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Genetic variability of Colombian populations of *Trypanosoma cruzi* and *Trypanosoma rangeli*

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ABSTRACT

This paper presents our study of genetic variability of *Trypanosoma cruzi* and *Trypanosoma rangeli* strains isolated from different Colombian biological hosts, using multilocus enzyme electrophoresis for 15 enzyme systems and electrophoretic analysis of kinetoplast DNA (kDNA) digested with EcoRI and MspI endonucleases. Isoenzyme profiles were used to determine genotypes for each of the strains. Populations of *T. cruzi* and *T. rangeli* were found to have a polymorphic average of 86.7% and 26.7%, respectively.
Schyzodeme analysis showed high variability for *T. cruzi*, since its genetic distance values were found to be greater than 50%, considerably higher than those previously reported for several *T. cruzi* strains from other countries. These results suggest that Colombian strains should be considered as genetically independent entities and are worth studying independently from each other to clearly establish their biological and clinical characteristics.

Key terms: genetic polymorphism, heterozygosity, schyzodeme, *Trypanosoma cruzi*, *Trypanosoma rangeli*, zymodeme.

INTRODUCTION

Trypanosoma cruzi is a hemoflagellate protozoan of major medical concern. It affects 16 to 18 million people in Central and South America (WHO, 1991), where it has become a public health risk in many countries. In Colombia, it affects about 3.3% of the population, though almost 10% are at risk of infection (Schmunis, 1991).

Strains of *T. cruzi* isolated from different mammals and vectors are morphologically indistinguishable and frequently referred to as a single pathogenic entity in humans. However, population analysis reveals differences in their biological behaviour, epidemiology and clinical profiles, suggesting that *T. cruzi* is genetically heterogeneous (Miles *et al.* 1978). This highlights the need for identification of population differences using stable molecular markers.

Zymodeme and schyzodeme analyses have been used for the genetic characterization of Trypanosoma strains to establish those properties which are potentially associated with the heterogeneity of the parasite and which are features of Chagas Disease. For example, starch gel electrophoretic analysis (Miles *et al*, 1980) for more than 15 isoenzymes classified Brazilian *T. cruzi* populations into three main zymodeme groups: Z1, restricted to wild vectors and animals; Z2, restricted to humans and domestic animals; and Z3, only wild animals. Using the same experimental approach in a study of 524 *T. cruzi* strains from a broad geographical range, Tibàyrenc and Ayala (1988) reported greater polymorphism and identified 43 new and different zymodemes. Andrade *et al.*, (1983) and De Lucadoro *et al.*, (1993) have analyzed the genetic structure of Argentinean and Brazilian *T. cruzi* populations and found a strong correlation between the genetic structure of the parasite and its geographic origin in these countries.

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Kinetoplast DNA digestion has been used to analyze the genetic structure of *T. cruzi*. Paraguayan *T. cruzi* populations have been classified into four main parasite groups (Mimori *et al.*, 1992) and Carreño *et al.*, (1987) have shown that there are similarities between groups of schyzodeme and zymodeme in Chilean *T. cruzi* populations.

In Colombia, Saravia *et al.*, (1987) studied 54 strains of *T. cruzi* and identified 16 different groups. However, Holguín *et al.*, (1987) found low genetic variability in Colombian strains of *T. rangeli* from domiciliary and sylvatic cycles.

In this paper we report our findings of a study of the genetic structure of Colombian *T. cruzi* and *T. rangeli* strains using qualitative and quantitative zymodeme and schyzodeme electrophoretic analysis.

MATERIALS AND METHODS

Trypanosoma cruzi and Trypanosoma rangeli strains

Strains of *Trypanosoma cruzi*, Coy4-tol, Gp-ant, Gal61-suc, Aq1-ant, and *Trypanosoma rangeli*, Sc3-ant and Gal57-suc were isolated and identified at the Chagas Laboratory of the University of Antioquia. Controls for classifying populations by zymodeme analysis were *Trypanosoma cruzi*, Dm-28c, Ev-77 and Pv-4, belonging to zymodeme Z1 (Saravia *et al.*, 1987) and Tulahuén and Cm-17 strains belonging to zymodeme Z2 and Z3, respectively. As a control for *T. rangeli*, the San Agustín strain was used (Saravia *et al.*, 1987). The International Center for Medical Research (Cali, Colombia), supplied these populations. The biological hosts for and the geographical origin of these strains are shown in Table I.

