Ann. Soc. belge Méd. trop.
1984, **64**, 239-248

GENERAL CLASSIFICATION OF THE ISOENZYMIC STRAINS OF *TRYPANOSOMA (SCHIZOTRYPANUM) CRUZI* AND COMPARISON WITH *T. (S.) C. MARINKELLEI* AND *T. (HERPETOSOMA) RANGELI*

by

M. TIBAYRENC[1] & D. LE RAY[2]
[1]*ORSTOM-IBBA, Embajada de Francia, Casilla 824, La Paz, Bolivie*
[2]*Laboratoire de Protozoologie, Institut de Médecine Tropicale Prince Léopold, Nationalestraat 155, B-2000 Antwerpen, Belgique*

Summary — Thirteen laboratory reference stocks of *Trypanosoma (Schizotrypanum) cruzi* were classified according to genetic distance by enzyme analysis of 14 loci. Three principal isoenzymic strains were recognized together with 6 lesser ones. These patterns fall within our previous classification, suggesting that by now the main bulk of *T. cruzi* enzymic variability has been recognized. Consequently it should be possible to identify any *T. cruzi* stock with certainty and to assess whether *cruzi*-like organisms belong to the *cruzi* complex.

T. (S) cruzi marinkellei and *T. (H.) rangeli* reference stocks were also compared. These two taxa showed to be different from the *T. cruzi* strains, but it was necessary to use a large set of enzymes before gaining evidence for this.

KEYWORDS : *Trypanosoma cruzi*; *T. cruzi marinkellei*; *T. rangeli*; Isoenzymic Strains; Genetic Distances.

Introduction

Isoenzymic studies on *Trypanosoma (Schizotrypanum) cruzi* were initiated by Toyé (1974). An intuitive analysis of the zymograms allowed Miles *et al.* (1977, 1978, 1980) to distinguish in Brazil three major isoenzymic strains, or « zymodemes ». These strains were classified by numerical taxonomy (Ready & Miles, 1980).

A genetic interpretation of the zymograms of a series of Bolivian isolates led us to the following conclusions : existence of diploidy (Tibayrenc, Cariou & Solignac, 1981a), lack of Mendelian sexuality at present (Tibayrenc *et al.*, 1981b) and possibility to classify strains according to their genetic distances (Nei, 1972; Tibayrenc, 1980; Tibayrenc & Miles, 1983; Tibayrenc *et al.*, 1983). Lanar, Levy & Manning (1981), by a study on *T. cruzi* DNA, postulated also that this organism had a diploid structure. These results are worth being compared to the ones reported previously by Tait (1980) for African trypanosomes using a similar methodology, and showing evidence for diploidy and presence of Mendelian sexuality.

This work presents a classification of the main laboratory reference stocks of *T. (S.) cruzi cruzi*, and a preliminary comparison with reference stocks of *T. (S.) cruzi marinkellei* and *Trypanosoma* (*Herpetosoma*) *rangeli*.

Material and methods

1) *Stocks :*

The main *T. (S.) cruzi* stocks used in reference laboratories are Tulahuen, Tehuentepec, Y and CL. These stocks and other studied herein are originated from a broad geographical range (table 1). We analysed also 2 *T. rangeli* reference stocks from monkey and from dog, as *T. rangeli* seems to be a complex of strains with a high genetic variability (Tibayrenc, unpublished data). Only one stock of *T. cruzi marinkellei* was considered.

TABLE 1

Stocks, origin and isoenzymic classification

Stock	Origin	Isoenzymic* classification
Trypanosoma cruzi cruzi :		
C8 (clone 1) *(Triatoma)*	Bolivia	1
WA 250 (Zymodeme I, clone)	Brazil	1
Tehuentepec *(Triatoma)*	Mexico	1 e
ITMAP 943 *(Aotus)*	Brazil	1 f
SC43 (clone 1) *(Triatoma)*	Bolivia	2
Tulahuen (clone FK11A)	Chile	2 a
ITMAP 240 (man)	Brazil	2 a
Maryland (raccoon)	USA	2 a
CL *(Triatoma*, clone)	Brazil	2 a
TU 107 *(Triatoma)*	Bolivia	2 c
Y (ITMAP 1923, clone)	Brazil	2 d
Esmereldo (Zymodeme II, clone)	Brazil	2 e
Can III (Zymodeme III, clone)	Brazil	3
T. cruzi marinkellei B7	Brazil	—
T. rangeli RGB (dog)	Venezuela	—
T. rangeli	Brazil	—

* Code numbers 1 a, b, c and 2 b are assigned to isoenzymic strains (Tibayrenc *et al.*, 1983) not found in the present study.

