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An Isoenzyme Survey of *Trypanosoma cruzi* Genetic Variability in Sylvatic Cycles from French Guiana

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LEWICKA, K., BRENIERE-CAMPANA, S. F., BARNABE, C., DEDET, J-P., AND TIBAYRENC, M. 1995. An isoenzyme survey of *Trypanosoma cruzi* genetic variability in sylvatic cycles from French Guiana. *Experimental Parasitology* **81**, 20–28. Twenty-seven trypanosomatidae stocks isolated from various hosts in French Guiana have been surveyed by Multilocus Enzyme Electrophoresis on cellulose acetate plates. The variability observed at 22 different enzyme systems was considerable, since 21 different enzyme profiles (zymodemes) could be distinguished. Clustering analysis and comparison with four laboratory reference stocks showed clearly that three stocks were distantly related from the rest and most probably cannot be included in the species *Trypanosoma cruzi*. All the other stocks were more related to the formerly described zymodeme I than to the formerly described zymodemes II and III. Genotype variability in this *T. cruzi* sylvatic population was notably higher than in domestic populations of the same parasite. This could suggest more frequent genetic exchange occurring in sylvatic cycles. Nevertheless, a population genetic analysis of the data showed a considerable linkage disequilibrium, which rather favors the hypothesis that *T. cruzi* has a basically clonal population structure in this ecosystem too.

INDEX DESCRIPTORS: Clonal structure; linkage disequilibrium; zymodeme I; *Didelphis marsupialis*; reservoir.

INTRODUCTION

The isozyme variability of *Trypanosoma cruzi*, the agent of Chagas' disease, has been widely explored (Miles *et al.* 1978; Romanha *et al.* 1979; Tibayrenc *et al.* 1981, 1986). Nevertheless, by comparison with the domestic cycles of Chagas' disease, apart from the works by Miles *et al.* (1981) in Brazil and by Saravia *et al.* (1987) in Colombia, relatively little is known about the genetic variability of *T. cruzi* strains isolated from wild cycles. The matter is of importance for two reasons: (i) Basic biology of *T. cruzi:* this parasite's population structure is believed to be basically clonal (Tibayrenc *et al.* 1981, 1986; Tibayrenc and Ayala 1988). Nevertheless, this clonal model does not rule out the possibility of occasional mating, which could be more frequent in the primitive habitat of the parasite (sylvatic cycles). (ii) An epidemiological point of view: sylvatic cycles, especially those from the Amazon basin, constitute a potential reservoir of reinfestation of anthropized areas that have benefited from control programs. It is hence important to have information about the specific genetic variability of wild *T. cruzi* strains, in order to perform a follow-up of the spread of these strains.

The present work addresses these questions by considering the isozyme variability and population genetics of *T. cruzi* in an Amazonian ecosystem, namely the rain forest of French Guiana.

MATERIALS AND METHODS

Origins of stocks. Table I summarizes the times of isolation, places, and hosts of the 31 stocks (27 freshly isolated ones + 4 reference stocks) under study. The 4 reference stocks have been selected in order to show a representative picture of *T. cruzi*'s overall genetic variability.

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TABLE I
Origins of the 31 *Trypanosoma cruzi* Stocks under Study and Zymodeme Assignation