Extract preparation and Isoenzyme Electrophoresis

Parasites were grown in LIT medium at 28°C, harvested by centrifugation at 3000g at 4°C for 10 minutes and stored at -70°C until the moment of use (Chiari and Camargo, 1983). For electrophoresis, parasites were lysed by suspension in a hyposmotic solution of EDTA, Ditiotreitol and -aminocaproic Acid at a final concentration of 2 mM each. The lysate solution was centrifuged at 15000g at 4°C for 30 minutes and the soluble fraction was stored at -70°C in 20 µl aliquots (Saravia *et al.*, 1987). Celullose acetate plates and thin starch gels were used in isoenzyme electrophoretic analysis, after Kreutzer and Souza (1981), Abderrazak *et al* (1993) and Miles *et al.*, (1980). The enzymes analyzed were phosphate glucose isomerase (PGI, E.C. 5.3.1.9), peptidase (PEP, E.C. 3.4.11.1), phosphate manose isomerase (PMI, E.C

TABLE I

Biological Hosts and Geographical Origin for the *T. cruzi* and *T. rangeli* Populations

Populations	Biological Host	Geographical Origin
T. rangeli Sc3-ant	Human	Antioquia
T. rangeli Gal57-suc	*Didelphis marsupialis*	Sucre
T. rangeli San Agustín	Human	Cundinamarca
T. cruzi Coy4-tol	*Rhodnius prolixus*	Tolima
T. cruzi Gp-ant	*Panstrogylus geniculatus*	Antioquia
T. cruzi Gal61-suc	*Wild Mouse*	Sucre
T. cruzi Aq1-ant	*Triatoma dispar*	Antioquia
T. cruzi Pv-4	*Rhodnius prolixus*	Cundinamarca
T. cruzi Ev-77	*Rhodnius prolixus*	Meta
T. cruzi Dm28c	*Didelphis marsupialis*	Venezuela
T. cruzi Tulahuén	Human	Chile
T. cruzi CM-17	*Dasypus sp.*	Meta

Antioquia, Cundinamarca, Meta, Sucre and Tolima are all Regions of Colombia.

5.3.1.8), phosphoglucomutase (PGM, E.C. 2.7.5.1), alanine amino tranferase (ALAT, E.C. 2.6.1.2), aspartate amino transferase (ASAT, E.C. 2.6.1.1), aconitase (ACON, E.C. 4.2.1.3), malate dehydrogenase (MDH, E.C. 1.1.1.37), isocitrate dehydrogenase (ICD, E.C. 1.1.1.42), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.4.9), Hexokinase (HK, E.C. 2.7.1.1), alcohol dehydrogenase (ADH, E.C. 1.1.1.2), glutamate dehydrogenase (GD, E.C. 1.4.1.2), pyruvate kinase (PK, E.C. 2.7.1.40) and malic enzyme (ME. E.C. 1.1.1.40).

Kinetoplast DNA (kDNA) Digestion

kDNA was obtained by chloroform-phenol extraction and the ethanol precipitation method (Gonçalves, 1984). Five micro-