2) *Cultivation and samples :*

All stocks but one were grown in Yaeger's LIT (cited by Castellani *et al.*, 1967) monophasic culture medium (3 ml medium per 10 ml test tube). Stock « Can III » representative of T. *cruzi* zymodeme III (Miles *et al.*, 1980) was able to grow in NNN biphasic culture medium only. Cells were harvested by centrifugation, lysed with a hypotonic enzyme stabilizer (Godfrey & Kilgour, 1976) and stored at $-70\,^{\circ}C$ until use. A single culture tube provided enough material to study the whole range of enzymes considered herein (table 2).

3) *Isoenzymes :*

Twelve enzyme systems were used (see table 2). The enzyme banding patterns obtained suggest that in ten of the enzymes, the products of a single locus are being observed, while in the case of ME and MDH the products of two loci for each enzyme are being observed. So the twelve enzyme systems allowed the study of 14 genetic loci. Table 2 describes the conditions of revelation. Most of the recipes were initiated according to Lanham *et al.* (1981) then underwent extensive modifications in order to

TABLE 2

Electrophoresis recipes

Enzyme	Tank buffer	Cell buffer	Distilled water	Developper buffer	Activator	Coenzyme	Linking enzyme	Substrate	Visualization method
PGM	A	A 1/4	2 ml	1 : 8 ml	$MgCl_2$ 1.0M 0.25 ml	NADP 5 mg (N5755)	G6PD 20μl (G8878)	Glucose 1 P 20 mg (G1259)	NBT 5 mg (N6876) PMS 3 mg (P9625)
GPI	A	A 1/9	2 ml	2 : 8 ml	$MgCl_2$ 1.0M 0.25 ml	NADP 5 mg (N5755)	G6PD 20μl (G8878)	Fructose 6P 10 mg (F3627)	NBT 5 mg (N6876) PMS 3 mg (P9625)
6PG	A	A 1/4	2 ml	1 : 8 ml	$MgCl_2$ 1.0M 0.25 ml	NADP 5 mg (N5755)	—	6 phosphogluconic acid 5 mg (P7877)	NBT 5 mg (N6876) PMS 3 mg (P9625)
ICD	A	A 1/4	2 ml	1 : 8 ml	$MgCl_2$ 1.0M 0.25 ml	NADP 5 mg (N5755)	—	DL-isocitrate 5 mg (I1252)	NBT 5 mg (N6876) PMS 3 mg (P9625)
G6P	B	B	2 ml	2 : 8 ml	$MgCl_2$ 1.0M 0.25 ml	NADP 5 mg (N5755)	—	Glucose 6P 5 mg (G7250)	NBT 5 mg (N6876) PMS 3 mg (P9625)
ME	A	A 1/4	2 ml	3 : 8 ml	$MgCl_2$ 1.0M 0.25 ml	NADP 5 mg (N5755)	—	Malate 1.0M pH7 0.6 ml (with malic acid M0875)	NBT 5 mg (N6876) PMS 3 mg (P9625)
MDH	B	B	4 ml	1 : 5 ml	—	NAD 5 mg (N7004)	—	Malate 1.0M pH7 1 ml (with malic acid M0875)	NBT 5 mg (N6876) PMS 3 mg (P9625)
GD NAD	A	A 1/4	—	—	—	NAD 5 mg (N7004)	—	Tris 0.05M, glutamate 1.0M 10 ml (with glutamic acid G1626)	NBT 5 mg (N6876) PMS 3 mg (P9625)
GD NADP	A	A 1/4	—	—	—	NADP 5 mg	—	Tris 0.05M, glutamate 1.0M 10 ml (with glutamic acid G1626)	NBT 5 mg (N6876) PMS 3 mg (P9625)
LAP	B	B 1/4	5 ml	4 : 5 ml	—	—	—	L-leucyl β naphtilamide (L0376) 2 mg	Fast black K salt (F7253) 5 mg
Pep A	B	B 1/4	8.5 ml	5 : 1.5 ml	$MnCl_2$ 0.1M 150 μl	—	L-aminoacid oxidase (A9253) 0.5 mg Peroxidase (P8125) 0,25 mg	L-leucylleucyl-leucine (L0879) 5 mg	3 amino 9 ethyl carbazole (A5754) 4 mg in 150 μl ethanol
Pep B	B	B 1/4	8.5 ml	5 : 1.5 ml	$MnCl_2$ 0.1M 150 μl	—	L-aminoacid oxidase (A9253) 0.5 mg peroxidase (P8125) 0,25 mg	L-leucyl-L-alanine (9250) 5 mg	3 amino 9 ethyl carbazole (A5754) 4 mg in 150 μl ethanol

Ten ml of revelation solution, to be mixed with 10 ml agarose 1.2 % at 60° C in plastic Petri dishes just before use.