Stock	Host	Geographic origin	Date of isolation	Zymodeme
A0055	*Philander opossum*	FRG	1982	1
A0080	*Didelphis marsupialis*	FRG	1982	1
A0083	*Didelphis marsupialis*	FRG	1982	2
A0087	*Didelphis marsupialis*	FRG	1982	2
A0092	*Didelphis marsupialis*	Acarouany	1982	1
A0096	*Didelphis marsupialis*	Cacao	1983	3
A0098	*Didelphis marsupialis*	Cacao	1983	4
A0099	*Didelphis marsupialis*	Cacao	1983	5
A0102	*Didelphis marsupialis*	Cacao	1983	6
A0105	*Didelphis marsupialis*	Cacao	1983	7
A0117	*Didelphis marsupialis*	FRG	1983	8
A0147	*Philander opossum*	Nancibo	1984	9
A0262	*Didelphis marsupialis*	FRG	1986	10
A0268	*Philander opossum*	Cacao	1986	11
A0269	*Philander opossum*	Cacao	1986	12
A0276	*Saimiri sciureus*	Cacao	1986	13
A0286	*Didelphis marsupialis*	Cacao	1987	14
A0290	*Didelphis marsupialis*	Cacao	1987	15
R0107	*Rhodnius prolixus*	Montjoly	1983	5
R0112	*Rhodnius prolixus*	Montjoly	1983	16
R0143	*Panstrongylus geniculatus*	Kaw	1984	17
R0145	*Panstrongylus geniculatus*	FRG	1984	18
R0150	*Rhodnius pictipes*	Cayenne	1984	18
R0173	*Rhodnius pictipes*	St George	1984	19
R0174	*Panstrongylus geniculatus*	Montjoly	1984	20
R0177	*Panstrongylus geniculatus*	Montjoly	1984	20
R0203	*P. rufotuberculatus*	Regina	1985	21
CanIII cl1Z3[a]	Human	Belém (Brazil)	?	27[b]
SC43 c12[a]	*Triatoma infestans*	Santa Cruz (Bolivia)	1981	39[b]
Teh cl1[a]	*Triatominae*	Mexico	?	12[b]
Tula FKIIA[a]	Human	Chili	?	43[b]

Note. A0055 to R0203, Guianese stocks.
[a] Reference stocks.
[b] As in Tibayrenc and Ayala, 1988.

Parasite extracts. Parasites were bulk-cultured in LIT liquid culture medium and harvested by centrifugation. Pellets were mixed with an equal volume of enzyme stabilizer (Godfrey and Kilgour 1976) and set on an ice bed for 20 min. They were then centrifuged again in an Eppendorf microfuge at 11,000*g* at 4°C for 10 min. Supernatants were divided into 20-μl aliquots and stored at −70°C for further multilocus enzyme electrophoresis (MLEE) analysis, while the pellets of broken cells were kept at −70°C for subsequent DNA analyses.

MLEE protocols. Protocols were performed according to Ben Abderrazak *et al.* (1993) with slight modifications. The Helena system was used (cellulose acetate electrophoresis). Eighteen different enzyme systems were surveyed, namely, aconitase (ACON, EC 4.2.1.3), alanine aminotransferase (ALAT, EC 2.6.1.2), diaphorase (DIA, EC 1.6.*.*.), esterase (EST, EC 3.1.1.1), glyceraldehyde 3-phosphate dehydrogenase (GAPD, EC 1.2.1.12), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glucose phosphate isomerase (GPI, EC 5.3.1.9), hexokinase (HK, EC 2.7.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), leucine aminopeptidase (LAP, EC 3.4.11. or 13.*), malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), mannose phosphate isomerase (MPI, EC 5.3.1.8), peptidases (substrates: L-leucyl-leucine–leucine and L-leucyl-L-alanine) (PEP, EC 3.4.11 or 13.*), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglucomutase (PGM, EC 2.7.5.1), and superoxide dismutase (SOD, EC 1.15.1.1.).

Estimation of overall genetic diversity. Observed heterozygosity, *Ho*, was estimated after the following formula: $Ho = ho / (N \times r)$, where *ho* is the total number of heterozygous genotypes in the sample, *N* is the number of isolates, and *r* is the number of assayed loci. Expected heterozygosity *H* was estimated after the formula

$$H = \sum_{1}^{r} h/r \quad \text{and} \quad h = 1 - \sum_{1}^{i} q_i^2,$$

where *h* is the expected heterozygosity at each locus, q_i is the frequency of the *i*th allele at a given locus, and *r* is the number of loci under study (see Pasteur 1985).

Estimation of phylogenetic divergence and clustering procedure. Jaccard's distance (Jaccard 1908) was used. It is estimated as follows: $D_j = 1 - (C / 2N - C)$, where *C* is the number of common bands between the two genotypes to be compared and *N* is the total number of observed bands in the two compared genotypes. Jaccard's distance ranges 0 to 1.

The UPGMA method (unweighted pair-group method with arithmetic averages) (Sokal & Sneath 1963) was used to cluster the zymodemes together according to their Jaccard distances. Dendrograms were computed by using the software kindly communicated by Serres and Roux (1986).