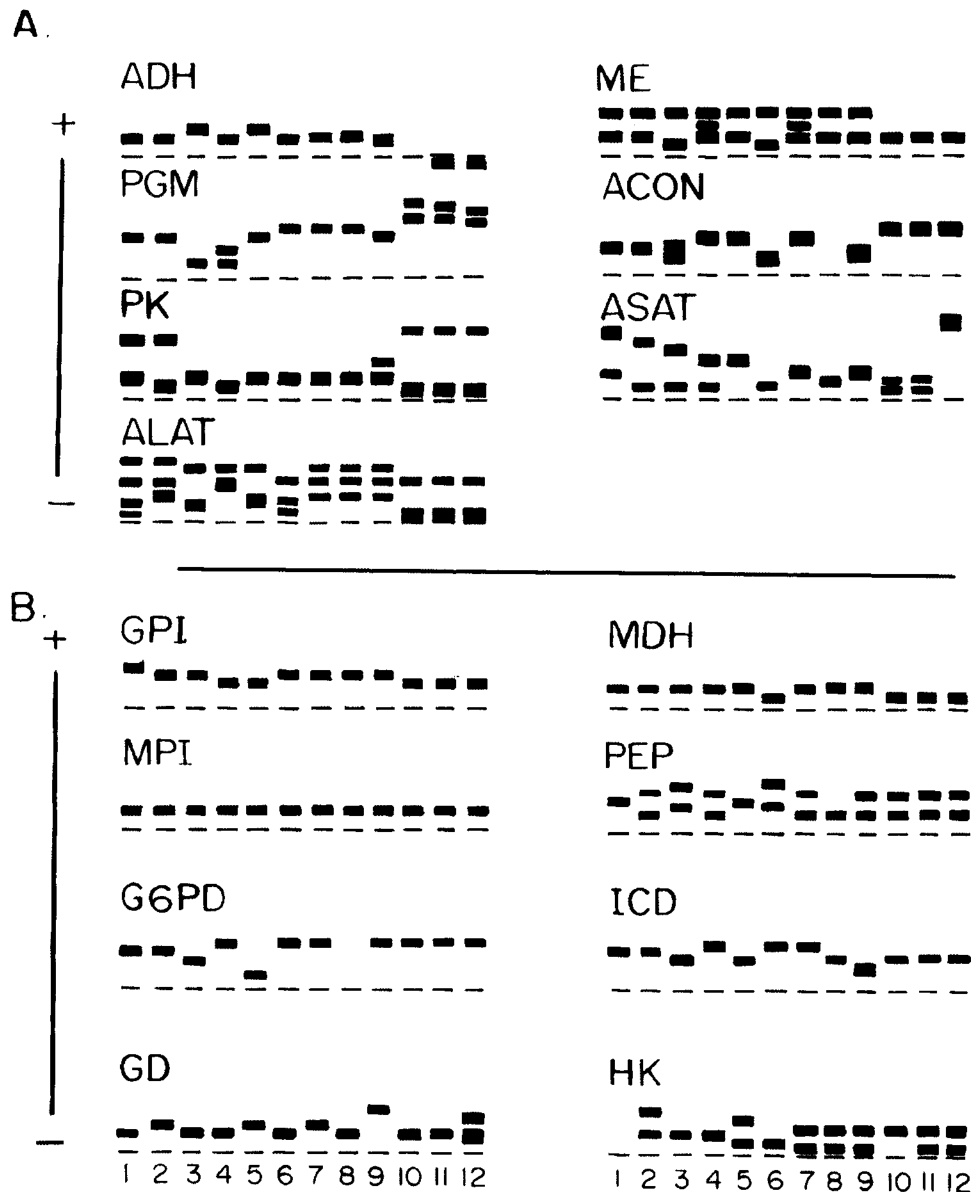

Figure 1. Isoenzyme representative electrophoretic profiles for *Trypanosoma cruzi* and *Trypanosoma rangeli*. A. Enzymes that distinguish *T. cruzi* from *T. rangeli*. B. Enzymes that did not distinguish between each species. *T. cruzi*: 1. Dm-28c, 2. Cm-17, 3. Aq-ant, 4. Gp-ant, 5. Gal61-suc, 6. Coy4-tol, 7. Pv-4, 8. Ev-77, 9. Tulahuén. *T. rangeli*: 10. Gal57-suc, 11. Sc3-ant, 12. San Agustín.

grams of kDNA from each population were taken and then digested with EcoRI and MspI enzymes (SIGMA Chem. Co.) at concentrations of 2 U/μg. Fragments were isolated by polyacrylamide gel electrophoresis at gradients from 4.5 to 10% and stained with silver nitrate as described Gonçalves *et al.*, (1984, 1990).

Data Analysis

Genotypes were determined by electrophoretic isoenzyme profiles, and genetic variability was measured using the BIOSYS-1 computer program (Swofford and Selander, 1989) which calculates average loci polymorphism (P), mean heterogeneity (H), genetic distances (D), and Nei's genetic identities (I). From a cross-population genetic distance matrix, a dendrogram was drawn using the UPGMA method (Crisci and Lopez, 1983), which in turn allowed us to identify genetically related groups. Observed genotype frequencies were compared to those which would have been expected if populations were at Hardy-Weinberg genetic equilibrium.

For schyzodeme analysis, the Jaccard Similitud Index (S) was used (Crisci and Lopez, 1983), a genetic distance matrix was calculated at D=1-S (Macedo *et al.*, 1993) and UPGMA dendrograms were drawn.

RESULTS

Isoenzyme Electrophoresis

Representative enzyme electrophoretic profiles for each of the populations are shown in Figures 1 and 2. On the whole, enzymatic migration was anodic, except for ADH in *T. rangeli*. ADH, ME, PGM, ASAT, ALAT, ACON, and PK enzymes made it possible to distinguish two species of Trypanosoma (Figs 1A and 2), which was not possible with the other enzymes (Figs 1B and 2).