Tank buffer : A = n° III Shaw & Prasad (1970); B = HELENA HR IS 0.075.

Developer buffer : 1 = Tris HCl 0.3M pH 8.0; 2 = Tris HCl 0.3M pH 7.0; 3 = Tris HCl 0.3M pH 7.4; 4 = Tris maleate pH 6.0 (Shaw & Prasad 1970); 5 = disodium phosphate 0.1M pH 7.4.

Chemicals : numbers between brackets refer to SIGMA catalogue. All electrophoreses carried out at 200 V for 20 mn.

improve the electrophoretic resolution. It is worth noting that, the tank buffer being the same, the more the cell buffer is diluted, the more the bands are well separated. Electrophoreses were carried out on cellulose acetate plates (HELENA laboratories).

4) *Genetic and statistical analyses :*

The present classification is based on our previous genetic hypotheses (Tibayrenc *et al.*, 1981 a & b). Nei's standard genetic distance (1972), based on isoenzymic pattern genetic interpretation, gives the average number of codon differences per gene between two populations (here two strains). Thus this measurement has a precise genetic meaning which is not the case of isoenzyme pattern numerical taxonomy as presented by Ready & Miles (1980). Calculations of Nei's standard genetic distance were performed on a microcomputer CASIO FX702P with a special programme composed at IBBA.

Results and discussion

1) *Classification of* T. cruzi *stocks according to genetic distance :*

The genetic distance and genetic similarity were calculated for each pairwise combination of the thirteen stocks examined and the values obtained are shown in table 3. In order to obtain Nei's coefficients, it is necessary to identify the alleles determining each enzyme electrophoretic pattern. Table 4 shows the genotypes inferred from the isoenzymic patterns. For a given enzyme, 4/4 for example means homozygote for the allele 4 and 1/3 means heterozygote for the alleles 1 and 3. For every enzyme, allele 1 is the electrophoretically fastest one (see for example photograph 1). On the basis of these values three very distinct groups can be distinguished namely groups 1, 2 and 3. Within the former two groups (1 and 2) one can distinguish several lesser isoenzymic strains giving a total of 9 distinct isoenzymic strains for the three groups (« isoenzymic strain » means an isoenzymic variant without considering its taxonomic or medical significance : Tibayrenc *et al.*, 1983). Group 3 is represented by one isoenzymic strain only (Mile's zymodeme III) and this observation is based on only one stock. Using the values of the genetic

TABLE 3

Matrix of genetic distances* (above the diagonal : genetic identity, below : genetic distance)

	1	1 e	1 f	2	2 a	2 c	2 d	2 e	3
1	—	0.92	0.78	0.20	0.20	0.15	0.15	0.15	0.07
1 e	0.08	—	0.85	0.20	0.20	0.15	0.15	0.15	0.15
1 f	0.25	0.16	—	0.28	0.28	0.15	0.15	0.15	0.15
2	1.63	1.63	1.29	—	0.90	0.55	0.58	0.55	0.17
2 a	1.63	1.63	1.29	0.11	—	0.67	0.69	0.65	0.19
2 c	1.91	1.91	1.91	0.60	0.40	—	0.95	0.70	0.15
2 d	1.93	1.93	1.93	0.55	0.37	0.06	—	0.76	0.14
2 e	1.91	1.91	1.91	0.60	0.43	0.35	0.27	—	0.15
3	2.62	1.93	1.93	2.16	1.65	1.93	1.95	1.93	—

* Genetic distance gives the average number of codon differences for gene between 2 populations (here : between 2 isoenzymic strains). See also figure 1, dendrogram of genetic distance.