Population genetic analysis. Population structure was explored by various complementary statistics. The tests estimate departures from panmixia, according to either departure from Hardy–Weinberg expectations (segregation of alleles at given loci) or linkage disequilibrium (recombination of genotypes among loci). The tests have been detailed elsewhere (Tibayrenc *et al.* 1990, 1991), together with the possible biases brought by geographical distance and natural selection. Table II summarizes the tests that have been actually used in the present work.

RESULTS

Genetic and genotypic diversity. Among the 18 enzyme systems tested, 4 made it possible to infer the activity of two different enzyme loci; namely, ALAT, EST, GDH, and ME. The locus in which the zone of activity had the fastest elecrophoretic migration was coded "1," while the other one was coded "2" (ex.: *Alat-1* and *Alat-2*). For the GDH system, *Gdh-1* and *-2* are specifically stained by using the specific coenzymes *Nad* and *Nadp*, respectively. The 18 enzyme systems therefore made it possible to evidence the activity of 22 different genetic loci. The profiles generated by the PEP systems (two different substrates) have been plotted together as a unique locus since some specificity overlaps can occur between these two kinds of peptidases. Plotting them together does not constitute a bias, but only a loss of information. Conversely, scoring redundant data as separate loci is a major bias for population genetic analysis. All loci proved to be variable (polymorphism rate = 1.0).

TABLE II
Population Genetic Criteria of Clonal Reproduction (after Tibayrenc, Kjellberg, and Ayala, 1990)

Label	Description
	Segregation (single locus data)
a	Fixed heterozygosity
b	Absence of segregating genotypes
c	Deviation from Hardy–Weinberg equilibrium
	Recombination (multi-locus data)
d	Overrepresented, widespread identical genotypes. Statistical tests: combinatorial (d1), Monte Carlo (d2)
e	Absence of recombinant genotypes
f	Linkage desequilibrium
g	Correlation between independent sets of genetic markers

In some stocks, the activity of certain loci was not detectable despite several assays. Such missing data are quoted 0 in Table III. Stocks for which several loci were not readable include A276 (11 missing loci), A269 (7 missing loci), and A83 and A87 (5 missing loci).

Genetic variability was interpreted when possible in terms of alleles, according to the hypothesis that *T. cruzi* is a diploid organism (Lanar *et al.* 1981; Tibayrenc *et al.* 1981). Nevertheless, at four loci, namely, *Dia, Hk, Pep,* and *Sod,* the patterns were too complex to be interpreted in terms of alleles. In this case, each distinct and reproducible pattern was considered a distinct genotype, the precise allelic composition of which remained unknown.

In the 27 Guianese stocks, the 22 variable loci made it possible to individualize 21 different zymodemes. The genotype diversity is therefore: 21/27 = 0.78. The genetic composition of the 21 zymodemes is given in Table III. Several indices of general genetic variability were estimated. For those loci which could be read in terms of alleles, the average number of different alleles per locus was 4.41 for the whole Guianese sampling and three for the "true" *T. cruzi* (see below).

TABLE III

Multilocus Genotypes of the 31 *Trypanosoma cruzi* Stocks Identified with 22 Polymorphic Loci

