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A genetic comparison between Brazilian and Bolivian zymodemes of *Trypanosoma cruzi*

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Summary

The enzyme profiles of three major Brazilian *Trypanosoma cruzi* zymodemes (Brazilian Z1, Brazilian Z2, Brazilian Z3) and of two principal Bolivian zymodemes were compared. Relationships were assessed both intuitively and by calculating genetic distances. One of the Bolivian zymodemes, Bolivian Z1, was closely related to the Brazilian Z1. The second Bolivian zymodeme, Bolivian Z2, was related to Brazilian Z2 but differed from all the Brazilian zymodemes in the occurrence of typical heterozygous isozyme patterns in five out of 12 enzyme loci. Parental stocks and clones of Bolivian Z2 had the same putative heterozygous patterns. The evidence from enzyme profiles on the ploidy of *T. cruzi* and the possibility of recombination was considered. The presence of putative heterozygous patterns in Bolivian Z2 supported the hypothesis that *T. cruzi* is diploid. The definition of *T. cruzi* as a single polytypic species or as a species complex was considered to be dependent on the presence or absence of genetic recombination between or within the zymodemes, which has not been demonstrated in the ecotopes so far examined.

Introduction

Enzymic heterogeneity of *Trypanosoma cruzi* was first demonstrated by MILES *et al.* (1977, 1978), who defined three principal Brazilian zymodemes. Numerical taxonomy was used to confirm that these three zymodemes were strictly delimited (READY & MILES, 1980). Two of the three zymodemes were subsequently shown to occur in Venezuela (MILES *et al.*, 1981a).

TIBAYRENC *et al.* (1981a and b) found two distinct Bolivian zymodemes of *T. cruzi*, and, on the basis of a tentative genetic analysis, proposed that *T. cruzi* was diploid but not reproducing sexually, at least in the Bolivian ecotopes examined. In this study we compare Brazilian and Bolivian *T. cruzi* strains by means of enzyme electrophoresis, seek further information on the ploidy of *T. cruzi*, and assess the taxonomic relationships between the *T. cruzi* zymodemes.

Materials and Methods

The *T. cruzi* stocks used in this comparative study of Brazilian and Bolivian zymodemes are summarized in Table I.

Stocks representing Brazilian zymodemes were clones. Further details of the origin of stock X10, representing Brazilian zymodeme (Z) 1, are given in SILVEIRA *et al.* (1979). X10 clone 1 was produced (by M.A.M.) at the

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Table I—*Trypanosoma cruzi* stocks examined

Stocks	Locality	Original Host
Brazilian		
X10 clone 1 (Brazilian Z1)	Belém, Pará State	Man
Esmeraldo clone 3 (Brazilian Z2)	São Felipe, Bahia State	Man
CAN III clone 1 (Brazilian Z3)	Belém, Pará State	Man
Bolivian		
C7 (Bolivian Z1)	Chiwisivi*	*Triatoma infestans*
C8 (Bolivian Z1)	Chiwisivi	*Triatoma infestans*
C8 clone 1		
133-79	Santa Cruz**	Man
0026-79	Santa Cruz	*Triatoma sordida*
C50 (Bolivian Z2)	Chiwisivi	*Triatoma infestans*
92-80 (Bolivian Z2)	Santa Cruz	Man
92-80 clone 1		
92-80 clone 2		
SC43 (Bolivian Z2)	Santa Cruz	*Triatoma infestans*
SC43 clone 1		
SC43 clone 2		

*60 km south of La Paz
**500 km south-east of La Paz

Table II—Occurrence of allozymes in Brazilian and Bolivian *Trypanosoma cruzi* zymodemes