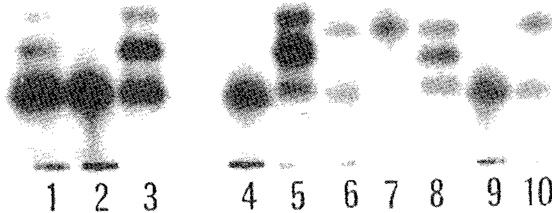
TABLE 4

Genotypes Inferred from the enzyme patterns

Isoenzymic strain	1	1 e	1 f	2	2 a	2 c	2 d	2 e	3
Enzyme									
PGM.E.C.2.7.5.1	1/1	1/1	1/1	2/5	2/5	4/6	5/5	5/5	3/3
GPI.E.C.5.3.1.9	6/6	6/6	6/6	2/5	3/5	3/3	3/3	1/3	4/4
6PG.E.C.1.1.1.44	3/3	3/3	3/3	1/3	1/3	1/1	1/1	2/2	4/4
ICD.E.C.1.1.1.42	1/2	1/2	1/2	3/4	3/4	4/4	4/4	4/4	3/3
G6P.E.C.1.1.1.49	4/4	4/4	4/4	3/3	3/3	2/2	2/2	1/1	5/5
ME1.E.C.1.1.1.40	3/3	2/2	2/2	1/1	1/1	1/1	1/1	1/1	2/2
ME2.E.C.1.1.1.40	2/2	2/2	4/4	3/3	3/3	3/3	3/3	3/3	1/1
MDH1.E.C.1.1.1.37	2/2	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3
MDH2.E.C.1.1.1.37	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
GDNad + E.C.1.2.1.2	3/3	3/3	3/3	1/1	2/2	2/2	2/2	2/2	2/2
GDNadp + E.C.1.4.1.2	1/1	1/1	1/1	2/2	2/2	3/3	3/3	3/3	4/4
Pep A E.C.3.4.11	1/1	1/1	3/3	3/3	3/3	4/4	4/4	2/2	5/5
Pep B E.C.3.4.11	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
LAP E.C.3.4.11	1/1	1/1	1/1	2/2	2/2	2/2	2/2	2/2	3/3

Examples : genotype 3/3 = homozygote for the allele N° 3; genotype 1/2 = heterozygote for the alleles N° 1 and 2. For each enzyme, the allele N° 1 is the faster one on electrophoresis. (See also figure 2, isoenzymic patterns).

PGM = Phosphoglucomutase; GPI = Glucose phosphate isomerase; 6PG = 6 phosphogluconate deshydrogenase; ICD = Isocitrate deshydrogenase; G6P = Glucose 6 phosphate deshydrogenase; ME = Malate deshydrogenase Nadp + or malic enzyme; MDH = Malate deshydrogenase Nad +; GD = Glutamate deshydrogenase; PEP = Peptidase; LAP = leucine aminopeptidase.



Photograph 1.

Zymogram for GPI enzyme. As GPI is a dimeric enzyme, heterozygous samples show 3-banded patterns.

Samples 2, 4 and 9 = homozygote 4/4 (for the same allele n° 4) (izoenzymic strain 1).

Sample 5 = heterozygote 1/3 (for 2 different alleles n° 1 and 3) (isoenzymic strain 2).

Sample 8 = heterozygote 2/3 (isoenzymic strain 2 a).

Sample 7 = homozygote 2/2 (isoenzymic strain 2 c).

Samples 1 and 3 = mixed stocks (strains 1 and 2).

Samples 6 and 10 = mixed stocks (strains 1 and 2 c).

distances obtained, a dendrogram has been constructed (UPGMA clustering method) illustrating the relationships between these groups (fig. 1). One can notice that in the present work, clusterings of the main groups are different than in previous studies (Ready & Miles, 1980; Tibayrenc & Miles, 1983). This is due to the fact that improved electrophoresis techniques have revealed additional allelic differences for zymodeme III in front of groups 1 and 2.