	6Pgd	*Acon*	*Alat1*	*Alat2*	*Dia*	*Est2*	*G6pd*	*Gapd*	*Gdh1*	*Gdh2*	*Got*	*Gpi*	*Hk*	*Idh*	*Lap*	*Mdh*	*Me1*	*Me2*	*Mpi*	*Pep*	*Pgm*	*Sod*
A0055	*4/6*	*2/2*	*2/2*	*2/2*	5;8	*2/2*	*7/7*	*3/3*	*3/3*	*3/3*	4	*5/5*	2;6;9	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
A0080	*4/6*	*2/2*	*2/2*	*2/2*	5;8	*2/2*	*7/7*	*3/3*	*3/3*	*3/3*	4	*5/5*	2;6;9	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
A0083	*1/1*	*1/1*	*3/3*	*0*	1;2;5;11	*2/2*	*1/1*	*1/1*	*0*	*0*	0	*1/1*	0	*2/2*	*1/2*	*1/1*	*3/3*	*1/1*	*1/1*	1;4;9	*2/2*	1;2;3;4;5;6;9;12
A0087	*1/1*	*1/1*	*3/3*	*0*	1;2;5;11	*2/2*	*1/1*	*1/1*	*0*	*0*	0	*1/1*	0	*2/2*	*1/2*	*1/1*	*3/3*	*1/1*	*1/1*	1;4;9	*2/2*	1;2;3;4;5;6;9;12
A0092	*4/6*	*2/2*	*2/2*	*2/2*	5;8	*2/2*	*7/7*	*3/3*	*3/3*	*3/3*	4	*5/5*	2;6;9	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
A0096	*0*	*3/3*	*3/3*	*3/3*	5;7	*2/2*	*5/5*	*4/4*	*0*	*3/3*	4	*5/5*	2;5;8	*3/5*	*4/4*	*2/2*	*3/3*	*2/2*	*4/4*	2;8	*6/6*	7;8;9;12
A0098	*0*	*3/3*	*3/3*	*3/3*	5;7	*2/2*	*5/5*	*2/3*	*0*	*3/3*	4	*5/5*	2;5;8	*3/3*	*4/4*	*2/2*	*2/6*	*2/2*	*3/3*	2;6	*6/6*	7;8;9;12
A0099	*6/6*	*3/3*	*2/2*	*3/3*	5;8	*3/3*	*6/6*	*4/4*	*3/3*	*3/7*	1;4	*5/5*	1;4;7	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
A0102	*6/6*	*3/3*	*2/2*	*3/3*	5;8	*2/2*	*7/7*	*4/4*	*3/3*	*3/3*	1;5	*6/6*	1;4;7	*3/3*	*3/3*	*2/2*	*2/2*	*2/2*	*3/3*	2;8	*5/5*	7;8;9;11;12
A0105	*6/6*	*3/3*	*2/2*	*2/2*	5;8	*4/4*	*6/6*	*4/4*	*3/3*	*3/3*	1;4	*5/5*	2;6;9	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	4;7	*8/8*	6;7;8;9;12
A0117	*6/6*	*3/3*	*2/2*	*3/3*	5;9	*3/3*	*6/6*	*4/4*	*3/3*	*3/3*	1;4	*5/5*	2;5;8	*4/4*	*4/4*	*2/2*	*3/3*	*2/2*	*4/4*	4;7	*5/5*	7;8;9;12
A0147	*6/6*	*3/3*	*3/3*	*3/3*	5;9	*3/3*	*5/5*	*4/4*	*3/3*	*3/3*	1;4	*5/5*	2;5;8	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*4/4*	4;9	*6/6*	7;8;9;12
A0262	*0*	*2/2*	*2/2*	*2/2*	4;7	*2/2*	*5/5*	*4/4*	*0*	*1/1*	4	*5/5*	2;6;9	*4/4*	*3/3*	*2/2*	*1/1*	*1/1*	*2/2*	2	*4/4*	7;8;9;12
A0268	*0*	*3/3*	*2/2*	*2/2*	5;7	*2/2*	*7/7*	*4/4*	*0*	*3/3*	3	*5/5*	2;6;9	*4/4*	*3/3*	*2/2*	*1/1*	*1/1*	*3/3*	2;9	*3/3*	7;8;9;12
A0269	*0*	*0*	*2/2*	*2/2*	0	*2/2*	*0*	*4/4*	*0*	*3/3*	3	*5/5*	0	*4/4*	*3/3*	*2/2*	*1/1*	*1/1*	*3/3*	2;7	*0*	7;8;9;12