		BRZ1	BRZ2	BRZ3	BOLZ1	133-79	0026-79	BOLZ2
ADH	1	0	0	0	0	0	1	0
	2	1	0	0	1	1	0	0
	3	0	1	1	0	0	0	1
MDH	1	1	1	1	1	1	1	1
ME	1	0	1	1	0	0	0	1
Locus A	2	1	0	0	1	1	1	0
ME	1	0	0	1	0	0	0	0
Locus B	2	1	0	0	1	1	1	0
	3	0	1	0	0	0	0	1
ICD	1	0·5	0	0	0·5	0·5	0·5	0
	2	0	0	1	0	0	0	0·5
	3	0·5	0	0	0·5	0·5	0·5	0
	4	0	1	0	0	0	0	0·5
6PGDH	1	0	1	0	0	0	0	0·5
	2	0	0	0	0	0	0	0·5
	3	1	0	0	1	1	1	0
	4	0	0	1	0	0	0	0
G6PD	1	0	1	0	0	0	0	0
	2	0	0	0	0	1	1	1
	3	1	0	0	1	0	0	0
	4	0	0	1	0	0	0	0
GD	1	1	1	1	1	1	1	1
HK	1	0	0	1	?	?	?	?
	2	1	0	0	?	?	?	?
	3	0	1	0	?	?	?	?
PK	1	1	1	1	1	1	1	1
PGM	1	1	0	0	1	1	1	0
	2	0	0	0	0	0	0	0·5
	3	0	0	1	0	0	0	0
	4	0	1	0	0	0	0	0·5
PEP	1	0	1	0	?	?	?	?
	2	1	0	0	?	?	?	?
	3	0	0	1	?	?	?	?
MPI	1	1	0	0	0	0	0	0·5
	2	0	1	0	1	0·5	1	0·5
	3	0	0	1	0	0	0	0
	4	0	0	0	0	0·5	0	0
GPI	1	0	0	0	0	0	0	0·5
	2	0	1	0	0	0	0	0
	3	0	0	1	0	0	0	0·5
	4	1	0	0	1	1	1	0

(a) Abbreviations: enzyme as stated in the text (Materials and Methods); stocks and clones, BRZ1 = Brazilian Z1, BOLZ1 = Bolivian Z1 etc.
(b) Allozymes numbered 1, 2, 3, 4 in order of decreasing anodal mobilities
(c) Allozyme frequency scored as 1·0 when a single isozyme was present in a given zymodeme, as 0·5 when isozyme patterns were double or more complex and as 0 when the allozyme was absent.

Instituto Evandro Chagas, by cloning directly from infected xenodiagnosis bug faeces into culture. A full account of the origins and characteristics of Esmeraldo[1] clone 3, representing Brazilian Z2 and CAN III clone 1, representing Brazilian Z3, is given elsewhere (MILES *et al.*, 1980; DVORAK *et al.*, 1980).

[1]Esmeraldo, the correct spelling of the name of the patient = Esmereldo of earlier publications.

The stock C7 was selected to represent one of the major Bolivian zymodemes, Bolivian Z1, and stocks 92-80 and C50 to represent the second Bolivian zymodeme, Bolivian Z2, from the 110 stocks originally examined, with four enzymes, by TIBAYRENC & DESJEUX (1983). The stocks 0026-79 and 133-79 were included as they exhibited some divergence from Bolivian Z1 (Table II).

To investigate the relationship between enzyme profiles of parental Bolivian stocks and clones derived from them, three