Cellulose acetate plates gave better resolution for ME, GD, ICD, G6PD and PGI enzymes, while the starch gel electrophoresis method increased resolution for ALAT, ASAT, ADH, HK, PK, PGM, MDH and PEP enzymes. Low PMI and ACON activity was recorded with both methods (data not shown).

ME for *Trypanosoma cruzi* was the only enzyme found to be active in two loci (ME-1 fastest migration and ME-2 slowest migration). Locus ME-1 produced homozygote and heterozygote electrophoretic profiles, while ME-2 produced only homozygote profiles (Figs 1A and 2). Other enzymes showed only one active zone, from which it can be inferred that they are coded

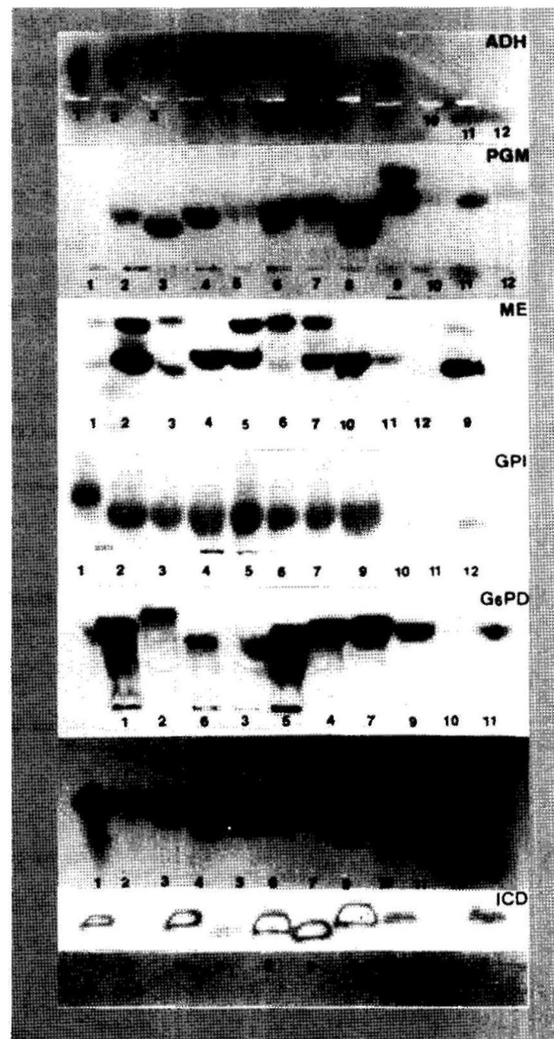

Figure 2. Electrophoretic profiles of some of the isoenzymes of Colombian *Trypanosoma cruzi* and *T. rangeli* populations. *T. cruzi*: 1. Dm-28c, 2. Cm-17, 3. Aq-ant, 4. Gp-ant, 5. Gal61-suc, 6. Coy4-tol, 7. Pv-4, 8. Ev-77, 9. Tulahuén. *T. rangeli*: 10. Gal57-suc, 11. Sc3-ant, 12. San Agustín.

for by only one locus. However, ADH, ACON, PGI, PMI, MDH, G6PD, ICD, and GD enzymes produced one-band electrophoretic profiles, from which it can be concluded that populations are homozygous for the respective loci, whereas the rest were observed to be heterozygote populations (Fig 1).

Results for *T. rangeli* showed that each of the enzyme systems analyzed is controlled by only one locus. Malic enzyme was not active in locus ME-1 and was homozygote in locus ME-2 (Figs 1 and 2). The three *T. rangeli* sampled populations were homozygote for ADH, G6PD, PGI, MDH, ACON, PMI, and ICD enzymes, while the other enzymes were heterozygote. Polymorphic differentiation was based only on PGM and ASAT, which distinguished the three populations as separate.

T. cruzi and T. rangeli Polymorphism

Genotypes were determined by electrophoretic profiles for each of the loci according to the nomenclature proposed by Miles *et al.*, (1985). In *T. cruzi*, highly polymorphic enzymes had seven (PEP, ASAT), and eight alleles (ALAT). The only totally monomorphic enzyme found, with just one allele (homozygote genotype 1/1), was PMI. In *T. rangeli*, ASAT and PGM enzymes had four and five alleles respectively, making them the most polymorphic enzymes.