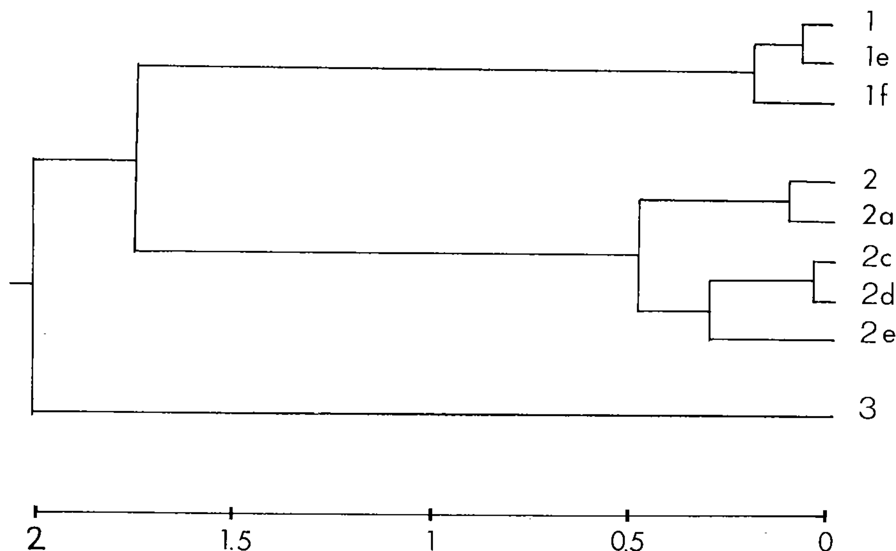


Figure 1.
Dendrogram of genetic distances among the isoenzymic strains.
Only the lowest values (for examples 0.08GD between 1 and 1 e) are exact;
higher values were calculated by arithmetical mean. See also table 3, matrix of genetic distances.

By comparing the present results with previous ones (Ready & Miles, 1980; Tibayrenc & Miles, 1983; Tibayrenc *et al.*, 1983) it is suggested that the principal variants have now been recognized. Our results show the same major groupings as in Ready & Miles's work (1980) but with a greater intragroup variability and numerous lesser strains (especially heterozygous ones). On the other hand, the geographical range considered is huge, especially in the present work. This would allow to determine any stock with safety in the future, including *cruzi*-like organisms. In order to standardize electrophoretic studies, we propose to use as reference samples one or more of the well documented stocks studied here : Miles' zymodemes, Tulahuen, Tehuentepec, Y, CL.

Theoretically genetic distance relates to the average number of codon differences per gene between two populations or taxa (Nei, 1972). Biochemical evolution is roughly correlated to time and represents a « molecular clock » (Wilson, Carlson & White, 1977). So genetic distance is proportional to the time of divergence between two taxa. Nevertheless, organisms devoid of sexuality constitute special cases. We have proposed elsewhere (Tibayrenc *et al.*, 1984 b) the hypothesis that *T. cruzi*

isoenzymic strains may have a recent evolutive origin (by clone generation from a sexual ancestor) as well as an ancient one (either by mitotic evolution or real biological speciation). In both instances, genetic distances show the biochemical relationships among the isoenzymic strains. But they are correlated to time only in the case of an ancient origin.

2) *Geographical distribution of isoenzymic strains :*

As seen in table 1 and previous work (Tibayrenc & Miles, 1983) some isoenzymic strains seem to be ubiquitous. For example, isoenzymic strain 2a was encountered in USA (Maryland stock), in Brazil (stocks CL and ITMAP 240), in Bolivia (Tibayrenc *et al.*, 1984 a) and in Chile (Tulahuen stock). Strain 1 and its relatives (Miles's zymodeme I) were observed in Amazon basin, in Bolivia (stock C8 cl 1) and in Mexico (Tehuentepec stock). The question of the original habitat of *T. cruzi* is an important one : Amazonian or Andean ? It is worth noting that the genetic variability of *T. cruzi* is especially high in Bolivia. As a rule, the genetic variability of a given species is higher at the centre of its geographical range of distribution. Obviously the question deserves additional studies on enzymic variability of *T. cruzi* over its whole geographical range.

3) *Ecological fitness of* T. cruzi :

Its seems to be huge. For example, isoenzymic strain 1 and its relatives were encountered in the Amazonian basin, where it undergoes a sylvatic transmission cycle (Miles *et al.*, 1980), as well as in Bolivia where it is found in a domestic habitat at high altitude (Tibayrenc & Desjeux, 1983).

4) *Comparison with* T. (S.) cruzi marinkellei *and* T. (H.) rangeli :

Due to the presence of numerous isoenzymic strains of *T. (S.) cruzi cruzi*, it was felt necessary to compare *T. (S.) cruzi marinkellei* and *T. (H.) rangeli* with the whole range of the *T. (S.) cruzi cruzi* strains. On the other hand, extensive data on isoenzymic variability of the taxa *T. (S.) cruzi marinkellei* and *T. (H.) rangeli* are lacking and it is perhaps tentamount to defining biochemically these taxa.