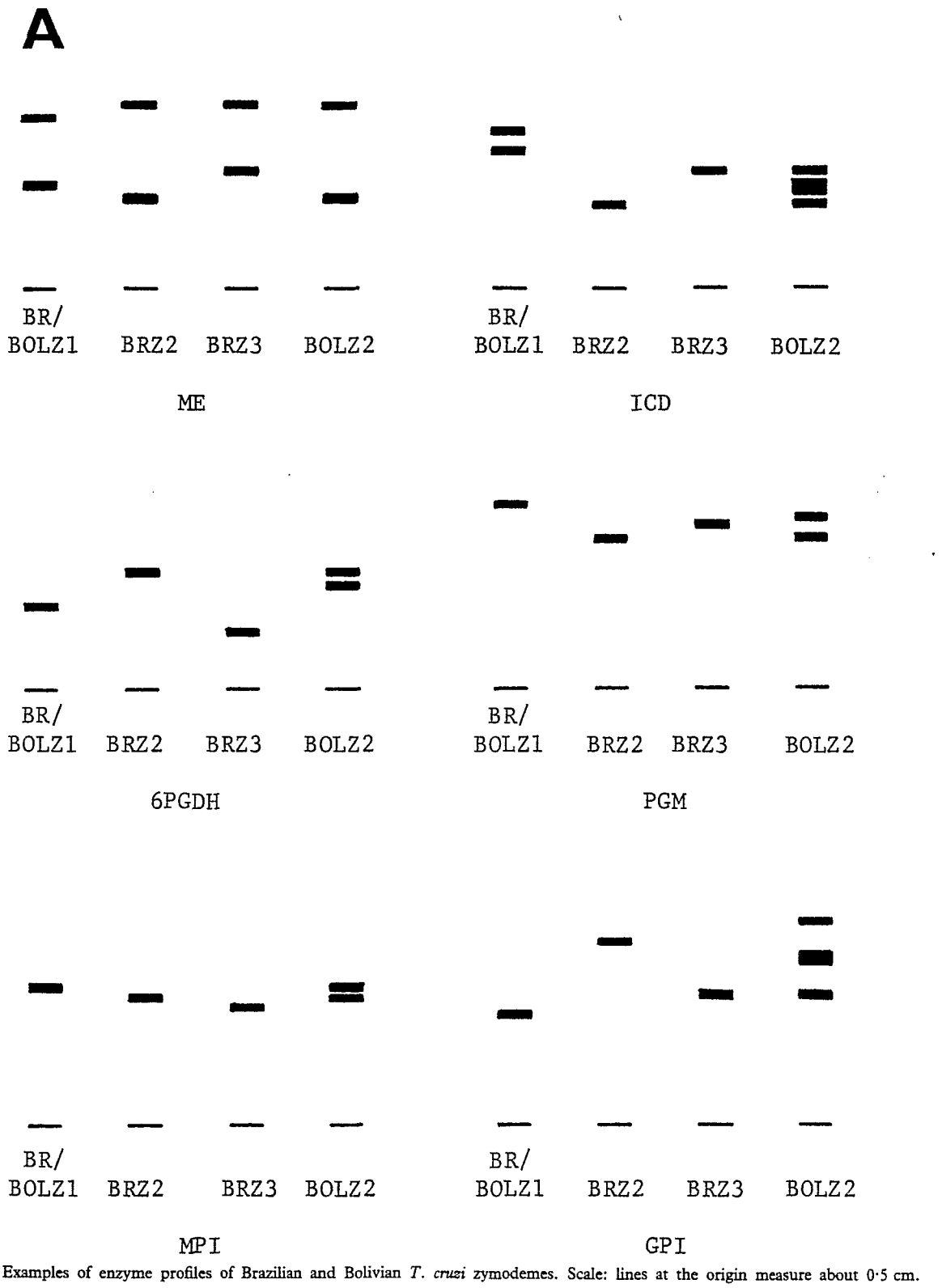

Fig. 1A. Examples of enzyme profiles of Brazilian and Bolivian *T. cruzi* zymodemes. Scale: lines at the origin measure about 0·5 cm.

Bolivian stocks were cloned from culture to culture (by M.A.M.) at the Instituto Evandro Chagas. One stock (C8) and one clone (C8 clone 1) of Bolivian Z1 and two stocks (92-80, SC43) and four clones (92-80 clones 1, 2, SC43 clones 1, 2) of Bolivian Z2 were chosen for examination from a total of nine Bolivian clones that were produced.

Brazilian stocks were grown initially in a biphasic blood-agar medium (MILES *et al.*, 1980) whilst Bolivian stocks were isolated and grown initially in GLSH liquid medium (TIBAYRENC & DESJEUX, 1983). All clones were produced in biphasic blood-agar medium (MILES *et al.*, 1980). Large volume cultures for the preparation of soluble