Genetic Variability

Mean observed and expected heterozygosity values, polymorphism and the mean number of alleles per locus for *T. cruzi* and *T. rangeli* populations are shown in Table II. *T. cruzi* heterozygosity was 13.3% with an average of 3.3 alleles per locus, and *T. rangeli* heterozygosity was 17.8% with an average of 1.4 alleles per locus. Assuming Hardy-Weinberg genetic equilibrium, the heterozygosity value observed for *T. cruzi* (0.133) is well below the expected value (0.503). On the contrary, observed and expected heterozygosity values for *T. rangeli* (0.178 and 0.156, respectively) were not statistically significant. Populations of *T. cruzi* were highly polymorphic (86.7%) while those of *T. rangeli* were not very polymorphic (26.7%).

Genetic Equilibrium

Observed genotype frequency values for *T. cruzi* and *T. rangeli* populations were compared to expected values, assuming Hardy-Weinberg genetic equilibrium. Thirteen polymorphic loci with $\chi^2 \geq 11.487$ and p less than 0.043 were found in *T. cruzi* populations, which rejects the genetic equilibrium hypothesis. However, four polymorphic loci with $\chi^2 \geq 7.00$ and p greater than 0.157 were found in *T. rangeli* populations, which supports the genetic equilibrium hypothesis.

Genetic Distance (D) and Genetic Identity (I)

Genetic similarity was calculated for nine *T. cruzi*, and three *T. rangeli* populations using Nei's genetic distance (D) and genetic identity (I) parameters. Figure 3 shows the dendrogram obtained from D values. A

TABLE II

Observed and Expected Mean Heterozygosity, Polymorphism and Mean Number of Alleles per locus for *T. cruzi* and *T. rangeli* populations.

Genetic Parameter	*T. cruzi*	*T. rangeli*
Observed Heterozygosity	0.133±0.06	0.178±0.085
Expected Heterozygosity	0.503±0.076	0.156±0.074
Polymorphism	86.7%	26.7%
Mean Number of Alleles per locus	3.3±0.5	1.4±0.2

± standard error

clear difference is observed between the two species of Trypanosoma that have D greater than 0.654. The *T. cruzi* strains that were genetically most closely related, with D=0.364, were Pv-4 and Gal61-suc, both of which were isolated from different vectors and regions. Gp-ant, Gal61-suc and Aq1-ant strains were closely related to the Pv-4 strain, domestic Colombian zymodeme Z1, with an average genetic distance of D=0.535, and were less related to Ev-77 strain, wild Colombian zymodeme Z1, with D=0.703. The Coy4-tol *T. cruzi* strain has very high genetic distances when compared to other *T. cruzi* strains (D=0.861), which is why it could not be classified under the groups mentioned above. All Colombian *T. cruzi* populations have very high genetic distances when compared to Venezuelan zymodeme Z1 strains, Dm-28c (D=0.832), Z2 (D=0.873), and Z3 (D=0.835).

Despite having been collected from different biological hosts and geographical regions, *T. rangeli* populations were found to be homogeneous, as D was relatively small (e.g., 0.079 between Sc3-ant and Gal57-suc).

Kinetoplast DNA Fragment Electrophoresis

Figure 4 shows the results of kDNA Restriction Fragment Length Polymorphism (RFLP) profiles obtained with the enzyme

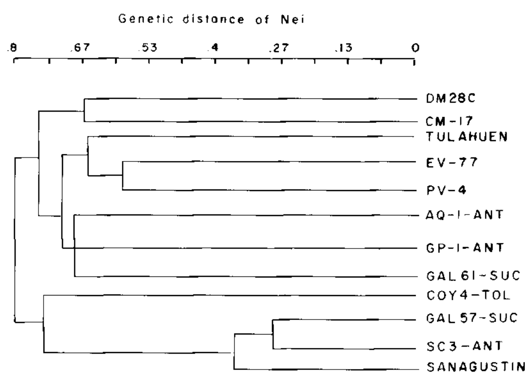

Figure 3. Dendogram summarizing the genetic distance of seven Colombian *Trypanosoma cruzi* and three *T. rangeli* populations using the Neis coefficient.