Isoenzymic studies on *T. cruzi marinkellei* were initiated by Baker *et al.* (1978) but without comparing this organism with *T. cruzi* isoenzymic strains. With the 3 reference stocks used here (2 *T. rangeli*, 1 *T. cruzi marinkellei*), some enzymes allowed to distinguish these 2 taxa from all the *T. cruzi* isoenzymic strains (see table 5 and fig. 2) identified so far. It is important to use a large set of enzymes for obtaining easy and sure determinations as some differences are very slight and difficult to use for routine diagnosis. At this stage we prefer not to give any genetic interpretation of these comparisons. As a matter of fact, genetic distance calculations become less valuable when the organisms being compared have few relationships (Ayala, 1975) : in this case « common » alleles may represent in reality different proteins sharing the same electrophoretic mobility. *T. (S.) cruzi marinkellei* compared to *T. cruzi cruzi* expressed only one 6PG « common » allele (shared with *T. cruzi cruzi* isoenzymic strains 2, 2a and 2c) and one G6P « common » allele (shared with strain 3). Such a relationship would correspond to a genetic distance higher than 2.5 between the two organisms.

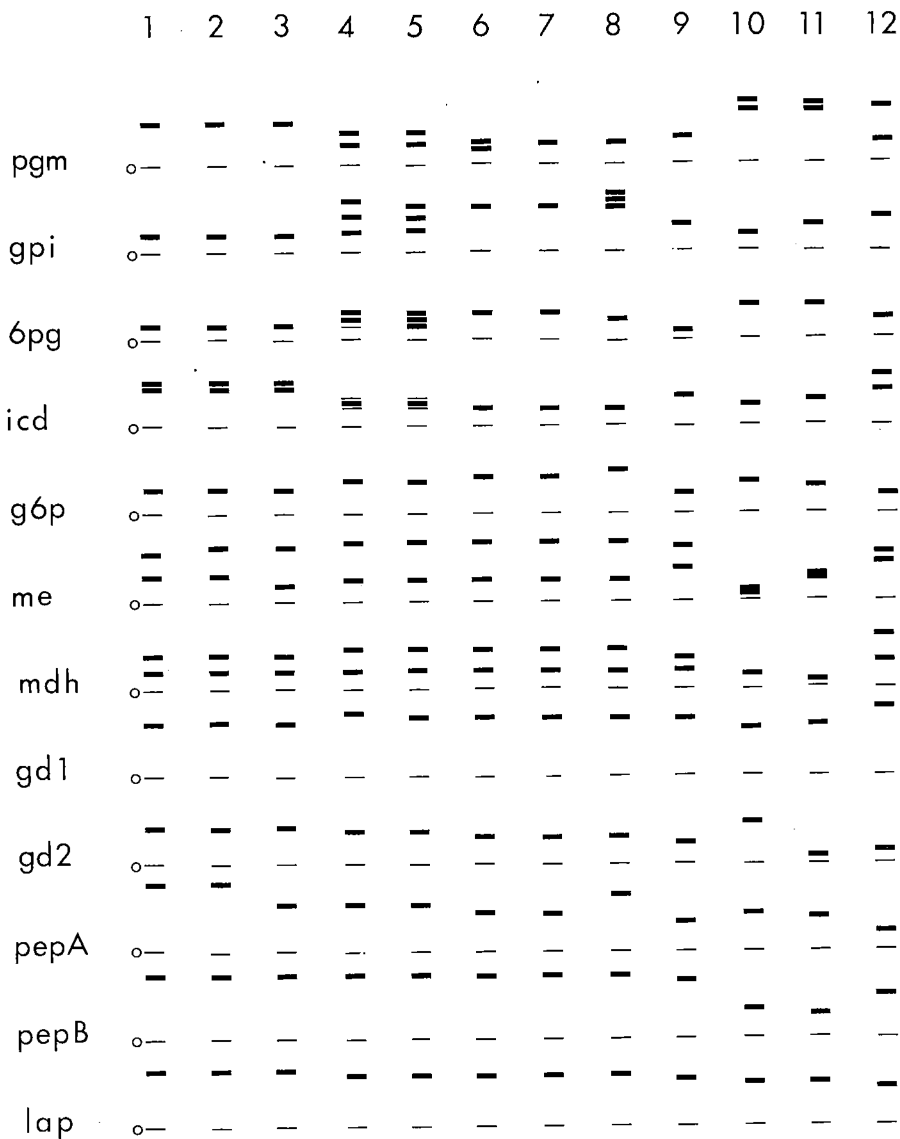


Figure 2.

Isoenzymic patterns. From 1 to 9 : *T. cruzi* isoenzymic strains
(1 = IS 1; 2 = 1 e; 3 = 1 f; 4 = 2; 5 = 2 a; 6 = 2 c; 7 = 2 d; 8 = 2 e; 9 = 3);
10 = *T. rangeli* from dog; 11 = *T. rangeli* from monkey; 12 = *T. cruzi marinkellei*;
o = origin of migration.