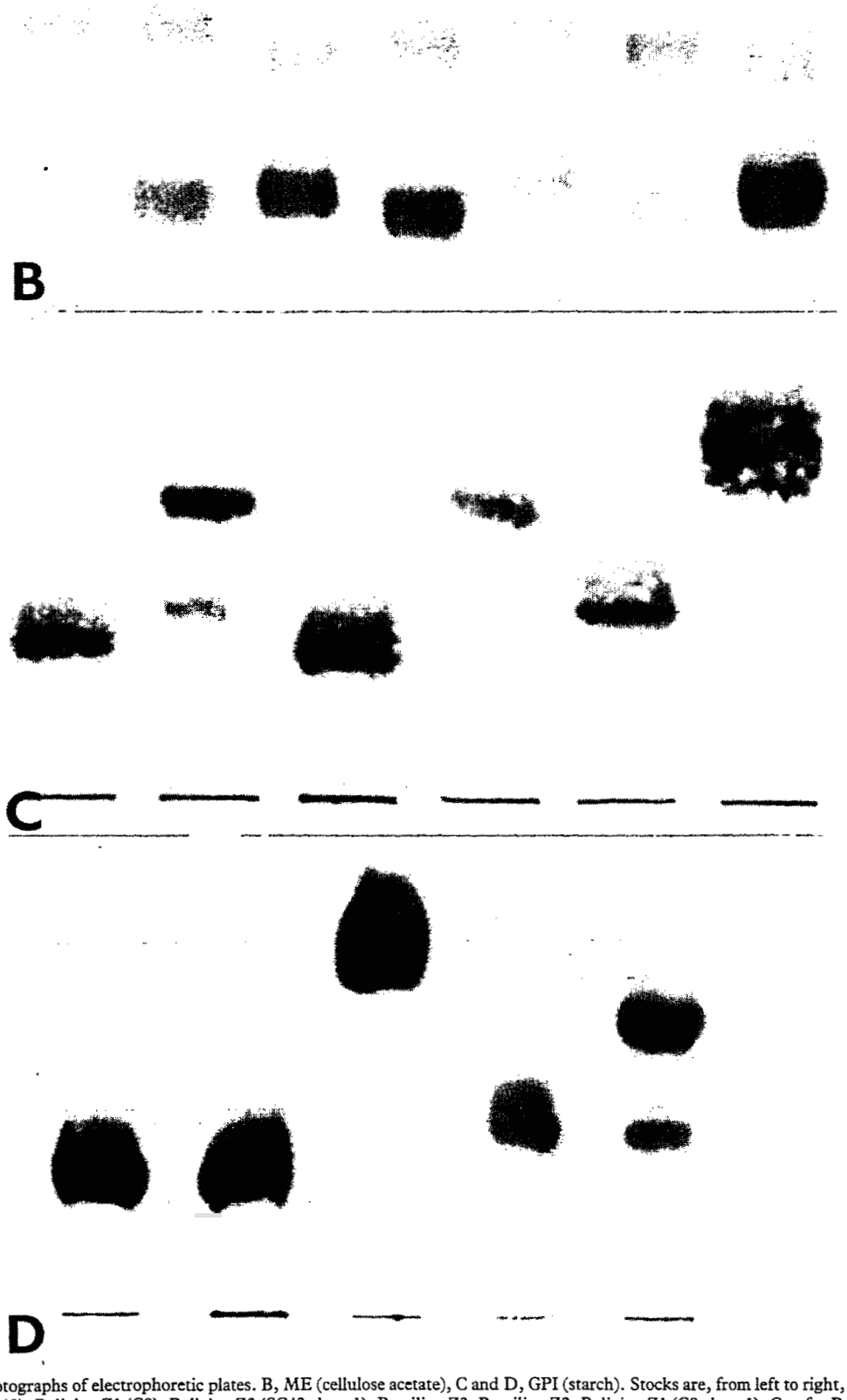

Fig. 1B-D. Photographs of electrophoretic plates. B, ME (cellulose acetate), C and D, GPI (starch). Stocks are, from left to right, B: Brazilian Z1, Bolivian Z2 (SC43), Bolivian Z1 (C8), Bolivian Z2 (SC43 clone 1), Brazilian Z3, Brazilian Z2, Bolivian Z1 (C8 clone 1); C as for B but omitting the last stock; D: Brazilian Z1, Bolivian Z1, Brazilian Z2, Brazilian Z3 and Bolivian Z2. There is clearly heterogeneity, on cellulose acetate electrophoresis, in the mobilities of the more anodic ME isozymes (Fig. 1B). The most consistent differences were between Bolivian Z1 and Bolivian Z2, Brazilian Z2, Brazilian Z3. The differences between Brazilian Z1 and Bolivian Z1 were not regarded as significant in this particular study (see Table II, ME Locus A). As illustrated in this Figure further work may demonstrate that there are significant differences in the mobilities of the more anodic ME isozymes of Brazilian Z1 and Bolivian Z1. Scale: lines at the origin measure about 0·5 cm.