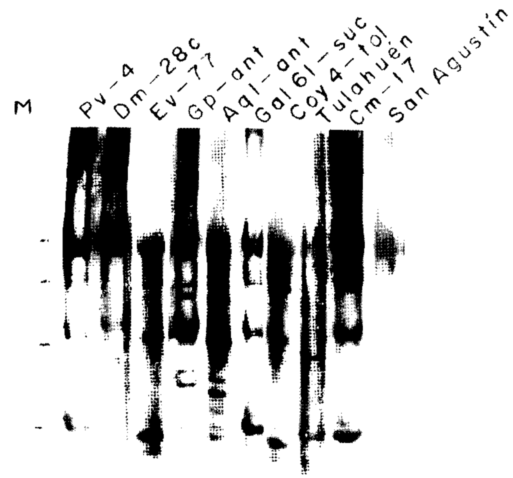

Figure 4. Schyzodeme profiles of Colombian *Trypanosoma cruzi* and *T. rangeli* populations obtained with the enzyme EcoRI. Polyacrylamide gradient gel electrophoresis and staining were performed as described in Materials and Methods Section. Molecular weight markers are from (top to bottom) 1353, 872, 603 and 310 pb.

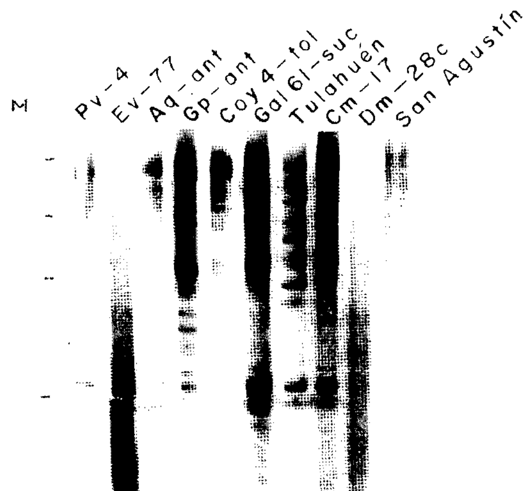

Figure 5. Schyzodeme profiles of Colombian *Trypanosoma cruzi* and *T. rangeli* populations obtained with the enzyme MspI. Polyacrylamide gradient gel electrophoresis and staining were performed as described in Materials and MethodsSection. Molecular weight markers are from (top to bottom) 1353, 872, 603 and 310 pb.

Eco RI, while Figure 5 shows the kDNA RFLP profiles with the enzyme Msp I. It is possible to detect quite different kDNA RFLP in each *T. cruzi* sample. From genetic distance analysis, we can deduce that none of the *T. cruzi* populations is genet

ically related. The only exception was found using Eco RI enzyme between Aq1-ant and Gp-ant populations (D=0.363), both of which were isolated from different hosts and regions (Fig 6). This was not confirmed by using Msp I (Fig 7). These results show a clear interspecific difference between *T. cruzi* and *T. rangeli*.

DISCUSSION

Our study is the first investigation of genetic structure performed in Colombian *T. cruzi* populations using zymodeme and schyzodeme analysis. Isoenzyme electrophoretic profiles obtained with starch gel electrophoresis and cellulose acetate plating complemented this study.

An isoenzyme polymorphism of 86.7% was obtained using zymodeme analysis, which indicates the high genetic variability of this species. The sampled populations did not classify under any of the three zymodemes proposed by Miles, even though they are genetically similar to the Pv-4 strain, previously classified as zymodeme Z1. However, in a study of 54 eastern Colombian strains of *T. cruzi*, Saravia *et al.*, (1987) classified parasite strains into 16 different groups, similar to zymodeme Z1 and Z3. Similarly, De Lucadoro *et al.*, (1993) observed 80% genetic polymorphism in 95 Argentinean *T. cruzi* strains, classified them into 12 groups, and obtained very high genetic distances compared to the three zymodemes proposed by Miles. Tibayrenc and Ayalas study (1988) of 524 *T. cruzi* strains from a broad geographical range, found a polymorphism of 93.3% and 43 different zymodemes with genetic distances between 0.017 and 2.015 (average of 0.757), which provides further evidence of the high genetic variability of the populations of this parasite. The Colombian *T. cruzi* populations analyzed in this study are highly divergent, since their genetic distance values (0.364 to 1.153) are higher than those obtained by other authors for *T. cruzi* in other countries.

Taxonomically, Colombian *T. cruzi* strains are classified as zymodeme Z1, which is characteristic of parasites from countries north of the Amazon Basin. Widmer *et al.*, (1985), and Ebert (1985) found Colombian *T. cruzi* to have low genetic variability and classified them as zymodeme Z1. These authors analyzed very few loci and worked only with domiciliary and peri-domiciliary strains. In this study we analyzed sylvatic and domestic Colombian strains, thus a more diverse parasite stock. In addition, we analyzed 16 different loci, allowing us to unequivocally identify zymodemes, which is a difficult task when only a few loci are analyzed.