A0276	*0*	*0*	*0*	*1/1*	3	*5/5*	*2/2*	*0*	*0*	*3/3*	6	*0*	0	*0*	*8/8*	*3/3*	*0*	*2/2*	*0*	11;13	*0*	7;8;9;11;12
A0286	*0*	*2/2*	*2/2*	*3/3*	4;6	*2/2*	*7/7*	*4/4*	*0*	*2/2*	4	*5/5*	0	*4/4*	*4/4*	*2/2*	*1/1*	*1/1*	*3/3*	2;6;9	*4/4*	7;8;9;12
A0290	*0*	*2/2*	*2/2*	*3/3*	4;6	*3/3*	*7/7*	*4/4*	*0*	*2/2*	4	*5/5*	0	*4/4*	*4/4*	*2/2*	*1/5*	*1/1*	*3/3*	2;6	*3/3*	7;8;9;12
R0107	*6/6*	*3/3*	*2/2*	*3/3*	5;8	*3/3*	*6/6*	*4/4*	*3/3*	*3/7*	1;4	*5/5*	1;4;7	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
R0112	*6/6*	*3/3*	*2/2*	*3/3*	5;8	*3/3*	*7/7*	*4/4*	*3/3*	*4/8*	1;4	*5/5*	1;4;7	*4/4*	*4/4*	*2/2*	*3/3*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
R0143	*6/6*	*3/3*	*2/2*	*3/3*	5;10	*2/2*	*6/6*	*4/4*	*3/3*	*3/3*	1;4	*5/5*	1;4;7	*4/4*	*4/4*	*2/2*	*3/3*	*3/3*	*3/3*	3;10	*5/5*	7;8;9;12
R0145	*6/6*	*3/3*	*2/2*	*3/3*	5;8	*2/2*	*6/6*	*4/4*	*3/3*	*5/9*	1;4	*5/5*	1;4;7	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
R0150	*6/6*	*3/3*	*2/2*	*3/3*	5;8	*2/2*	*6/6*	*4/4*	*3/3*	*5/9*	1;4	*5/5*	1;4;7	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	3;10	*5/5*	7;8;9;12
R0173	*5/5*	*3/3*	*2/2*	*3/3*	5;9	*2/2*	*4/4*	*4/4*	*3/3*	*2/2*	1;4	*5/5*	1;4;7	*3/3*	*4/4*	*2/2*	*2/2*	*2/2*	*3/3*	2;9	*3/3*	7;8;9;11;12
R0174	*5/5*	*3/3*	*2/2*	*3/3*	5;8	*2/2*	*5/5*	*4/4*	*3/3*	*2/2*	1;5	*5/5*	1;4;7	*3/3*	*4/4*	*2/2*	*2/2*	*1/1*	*3/3*	2;7	*3/3*	7;8;9;11;12
R0177	*5/5*	*3/3*	*2/2*	*3/3*	5;8	*2/2*	*5/5*	*4/4*	*3/3*	*2/2*	1;5	*5/5*	1;4;7	*3/3*	*4/4*	*2/2*	*2/2*	*1/1*	*3/3*	2;7	*3/3*	7;8;9;11;12
R0203	*5/5*	*3/3*	*2/2*	*3/3*	5;8	*2/2*	*5/5*	*4/4*	*2/2*	*2/2*	1;4	*4/4*	1;4;7	*3/3*	*4/4*	*2/2*	*2/2*	*1/1*	*3/3*	4;7	*3/3*	7;8;9;12
Teh	*6/6*	*3/3*	*2/2*	*1/1*	3;5;8	*2/2*	*4/4*	*4/4*	*3/3*	*3/3*	1;4	*5/5*	3;6;10	*4/4*	*4/4*	*2/2*	*2/2*	*2/2*	*4/4*	2;6	*5/5*	7;8;9;12
SC43	*3/6*	*3/3*	*2/2*	*2/2*	5;8	*1/1*	*4/4*	*5/5*	*1/1*	*2/2*	2	*2/4*	2;6;9	*6/6*	*6/6*	*3/3*	*1/1*	*3/3*	*0*	4;10	*7/10*	7;8;10
Tula	*3/6*	*4/4*	*2/2*	*3/3*	5;9	*2/2*	*3/3*	*0*	*2/2*	*5/5*	0	*3/4*	3;6;10	*6/6*	*6/6*	*2/2*	*1/1*	*3/3*	*3/3*	4;9	*6/11*	7;8;10
Can III	*7/7*	*0*	*2/2*	*3/3*	0	*0*	*8/8*	*0*	*2/2*	*6/6*	2	*4/4*	0	*6/6*	*7/7*	*2/2*	*2/2*	*1/1*	*0*	7;12	*9/9*	0