enzymes were of modified Boné & Parent's liquid medium, including yeast extract but without serum supplement (MARTINI *et al.*, 1980). Stocks were harvested, lysed and soluble extracts were prepared by centrifugation as described by MILES *et al.* (1980). Supernatant aliquots, stored in liquid nitrogen as frozen droplets, were used for electrophoresis. Both starch-gel electrophoresis (SGE) and cellulose acetate electrophoresis (CAE) were employed. The following 17 enzymes were examined: alcohol dehydrogenase ($NADP^+$) (E.C.1.1.1.2, ADH); lactate dehydrogenase (E.C.1.1.1.27, LDH); malate dehydrogenase (E.C.1.1.1.37, MDH); malate dehydrogenase (oxaloacetate decarboxylating) ($NADP^+$) (E.C.1.1.1.40, ME); isocitrate dehydrogenase ($NADP^+$) (E.C.1.1.1.42, ICD); phosphogluconate dehydrogenase (E.C.1.1.1.44, 6PGDH); glucose 6-phosphate dehydrogenase (E.C.1.1.1.49, G6PD); glutamate dehydrogenase (E.C.1.4.1.2, GD); aspartate aminotransferase (E.C.2.6.1.1., ASAT); alanine aminotransferase (E.C.2.6.1.2., ALAT); hexokinase (E.C.2.7.1.1, HK); pyruvate kinase (E.C.2.7.1.40, PK); phosphoglycerate kinase (E.C.2.7.2.3, PGK); phosphoglucomutase (E.C.2.7.5.1, PGM); aminopeptidase (cytosol) (E.C.3.4.11.1, PEP); aconitate hydratase (E.C.4.2.1.3, ACON); mannosephosphate isomerase (E.C.5.3.1.8, MPI) and glucosephosphate isomerase (E.C.5.3.1.9, GPI). A full range of enzymes was used to compare stocks of Brazilian Z1, Z2 and Z3 with the Bolivian stocks C7, 92-80, C50, 0026-79 and 113-79. A selected group of polymorphic enzymes (ME, ICD, 6PGDH, G6PD, PGM, GPI) were used to compare the enzyme profiles of parental stocks and clones. The conditions for SGE were as described by MILES *et al.* (1980) and for CAE as given by LANHAM *et al.* (1981). The results with SGE and CAE were compared to assist interpretation and these patterns were further compared with those published by MILES *et al.*, (1980) for SGE, and LANHAM *et al.*, (1981) and TIBAYRENC *et al.* (1981a and b) for CAE.

Results

The results were analysed in three ways, firstly by making a visual comparison of the patterns observed in the representative stocks (intuitive analysis), secondly, by considering the evidence of the enzyme profiles on the enzyme subunit structures and the ploidy of *T. cruzi* and, thirdly by the tentative interpretation of the isozymes in terms of the alleles and loci determining them and the calculation of genetic distances between the zymodemes.

Examples of the enzyme patterns of the representative stocks are shown in Fig. 1 and the following features were apparent from intuitive analysis:

1. The same isozymes were seen in parental zymodeme stocks and in clones derived from them, excluding the possibility that multiple patterns were derived from mixed stocks. There was, however, some variability in the intensity of individual isozymes, amongst both parental stocks and clones. Examples of such variability are shown in Fig. 1 where the least anodic ME isozyme of Brazilian Z1 (Fig. 1B) and the most anodic GPI isozymes of Bolivian Z2 (Fig. 1D) are weak. Some of these differences probably reflect success in extracting isozymes, in the case of ME for example, where one isozyme is found in a particulate cell fraction and one in the cytosol (CANNATA *et al.*, 1979; CAZZULO *et al.*, 1980).
2. The three Brazilian zymodemes, Brazilian Z1, Brazilian Z2 and Brazilian Z3 were clearly defined, as reported previously (MILES *et al.*, 1980).
3. One of the principal Bolivian zymodemes, Bolivian Z1, represented by stock C7, was closely related to the Brazilian zymodeme Z1 (Table II).
4. The two Bolivian stocks 0026-79 and 133-79 were related to both Bolivian Z1 (C7) and the Brazilian zymodeme Z1 (Table II).
5. The second Bolivian zymodeme, Bolivian Z2, represented by stocks 92-80 and C50, differed from Brazilian Z1, Z2 and Z3. A particular feature of this zymodeme, as noted by TIBAYRENC *et al.* (1981a and b), was the presence of typical heterozygous patterns. Five out of 12 enzyme loci examined were apparently heterozygous, in the sense that two or more major isozymes were displayed (Table II, Fig. 1A). Furthermore, at least one component of all five heterozygous patterns was indistinguishable from single isozymes characteristic of either Brazilian Z1 (MPI), Brazilian Z2 (ICD, 6PGDH, PGM, MPI) or Brazilian Z3 (ICD, GPI). Thus the ICD pattern seen in Bolivian Z2 was the pattern expected in organisms heterozygous for alleles coding for the isozymes of Brazilian Z2 and Z3 (Fig. 1A). Three (6PGDH, PGM, MPI) of the five heterozygous patterns were double patterns with isozymes of approximately equal intensity and two (ICD, GPI) were triple with a central component of greater intensity than the two outer components. Double patterns implied that the enzymes 6PGDH, PGM and MPI are monomeric whilst for ICD and GPI three equivalent bands, with a central band of greater intensity, suggest two different alleles producing a dimeric enzyme with the two subunits combining at random.[1]