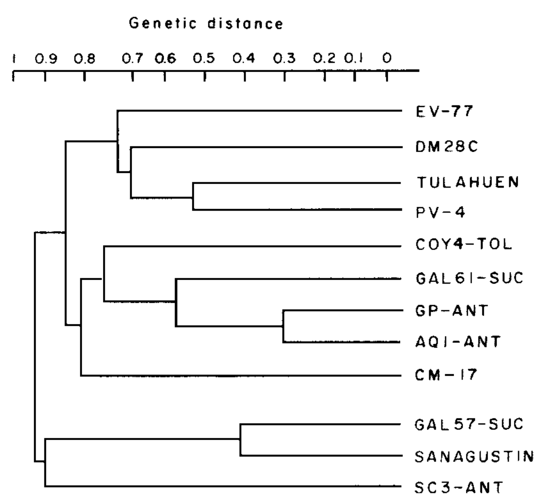

Figure 6. Dendogram of *Trypanosoma cruzi* and *T. rangeli* strains constructed by UPGMA from a Jaccards distance matrix obtained by kDNA electrophoresis digest with EcoRI enzyme.

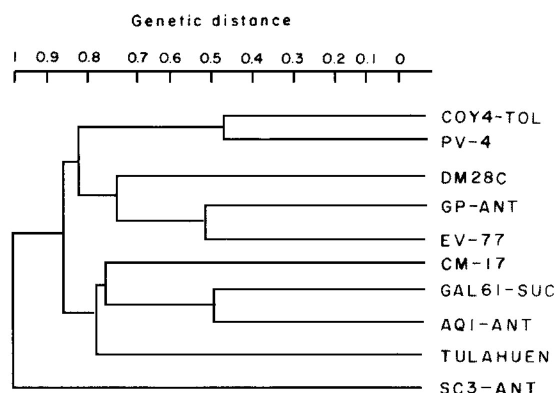

Figure 7. Dendogram of *Trypanosoma cruzi* and *T. rangeli* strains constructed by UPGMA from a Jaccards distance matrix obtained by kDNA electrophoresis digest with MspI enzyme.

Zymodeme analysis shows that the genetic structure of Colombian *T. cruzi* strains bears no relation to its biological or geographical origin. The Coy4-tol strain shows very high genetic distances when compared to Pv-4 and Ev-77, all being isolated from R. prolixus. Aq1-ant and Gp-ant strains, isolated in the Antioquia region, show genetic distances sufficiently high to consider them to be different zymodemes. De Lucadoro *et al.*, (1993) found that Argentinean strains were classified into 12 zymodemes, which were then sub-classified into three geographically related groups. They also observed overlapping between the wild and domestic transmission cycles, as they found the same zymodeme in different reservoirs from two different environments. Similarly, Andrade *et al.* (1983) determined that Brazilian zymodemes correspond closely to their geographical origin. In this study, we found that in sampled stocks, zymodemes are peculiar to each environment, host and region.

However, the observed heterozygosity value (Ho=0.133), against the expected value (He=0.503), supports the clonal structure hypothesis for *T. cruzi* populations, proposed by Tibayrenc and Ayala (1988) who observed a heterozygosity value of 0.058 against the expected value of 0.47. Similarly, in Argentina, De Lucadoro *et al.*, (1993) also observed a heterozygosity of 0.24, lower than the expected value of 0.43, which suggests that these strains have a more recent origin than that found by Tibayrenc since their loss of heterozygosity. From heterozygosity values obtained in this study, we suggest that the Colombian *T. cruzi* populations originated before the Argentinean strains, and in agreement with the Tibayrenc and Ayala hypothesis (1988), the high genetic variability and loss of heterozygosity observed lead us to assume that clonal evolution in *T. cruzi* is very ancient and that numerous clones have evolved independently of each other.