TABLE 5

Enzymes discriminating *Trypanosoma (S.) cruzi marinkellei* **and** *T. (H.) rangeli* **from the whole range of** *T. (S.) cruzi cruzi* **isoenzymic strains**

(1)	(2)
GD Nad +	GPI
GD Nadp +	ICD
LAP	PEP A
MDH	
ME	
PEP B	
PGM	

(1) = used for both *T. cruzi marinkellei* and *T. rangeli*.
(2) = used for *T. cruzi marinkellei* only (see also figure 2, isoenzymic patterns).

The differences between the two *T. (H.) rangeli* stocks are high. On 12 loci revealed, only 6PG and LAP showed common alleles. Most of the loci showed differences which are slight, but genetic distance calculations take in account only differences or identities, without considering distances between alleles. The genetic distance obtained between 2 *T. (H.) rangeli* stocks would be 1.79, which is comparable to the values observed between the main *T. (S.) cruzi* strains. The enzymic variability of *T. (H.) rangeli* seems to be high, and it is indispensable to perform further genetic studies on *T. (H.) rangeli* and *T. (S.) cruzi marinkellei*, and look at more isolates in order to obtain more reliable comparisons with *T. cruzi cruzi*.

Acknowledgements — *We are indebted to Dr M. A. Miles (London School of Hygiene and Tropical Medicine), who kindly provided clones of zymodemes I, II and III, and to Dr Fernando Plata (Institut Pasteur, Paris) for clones of Y and CL strains. We thank Dr A. Tait (Edinburgh) for discussion and advice. This study was carried out with the support of the French Technical Cooperation and of the Ministère de l'Industrie et de la Recherche (prolongation of support nº PVD/81/L-1423).*

Classification générale des souches isoenzymatiques de *Trypanosoma (Schizotrypanum) cruzi* **et comparaison avec** *T. (S.) c. marinkellei* **et** *T. (Herpetosoma) rangeli.*

Résumé — Treize stocks de référence de *Trypanosoma (Schizotrypanum) cruzi cruzi* ont été classés suivant leur distance génétique calculée sur la base de 14 loci. Trois souches isoenzymatiques principales ont été reconnues, de même que 6 souches mineures. Leurs profils correspondent à nos essais de classification antérieurs. Ce fait semble indiquer qu'à présent la plus grande partie des variants génétiques de *T. cruzi* a été identifiée. Ceci devrait permettre désormais de déterminer de façon sûre n'importe quel stock de *T. cruzi*, et de situer la position d'organismes de type *cruzi* vis-à-vis du complexe *cruzi*.

Après comparaison avec des stocks de référence de *T. (S.) cruzi marinkellei* et de *T. (H.) rangeli*, nous avons pu distinguer ces deux taxons de la série complète des souches isoenzymatiques de *T. cruzi*.

Algemene klassifikatie van de isoenzyme stammen van *Trypanosoma (Schizotrypanum) cruzi* **en vergelijking met** *T. (S.) c. marinkellei* **en** *T. (Herpetosoma) rangeli.*

Samenvatting — Dertien laboratorium referentiestocks van *Trypanosoma (Schizotrypanum) cruzi* werden geklassifieerd volgens genetische afstand b.m.v. enzymbepaling van 14 loci. Drie uitgesproken isoenzyme stammen werden waargenomen, samen met 6 van minder betekenis. Hun profiel kadert binnen onze vorige klassifikatie, hetgeen erop wijst dat momenteel het grootste deel van de genetische varianen van *T. cruzi* werd geïdentificeerd. Zulks zou voortaan moeten toelaten van om het even welke stock van *T. cruzi* met zekerheid te determineren, en van na te gaan of organismen van het *cruzi* type tot het *cruzi* complex behoren.

T. (S.) cruzi marinkellei en *T. (H.) rangeli* referentiestocks werden eveneens vergeleken. Deze twee taxa bleken te verschillen van de *T. cruzi* stammen, doch daartoe diende een volledige serie isoenzyme stammen te worden getest.

Received for publication on March 16, 1984.

REFERENCES

Ayala, F. J. (1975) : Genetic differentiation during the speciation process. Evolution. Biol., **8**, 1-78.

Baker, J. R., Miles, M. A., Godfrey, D. G. & Barrett, T. V. (1978) : Biochemical classification of some species of *Trypanosoma* (*Schizotrypanum*) from bats (Microchiroptera). Am. J. Trop. Med. Hyg., **27**, 483-491.