The tentative allocation of isozymes (allozymes) to alleles is given in Table II; the ploidy of the *T. cruzi* genome, genetic distances and taxonomic relationships between the zymodemes are discussed below.

Discussion

Evidence for diploidy

The heterozygous patterns of Bolivian Z2, particularly the approximately 1:2:1 distribution of intensity, in the enzymes GPI and ICD (Figs. 1A and 1C), which are known to be dimeric in man and other species (HOPKINSON *et al.*, 1976) suggest strongly that *T. cruzi* of Bolivian Z2 is not haploid but diploid or possibly tetraploid. By extrapolation it is probable that the organisms comprising the other *T. cruzi* zymodemes are also not haploid. Nevertheless it is still conceivable that all *T. cruzi* might be haploid, including Bolivian Z2, if the complex patterns are produced by duplications of loci on the haploid genome. This interpretation is improbable because it would require two mutational events. Firstly gene duplication and secondly mutation in one of the duplicated genes to produce a polypeptide coded by one locus that was electrophoretically distinguishable from the polypeptide coded by the other. For this to be the case every gene that determines the enzymes showing typical heterozygous patterns must have undergone gene duplication and subsequent point mutation.

A still more bizarre explanation could be proposed in which *T. cruzi* of Bolivian Z2, with heterozygous

[1]The two-banded ICD pattern of Brazilian Z1 and Bolivian Z1 (Fig. 1A) is difficult to reconcile with a dimeric ICD subunit structure. MILES *et al.* (1980) have pointed out however, that the enzyme shows a single band at lower voltages and the two bands seen, under the conditions used here, may reflect secondary modification.

patterns, is diploid and a product of the union of hypothetical haploid organisms. Bolivian Z2 would thus represent a heterozygote generated from two hemizygotes, all capable of prolonged independent mitotic division through many generations, alternating with periods of meiosis—such a phenomenon is known among the protozoa (SLEIGH, 1979). The respective positions of the isozymes, however, show that Bolivian Z2 cannot be a direct product of the other zymodemes encountered so far. Furthermore, if Bolivian Z2 is the product of the union of hemizygotes, to explain the consistent association of the heterozygous patterns (TIBAYRENC *et al.*, 1981b), subsequent meiosis must either occur without crossing over (e.g., one division meiosis), or the heterozygous enzymes must form a close linkage group, implying that *T. cruzi* has a very small number of chromosomes. This hypothesis is therefore unlikely to be correct, especially as SOLARI (1980) has circumstantial evidence of ten genetic units in the *T. cruzi* genome.

Finally one must allow that the complex patterns of Bolivian Z2 may not be genetically determined but occur as a result of secondary modification of the different enzymes. This is not a convincing explanation since the same, standardized procedure was used to prepare all *T. cruzi* stocks and the patterns, with the exception of minor variability in intensity, were consistently reproducible, with symmetrical two and three-banded patterns.