Note. Dia, Hk, Pep, and *Sod,* phenotypic interpretation. Other loci, genetic (allelic) interpretation. "0" means the lack of activity for enzyme under study, but has been interpreted as a missing value for the statistical tests.

Observed and expected heterozygosity for the "true" *T. cruzi*, *H* (expected heterozygosity), was 0.394, while *Ho* (observed heterozygosity) was only 0.035. Hence a severe general deficit of heterozygotes is apparent, which is confirmed by Hardy–Weinberg statistics (see below).

Phylogenetic diversity. According to the UPGMA dendrogram computed from Jaccard's distance matrix (see Fig. 1), three stocks, namely, A83, A87, and A276, obviously fall outside the rest of the Guianese sample and from the *T. cruzi* reference stocks. It is probable that they do not belong to the species *T. cruzi* (see Discussion).

In the rest of the sampling (Guianese stocks + reference stocks), all Guianese stocks are clustered together with the Tehuantepec reference stock, while the three other reference stocks fall outside (see Fig. 1).

Population genetic analysis. The tests have been performed on only those Guianese stocks attributed to the species *T. cruzi* (see below). The *f* test of linkage disequilibrium has been performed by taking as a unit (i) each stock or (ii) only each distinct genotype. All tests (segregation and recombination) were statistically highly significant.

Departures from Hardy–Weinberg expectations (segregation tests) have been estimated by χ^2 statistics. For all loci, the *P* level of significance was $<10^{-3}$, except for *Gapd*, *Lap*, and *6Pgd*, for which it was between 10^{-2} and 10^{-3}.

All linkage disequilibrium statistics (recombination tests; see Tibayrenc *et al.* 1990) were highly significant. Under the null hypothesis of lack of departure from panmictic expectations, the probability of recording the most abundant genotype with a size as high or higher than actually observed (three stocks) is 4.6×10^{-13} (test d1). The probability of observing any genotype with a size as high or higher than the dominant genotype's is 5×10^{-4} (test d2). The probability of observing as few or fewer different genotypes than actually observed is 8×10^{-4} (test e). The probability of observing as high a linkage disequilibrium as actually observed is $<10^{-4}$ (by taking as a unit either the stocks or the different genotypes).

Discussion

Taxonomical considerations. This Guianese sample of sylvatic trypanosomatid stocks obviously shows two different kinds of isolates. The first one, which is represented by only three stocks (A83, A87, and A276), most probably is not related to the species *T. cruzi* and represents a distinct phylogenetic lineage. This result is inferred from both the matrices of Jaccard's distances and the UPGMA dendrogram (see Fig. 1). These three stocks could belong to the species *Trypanosoma* (*Herpetosoma*) *rangeli*, although this has to be confirmed by comparing them with reference stocks. It is worth noting that the A276 stock shows no activity for the locus *Me-1*, a peculiarity that has been considered characteristic of *T. rangeli* by Brenière *et al.* (1993).

Phylogenetic inferences. The other Guianese stocks can be attributed to *T. cruzi* and, more specifically, appear to be phylogenetically more related to the formerly described zymodeme I (Miles *et al.* 1978) than to the formerly described zymodemes II and III (Miles *et al.* 1978). Indeed, in the dendrogram, they are clustered together with the Tehuantepec reference stock and not with the other reference stocks, namely, Can III (which is attributed to zymodeme III; Miles *et al.* 1978), SC43, and Tula, which are more related to zymodeme II (Tibayrenc *et al.* 1986). Since Tehuantepec is itself related to zymodeme I (Tibayrenc *et al.* 1986), it can be concluded that all these Guianese *T. cruzi* stocks are related to this zymodeme, which confirms former results obtained on a more limited number of Guianese stocks (Dédet *et al.* 1985). Our results are hence different from the ones obtained by Saravia *et al.* (1987) on Colombian sylvatic *T. cruzi* stocks. Indeed these authors did observe in that country the presence of stocks related to zymodeme III together with stocks related to zymodeme I.

Although these Guianese stocks appear more related to zymodeme I, it must be emphasized