Castellani, O., Ribeiro, L. V. & Fernandes J. F. (1967) : Differentiation of *Trypanosoma cruzi* in culture. J. Protozool., **14**, 447-451.

Dedet, J. P., Chippeaux, J. P., Goyot, P., Pajeot, F. X., Tibayrenc, M., Geoffroy, B. & Gosselin, H. (1984) : Les hôtes naturels de la maladie de Chagas en Guyane française. Endémicité élevée de *Trypanosoma cruzi* zymodème 1 chez les marsupiaux sauvages. Ann. Parasit., sous presse.

Godfrey, D. G. & Kilgour, V. (1976) : Enzyme electrophoresis in characterizing the causative agent of Gambian Trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg., **71**, 217-225.

Lanar, D. E., Levy, L. S. & Manning, J. E. (1981) : Complexity and content of the DNA and RNA in *Trypanosoma cruzi*. Mol. Biochem. Parasitol., **3**, 327-341.

Lanham, S. M., Grendon, J. M., Miles, M. A., Povoa, M. & de Souza, A. A. (1981) : A comparison of electrophoretic methods for isoenzyme characterization of Trypanosomatids. I. Standard stocks of *Trypanosoma cruzi* zymodemes from northeast Brazil. Trans. R. Trop. Med. Hyg., **75**, 742-750.

Miles, M. A., Lanham, S. M., de Souza, A. A. & Povoa, M. (1980) : Further enzymic characters of *Trypanosoma cruzi* and their evaluation for strain identification. Trans. R. Soc. Trop. Med. Hyg., **74**, 221-237.

Miles, M. A., de Souza, A. A., Povoa, M., Shaw, J. F., Lainson, R. & Toyé, P. J. (1978) : Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochtonous patients with Chagas's disease in Amazonian Brazil. Nature, **272**, 819-821.

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. Trans. R. Soc. Trop. Med. Hyg., **71**, 217-225.

Nei, M. (1972) : Genetic distance between populations. Am. Natural., **106**, 283-292.

Ready, P. D. & Miles, M. A. (1980) : Delimitation of *Trypanosoma cruzi* zymodemes by numerical taxonomy. Trans. R. Soc. Trop. Med. Hyg., **74**, 238-242.

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

Tait, A. (1980) : Evidence for mating and diploidy in Trypanosomes. Nature, **287**, 536-537.

Tibayrenc, M. (1980) : Application of the calculations of genetic distances for flagellate systematics. Cah. ORSTOM sér. Ent. méd. & Parasitol, **18**, 301-302.

Tibayrenc, M., Cariou, M. L. & Solignac, M. (1981a) : Interprétation génétique des zymogrammes de flagellés des genres *Trypanosoma* et *Leishmania*. C. R. Acad. Sci. 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 taxinomiques. C. R. Acad. Sci. Paris, **293**, 207-209.

Tibayrenc, M., & Desjeux, P. (1983) : The presence in Bolivia of two distinct zymodemes of *Trypanosoma cruzi*, circulating sympatrically in a domestic transmission cycle. Trans. R. Soc. Trop. Med. Hyg., **77**, 73-75.

Tibayrenc, M., Echalar, L., Brénière, 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 taxonomique et immunologique des différentes isoenzymes. C. R. Acad. Sci. Paris, **296**, 721-726.

Tibayrenc, M., Echalar, L., Dujardin, J. P., Poch, O. & Desjeux, P. (1984a) : The microdistribution of isoenzymic strains of *Trypanosoma cruzi* in Southern Bolivia; new isoenzyme profiles and further arguments against Mendelian sexuality. Trans. R. Soc. Trop. Med. Hyg., sous presse.

Tibayrenc, M., Le Ray, D., Solignac, M., Cariou, M. L. & Desjeux, P. (1984b) : Les souches isoenzymatiques de *Trypanosoma cruzi* : origine récente ou ancienne, homogène ou hétérogène ? C. R. Acad. Sci. Paris, sous presse.

Tibayrenc, M. & Miles, M. A. (1983) : A genetic comparison between Brazilian and Bolivian zymodemes of *Trypanosoma cruzi*. Trans. R. Soc. Trop. Med. Hyg., **77**, 76-83.

Toyé, P. J. (1974) : Isoenzyme variation in isolates of *Trypanosoma cruzi*. Trans. R. Soc. Trop. Med. Hyg., **68**, 147.

Wilson, A. C., Carlson, S. S. & White, T. J. (1977) : Biochemical Evolution. Ann. Rev. Biochem., **46**, 573-639.