Origin of heterozygous enzyme patterns

The heterozygous patterns, such as those observed in Bolivian Z2, could have arisen simply by mutation of one allele at each locus of the enzymes concerned. The occurence of heterozygous patterns in five out of 12 enzymes however, without apparent intermediates having fewer heterozygous patterns, argues against this. Sharing of enzyme bands suggests that organisms such as those comprising Brazilian Z2 and Z3 may have contributed, through some sort of recombinational event, to the genetic constitution of the putatively heterozygous Bolivian Z2. There is no evidence in favour of gene exchange occurring currently, either between or within the *T. cruzi* zymodemes. This is in marked contrast to the situation with the African trypanosome, *T. brucei*, where heterozygous patterns also indicate diploidy but close agreement between allozyme frequencies and predicted Hardy-Weinberg equilibria suggest random mating (GIBSON *et al.*, 1980; TAIT, 1980). Our results do not preclude the occurrence of genetic recombination in *T. cruzi* elsewhere in South American ecotopes. Indeed, in view of the relatively recent development of endemic Chagas' disease in artificial ecotopes, by movement of vector and human populations, it is quite possible that sexual reproduction occurs in relic cycles of transmission. There is no evidence of this from the Amazon basin (MILES *et al.*, 1981b) but vast endemic regions involving *Triatoma infestans*, the vector with which heterozygous *Trypanosoma cruzi* patterns are associated here, remain to be studied.

Whether hererozygous patterns have arisen by mutation or a recombinational event the fixed nature of the heterozygosity can be explained by the isolation and expansion of a few clonal populations, the so-called founder effect, and/or the selection, through environmental bottlenecks, of those strains with more favourable isozyme constitutions. The founder effect is certainly likely to operate in a domestically transmitted disease where individual triatomine bugs or infected mammals may introduce *T. cruzi* to new dwellings. An alternative explanation of the consistent association of heterozygous patterns, is that Bolivian Z2 is a tetraploid hybrid strain, derived from two as yet unrecognized diploid parental zymodemes. If the diploid parents carried different alleles the tetraploid would exhibit heterozygosity, genetically isolated, or fixed, by failure of chromosome pairing during meiosis.

In summary we conclude that, in the ecotopes examined there is no genetic exchange between the two Bolivian zymodemes, the organisms comprising Bolivian Z2, which exhibits the most complex isozyme patterns, are probably diploid (possibly tetraploid) and do not undergo genetic exchange within the zymodeme.

Accepting that all the zymodemes are diploid but that there is as yet no evidence of genetic recombination, we may make a tentative genetic interpretation of the observed enzyme patterns, and use this as a basis for calculations of genetic distance.

Genetic distance and taxonomy

Numerical taxonomic methods have demonstrated that the three Brazilian *T. cruzi* zymodemes are clearly defined and widely separated by enzyme characters (READY & MILES, 1980). A simple method has been proposed by NEI (1972) to estimate relationships on the basis of electrophoretic data : his formula for "standard genetic distance" is $DS = -\log_e I$ where I gives the mean degree of genetic identity and D is a measure of the number of codon differences per gene between two populations. This index can apparently be used as an approximate "molecular clock" to measure rate of protein evolution at least over long periods of time and with a given class of proteins, independently of frequency of generations (WILSON *et al.*, 1977) and of speciation rate (AVISE & AYALA, 1975). Of several formulae proposed, Nei's for genetic distance seems to correlate

	BRZ1	BRZ2	BRZ3	BOLZ1	133-79	0026-79	BOLZ2
BRZ1	0						
BRZ2	1·36	0					
BRZ3	1·36	0·88	0				
BOLZ1	0·09	1·08	1·36	0			
133-79	0·17	1·20	1·35	0·12	0		
0026-79	0·30	1·08	1·36	0·20	0·12	0	
BOLZ2	1·09	0·29	0·58	1·09	0·89	0·84	0

Fig. 2. Genetic distances estimated with 12 loci.

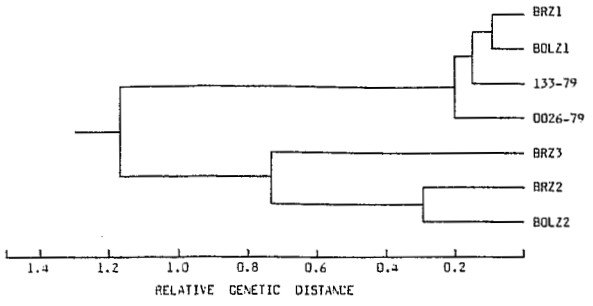

Fig. 3. Dendrogram based on genetic distances in Fig. 2. Constructed according to the method of NEI (1975) only closest values are exact, others are mean values, which gives slight deformation.

best with time (CHAKRABORTY & TATENO, 1976) although it is calculated ideally from a large number of loci (GORMAN & RENZI, 1979). Most studies of genetic distances concern sexual organisms but they can also be used for organisms where mode of reproduction might be asexual; TIBAYRENC (1980) has proposed their use in flagellate systematics. The genetic distances, calculated according to Nei, from our electrophoretic data, for representative stocks of the *T. cruzi* zymodemes, are shown in Fig. 2. A dendrogram, prepared from these values is shown in Fig. 3; such dendrograms do not necessarily reflect accurately evolutionary history (THROCKMORTON, 1977).