Another aspect, which supports clonal structure in these populations, is the absence in some genotypes of loci with more than one allele present, which might have been expected assuming independent genetic segregation. However, Carrasco *et al.*, (1996) have suggested a possible genetic exchange in sylvatic populations of *T. cruzi*, where they observed frequency values identical to those predicted by Hardy-Weinberg distribution. These results were confirmed by RAPD profiles and indicate that genetic exchange is important

Table III. Genetic distances (diagonal above) and genetic identity (diagonal below) for *T. cruzi* and *T. rangeli* population

	Trypanosoma cruzi									*Trypanosoma rangeli*		
Population	Dm-28c	Cm-17	Pv-4	Ev-77	Tulahuen	Coy4-tol	Aq1-ant	Gal61-suc	Gp-ant	Gal57-suc	Sc3-ant	Sanagustin
Dm-28c		0.540	0.882	0.595	0.889	0.824	1.153	0.676	0.865	0.847	0.851	0.915
Cm-17	0.583		0.748	0.787	0.712	0.769	1.044	0.932	0.730	1.026	1.159	1.118
Pv-4	0.414	0.473		0.372	0.387	0.577	0.711	0.364	0.488	1.029	1.214	1.115
Ev-77	0.552	0.455	0.690		0.657	0.609	0.952	0.676	0.577	0.847	0.654	0.828
Tulahuen	0.411	0.491	0.679	0.518		1.117	0.916	0.674	0.788	0.993	1.178	1.198
Coy4-tol	0.439	0.463	0.561	0.544	0.327		0.934	0.882	0.767	0.788	0.750	0.853
Aq1-ant	0.316	0.352	0.491	0.386	0.400	0.393		0.563	0.560	1.442	1.404	1.590
Gal61-suc	0.509	0.394	0.695	0.509	0.509	0.414	0.569		0.533	1.208	1.295	1.458
Gp-ant	0.421	0.482	0.614	0.551	0.455	0.464	0.571	0.587		1.174	1.135	1.280
Gal67-suc	0.429	0.359	0.357	0.429	0.370	0.455	0.236	0.299	0.309		0.079	0.141
Sc3-ant	0.427	0.314	0.297	0.520	0.308	0.472	0.246	0.274	0.321	0.924		0.147
San Agustin	0.401	0.327	0.328	0.437	0.302	0.426	0.204	0.233	0.278	0.868	0.863	

to the generation of genetic diversity in *T. cruzi*.

In *T. rangeli* populations, loss of heterozygosity was not observed, and the loci analyzed for both populations are at Hardy-Weinberg genetic equilibrium. The low number of populations analyzed makes it difficult to give any explanation of the genetic structure found for this species which is sympatric to *T. cruzi*. Further study of a greater number of populations is required.

Though isolated from different hosts and regions, *T. rangeli* populations show a low genetic variability using zymodeme analysis. A polymorphism of 26.7% and a genetic distance between the three strains of 7.9% were observed. However, with schyzodeme analysis, populations were more than 80% different, though Holguín *et al.*, (1987) did not observe isoenzyme polymorphism when analyzing 14 loci in Colombian populations of this species. With other techniques, such as PFGE, Henriksson *et al.*, (1996) studied 16 different samples of *T. rangeli* and showed that populations are highly polymorphic in their molecular karyotype.

As for zymodeme analysis, the genetic variability determined by kDNA analysis was very high, and differences greater than 50% were found. Thus, Colombian *T. cruzi* strains can not be classified in schyzodeme groups as has been done in other countries. For example, Mimori *et al.*, (1992) classified eleven Paraguayan *T. cruzi* strains, digested with EcoRI and MspI into four groups, which were all very similar. In the same way, Carreño *et al.*, (1987) classified 18 Chilean strains into three schyzodeme groups, which correlated well with the three schyzodemes found in Chile. In this study, we did not observe any agreement between zymodeme and schyzodeme profiles. It is important to note that zymodeme analysis is based on basal metabolic genes that mutate in neutral zones, while schyzodeme analysis is based on mitochondrial DNA that has a high mutation rate.

With the exception of Gp-ant and Aq1-ant strains (from the Antioquia region of Colombia) with a 35% difference in genetic distance, all Colombian strains show genetic distances greater than 70% when studied with EcoRI enzyme. With MspI, genetic distances are greater than 50% and show a high polymorphism. Using EcoRI and MspI enzymes, Solari *et al.*, (1992) found a homology of 44.6% and 34% respectively in Bolivian *T. cruzi* strains and some Chilean strains showed homologies of 52% when analyzed with any of these enzymes. A homology of 67% was found when Argentinean, Chilean and Brazilian strains were compared with the abovementioned technique.

Clonality and absence of sexual recombination in Trypanosoma, and high genetic variability are found in Colombian *T. cruzi* populations. These are findings that merit major biological and medical concern because they suggest that Colombian *T. cruzi* populations should be considered as natural clones and consequently as independent genetic entities.

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