immunogénique des différentes isoenzymes. *Comptes-rendus de l'Académie des Sciences, Paris*, **296**, 721-726.

Accepted for publication 8th November, 1983.