Based on the 12 loci in Table II some of the calculated genetic distances are extremely high, for example 1·36 between Brazilian Z1 and Brazilian Z2 and Brazilian Z1 and Brazilian Z3 and 0·88 between Brazilian Z2 and Brazilian Z3. These values rise to 1·52 and 1·03 respectively using data for the 14 loci available for the Brazilian zymodemes. These estimates may be considered minimal as we did not include ASAT and ALAT although these enzymes have been shown to distinguish the three Brazilian zymodemes (MILES *et al.*, 1980), and because classical methods for electrophoresis, used here, are considered to underestimate the degree of variability (COYNE, 1976).

It is interesting to compare the genetic distances between the *T. cruzi* zymodemes with those of other organisms. Genetic distances between taxa generally increases with the rank of the taxa being compared and between distinct species values are usually less than 1·0. FERGUSON (1980) gives a mean genetic distance of 0·645 for examples of invertebrate, vertebrate and plant species. The values observed for *T. cruzi* zymodemes are thus comparable to those recorded for well differentiated species, for example *Drosophila* spp. 1·04 and 1·3 (ZIMMERMAN *et al.*, 1978; CRONIN *et al.*, 1981), and exceed most values for subspecies, for example, *Drosophila* subspp. 0·255 (ZIMMERMAN *et al.*, 1978); *Gorilla* subspp. 0·75 (CRONIN *et al.*, 1981)—but the latter high value for *Gorilla* subspp. was based on specifically selected, rapidly evolving loci. In addition, although MILES *et al.* (1981c) did not calculate genetic distances, the level of genetic divergence between *Leishmania mexicana* and *L. braziliensis* appears similar to that between *T. cruzi* zymodemes. This does not prove that *T. cruzi* zymodemes represent distinct species, only that genetic divergence between the zymodemes is very high. If *T. cruzi* should prove to be a sexual organism the problem of definition of zymodemes as species hinges on the presence or absence of genetic exchange between them. If significant genetic exchange does not occur between zymodemes, but only within them, it would be a sound basis to treat *T. cruzi* as a complex of species. If significant recombination between zymodemes does occur it will demonstrate that *T. cruzi* is a single polytypic species, but, if this is so, the high values for genetic distance will be difficult to explain. At present a pragmatic approach is advisable, considering the zymodemes as different entities in epidemiological, clinical and immunological studies. In populations of *T. brucei* undergoing random mating, where zymodemes, defined by single rather than sets of isozyme differences (WHO, 1978; MILES *et al.*, 1981b), may correspond with the level of individual variation, the treatment of all zymodemes as separate entities is questionable and the zymodeme concept requires re-evaluation (MILES, in press).

The very close relationship between the major Bolivian zymodeme, Bolivian Z1, represented by stock C7, and the Brazilian Z1 is obvious both intuitively and from the genetic distance calculations (Figs. 1, 2, 3). Stocks 0026-79 and 133-79 are also clearly related to both Brazilian Z1 and Bolivian Z1 (Figs. 2, 3). The second principal Bolivian zymodeme, Bolivian Z2, is most closely related to Brazilian Z2 (Figs. 1, 2, 3). A more precise understanding of the relationships between the Brazilian and Bolivian zymodemes is not possible without additional epidemiological and experimental data.

This study provides further evidence on the haploid/diploid status of the *T. cruzi* genome and reveals an example of apparently fixed heterozygosity within the Bolivian Z2. A continuation of this analysis, perhaps using simpler electrophoretic methods (LANHAM *et al.*, 1981) will determine whether *T. cruzi* is a sexual organism, may clarify the adaptive significance, if any, of the heterozygosity and, in conjunction with clinical studies and antigenic characterization of zymodemes, allow re-evaluation of the circumstantial evidence (MILES *et al.*, 1981a), which link the infecting zymodeme and the form of Chagas' disease in man.

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