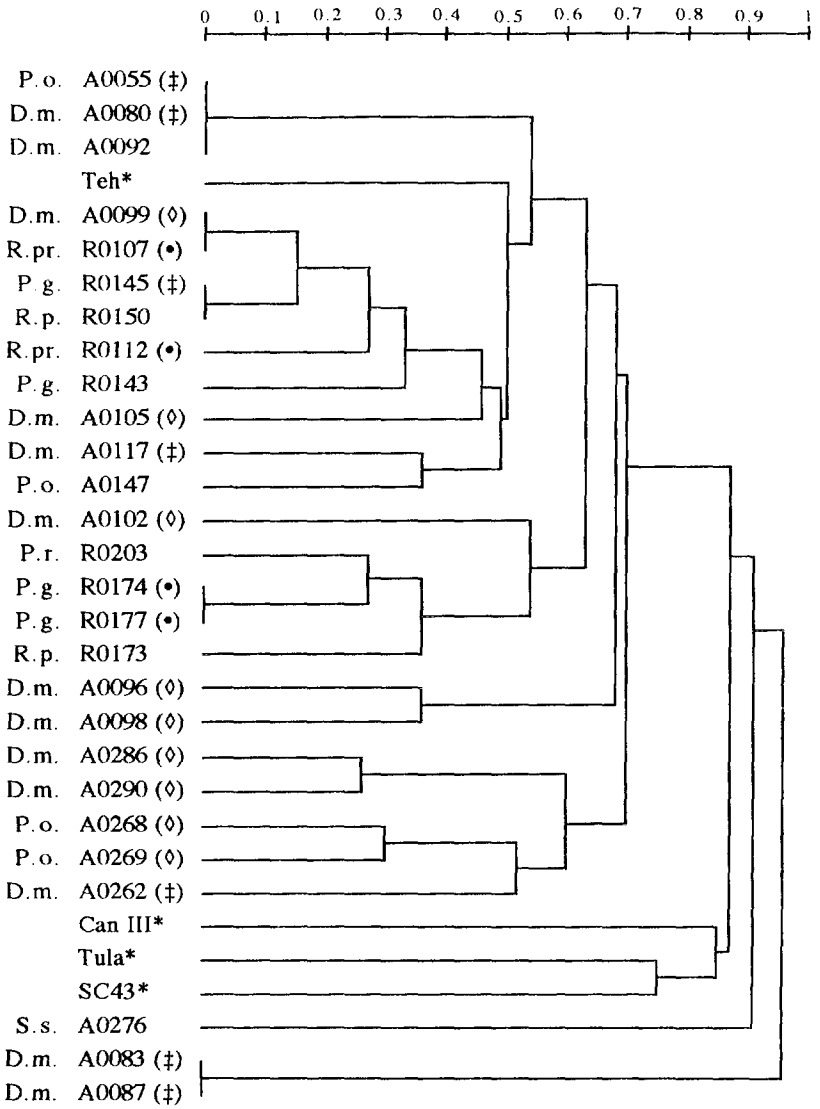

FIG. 1. Dendrogram of the 31 *Trypanosoma cruzi* stocks constructed by UPGMA from a Jaccard's distance matrix, obtained by multilocus enzyme electrophoresis. Hosts and principal geographical origins are indicated for each Guianese stock. D.m., *Didelphis marsupialis;* P.g., *Panstrongylus geniculatus;* R.p., *Rhodnius pictipes;* R.pr., *Rhodnius prolixus;* S.s., *Saimiri sciureus.* (‡), FRG; (◊), Cacao; (●), Montjoly; *, Reference stocks.

that they show considerable genetic diversity (see Fig. 1). This further confirms that the formerly described zymodeme I (Miles *et al.* 1978) represents at best a considerably heterogeneous phylogenetic lineage (Tibayrenc *et al.* 1986). It would be quite misleading to consider the stocks related to this zymodeme a unique genetic class.

Population structure. To our knowledge, this is the first time that a population genetics analysis has been performed on a fair sample of sylvatic *T. cruzi* stocks. Basically clonal population structure in *T. cruzi* (Tibayrenc *et al.* 1981, 1986) had been documented until now mostly on stocks taken from domestic cycles. It could be suspected that genetic recombination

is more abundant in sylvatic cycles, which constitute the original ecological niche of *T. cruzi* (Tibayrenc and Ayala 1988). The present results bring little support to this hypothesis. Indeed all population genetics tests show considerable departure from panmictic expectation, which is circumstancial evidence for clonal population structure or at least for the existence of discrete genetic lineages within the population under study.

Although taken from only one country, this sample is not rigorously sympatric. Hence it could be feared that geographical distance leading to allelic frequency differences among localities by genetic drift would be responsible for the observed departures from panmixia. Although this might and probably does account for some of the departures from panmixia observed, three states of affairs suggest that it is not a satisfactory overall explanation for our results.

First, at several loci, an excess of heterozygotes is apparent by comparison with theoretical expectations. For example, at the *Gdh-2* locus, the heterozygous genotype is represented two times (expected size: 0.08). The probability of this result, estimated by combinatorial analysis (d1 test) is 3×10^{-3}. Now, if geographical distance and genetic drift were responsible for departure from panmixia, the converse result (deficit of heterozygotes) should be observed (Wahlund effect: see Tibayrenc *et al.* 1991).

Second, it should be noticed that out of the four genotypes that have been sampled more than one time (see Table I), three were sampled over long geographical distances. For example, when the dominant genotype (three stocks) is considered, two of the stocks attributed to it were sampled in the FRG locality, while the third one was found in Acarouany, close to the Surinamese border, more than 150 km away. It has been shown (Tibayrenc *et al.* 1991) that when geographical distance is responsible for departures from panmixia, the overrepresented genotypes have a tendency to be sampled from limited geographical areas.

Third, the zymodeme repartition shows no tendency to geographical structuration: on the contrary, a given locality can show the presence of extremely diverse zymodemes (see Fig. 1 and "epidemiological considerations" (below)).

Another interesting result comes from the f test, which is constantly highly significant, by taking as a unit either the stocks or the different genotypes. It has been proposed (Maynard Smith *et al.* 1993) that linkage disequilibrium in microbe natural populations sometimes is not due to true clonal evolution, but rather to short-term propagation of ephemeral clonal genotypes in a species that is basically sexual ("epidemic structure"). In these cases, linkage disequilibrium is expected to disappear by taking as a unit the genotype rather than the individual (stock). Since a different result was obtained here, it seems that this Guianese *T. cruzi* population has a "true" clonal population structure rather than an "epidemic" one. It should be noticed that in the study by Maynard Smith *et al.* (1993), *T. cruzi* was considered a "true" clonal species.

Although clonal structure appears to be a valuable working hypothesis for this sylvatic *T. cruzi* population, this result does not rule out the possibility of occasional mating. Acute statistics able to rigorously estimate the "dose" of sex in a predominantly clonal population are presently lacking. It is worth noting that the genotype diversity in this sample is notably higher than the one recorded in a set of Chilean stocks taken from domestic cycles and analyzed with the same methods (Neubauer 1992). Genotype diversity is 21/27 = 0.78. In Chile, genotype diversity is 0.56 (44 different zymodemes for 79 stocks). The difference is statistically significant ($P < 0.05$). It is impossible to know whether the higher genotype diversity recorded in the sylvatic stocks can be explained by "less scarce" genetic recombination or simply by higher clonal diversity. If the second hypothesis is true, two parameters that are not exclusive from one another could account for the difference observed between sylvatic and domestic cycles: first, the abundance of diversified ecological niches in the primary forest could favor the coexistence of various diversified clones. Second, anthropized *T. cruzi* populations could

have been founded by a limited number of natural clones (founder effect).

Epidemiological considerations. This work, by showing that the majority of the stocks under study can be attributed to *T. cruzi*, confirms the abundance of this parasite in the primary forest of French Guiana and the role of *Didelphis marsupialis* (see Table I) as an important reservoir (Dédet *et al.* 1985).

The limited number of stocks studied here makes it impossible to draw firm epidemiological conclusions. Nevertheless, some observations can be made.

First, it is apparent that the natural clones characterized by MLEE show no tendency to host specificity (see Fig. 1). Indeed a given host, such as *D. marsupialis*, can harbor extremely diverse *T. cruzi* clonal genotypes, as well as genotypes that were not attributed to *T. cruzi* (stocks A83 and A87). Similarly, *Philander opossum, Panstrongylus geniculatus, Rhodnius pictipes,* and *Rhodnius prolixus* harbored diversified genotypes. Conversely, a same *T. cruzi* genotype is shared by *D. marsupialis* and *R. prolixus* (stocks A99 and R107), by *D. marsupialis* and *P. opossum* (stocks A80 and A55), and by *R. pictipes* and *P. geniculatus* (stocks R150 and R145).

Second, as shown in Fig. 1, there is no tendency for given clonal genotypes to be confined to given geographical areas. On the contrary, a locality like Cacao shows the presence of various, distantly related genotypes, including the stock A276, which was not attributed to *T. cruzi*. Similarly, various genotypes were sampled at FRG and Montjoly (see Fig. 1).

Although the risk of Human Chagasic infection in French Guiana is limited, the results reached in the present study are helpful for improving our general knowledge of Amazonian Chagasic cycles and of *T. cruzi*'s basic biology and mating system.

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