Population Genetic Analysis of Colombian *Trypanosoma cruzi* Isolates Revealed by Enzyme Electrophoretic Profiles

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Although Colombia presents an enormous biological diversity, few studies have been conducted on the population genetics of Trypanosoma cruzi. *This study was carried out with 23 Colombian stocks of this protozoa analyzed for 13 isoenzymatic loci. The Hardy-Weinberg equilibrium, the genetic diversity and heterogeneity, the genetic relationships and the possible spatial structure of these 23 Colombian stocks of* T. cruzi *were estimated. The majority of results obtained are in agreement with a clonal population structure. Nevertheless, two aspects expected in a clonal structure were not discovered in the Colombian* T. cruzi *stocks. There was an absence of given zymodemes over-represented from a geographical point of view and the presumed temporal stabilizing selective phenomena was not observed either in the Colombian stocks sampled several times through the years of the study. Some hypotheses are discussed in order to explain the results found.*

Key words: *Trypanosoma cruzi* - population genetics - spatial autocorrelation - Colombia

In the present study, we show an extensive population genetics analysis, where the genetic heterogeneity and the spatial structure of *Trypanosoma (Schizotrypanum) cruzi* is analyzed throughout Colombia. This kind of study is of clinical importance since the extensive genetic polymorphism and heterogeneity of isolates may be related to the diverse pathology of the disease and its immunity, throughout the geographical distribution of the clonets (Dvorak 1984, Cosenza & Kroeger 1991). Although well kown in other Latin American countries (Brazil, Bolivia, Chile, for example), some population and spatial patterns of the *T. cruzi* clonets in Colombia are not very well known. The only previous studies reported for this country were those from Saravia et al. (1987), where 47 stocks were analyzed coming from the Piedmont region (Meta and Casanare departments); the inclusion of five Colombian stocks (MC50, MC52, MC53, MC60 and MC61) belonging exclusively to one zymodeme (19) in the study of Tibayrenc and Ayala (1988) and, more recently, the work of Márquez et al. (1998), where 28 new stocks coming from the Sucre, Antioquia and Tolima departments were analyzed. In the first work, 12 different zymodemes were detected (12/47 = 25.5%). In the second, the genetic variability shown in Colombia was lower, because only one zymodeme was detected from the five stocks analyzed (1/5 = 20%). In the third one, the amount of diverse zymodemes increased notably because 21 new zymodemes were detected out 28 stocks analyzed (21/28 = 75%). However, these stocks came from sylvatic origin and did not include stocks representing the domestic cycle of this parasite, and only represented a limited geographic fraction of Colombia. In our analysis, we included 23 stocks coming from seven different departments of Colombia (North Santander, Cesar, Antioquia, Casanare, Cundinamarca, Tolima, and Boyacá), and 20 new zymodemes were detected (20/23 = 87%). This evidences that the previous studies underrepresented the real genetic diversity in this neotropical country. It is remarkable that 18% of the Colombian territory (about 200,000 km^2) containing three million people is placed in the endemic area of distribution of *T. cruzi*, with almost one million people infected (3% of the total population of this country). The main population genetics results were in agreement with the hypothesis of a clonal structure, although recombination can be induced in vitro in several bacteria and other microorganisms, such as *Escherichia coli* (Ochman & Selander 1984). However, some spatial results showed the presence of various relevant geographic patterns, which shows the lack of one, or some, over-repre-

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sented zymodeme(s) over broad geographical areas. However, we show that the determination of spatial structure strongly depends on the statistic techniques employed. Additionally, the presumed temporal stabilizing selective phenomena are not observed either in the Colombian stocks sampled several times throught the period studied.

MATERIALS AND METHODS

Populations studied - Samples from people infected with *T. cruzi*, and animal samples from wild reservoirs, as well as vectors, were collected from 1985 to 1995 in North Santander, Cesar, Antioquia, Casanare, Cundinamarca, Tolima, and Boyacá, covering a significant fraction of the endemic geographical area of Colombia where this protozoan is present. The hosts and vectors, in which *T. cruzi* was obtained, were humans, *Rhodnius prolixus*, *Panstrongylus geniculatus* and the marsupial *Didelphis marsupialis*. Upon culture, isolation and characterization, 23 different stocks of *T. cruzi* were established. The areas of Colombia examined correspond to endemic areas of *T. cruzi* covering a broad range of altitudes, temperature and environmental conditions. Two reference stocks were additionally surveyed with regard to the Colombian stocks analyzed. One of these stocks comes from Bolivia (SO34), isolated from *Triatoma infestans*, and represents the Z1 zymodeme, predominant in Brazil and with a wild origin (Miles et al. 1984). The second one comes from Chile (MN), isolated from humans, and belongs to the Z2 zymodeme of typical domestic cycle. For overall comparison purposes, two samples of *Crithidia* sp. were analyzed along with the 23 Colombian stocks of *T. cruzi*. The stocks were isolated using a modified NNN culture.

Markers used - For the isoenzymatic analysis, 10^6 to 10^8 cells/ml from cultured stocks of *T. cruzi* were obtained by centrifugation at 1,000 g for 10 min at 4°C. Upon resuspension in sterile normal saline solution, the cell pellet was kept at -70°C. Electrophoresis in cellulose acetate was done according to the procedures of Tibayrenc et al. (1985). Thirteen different enzymatic markers were analyzed: glucose-6-phosphate isomerase (*Gpi*), malate dehydrogenase (*Mdh*), isocitrate dehydrogenase (*Idh*), phosphoglucomutase (*Pgm*), glutamate dehydrogenase NAD+ (*Gdh-Nad+*), glutamate dehydrogenase NADP+ (*Gdh-Nadp+*), malic enzyme 1 (*Me-1*), malic enzyme 2 (*Me-2*), glucose-6-phosphate dehydrogenase (*G6pdh*), peptidase 1 (*Pep-1*), peptidase 2 (*Pep-2*), 6-phosphogluconate dehydrogenase (6*pgdh*) and aspartate aminotranspherase (*Got*). The first 12 of these isoenzymes had also been analyzed by Tibayrenc and Ayala (1988) in their description of 43 American zymodemes, being the only difference with our set of polymorphic markers the use of *Got* instead of leucine aminopeptidase (*Lap*). In their study, they had also included aconitase and adenylate kinase, both of which resulted monomorphic and were not included in the profiles of the 43 zymodemes they defined. The comparison between their zymodemes and those presented in this analysis will therefore be possible to a large extent.

Population genetics procedures

Hardy-Weinberg equilibrium - To analyze the possible existence of Hardy-Weinberg equilibrium individually and collectively in the *T. cruzi* stocks analyzed, an unbiased estimator (f) based on the proportion of homozygotic alleles, which can be used as a measure of the coefficient of endogamy (Robertson & Hill 1984), was used. In this analysis, we assumed the hypothesis of diploidy in *T. cruzi.*

Heterozygosity and heterogeneity - The presence of stochastic or selective differential events could be detected in a given population if the heterogeneity of the H statistic (= expected heterozygosity) was too large. For this reason, this statistic was calculated for each stock analyzed. Additionaly, the G_{ST} statistic was obtained to analyze the degree of heterogeneity among the stocks studied.

Genetic distances - To analyze the genetic relationships among the *Trypanosoma* stocks, diverse genetic distance matrices were calculated (Nei's, Cavalli-Sforza & Edwards and Prevosti). Various dendrograms were built using diverse algorithms. There were the Single (Single-linkage clustering) and the WPGMA (Weighted Arithmetic Pair-Group Method) algorithms. The second procedure was applied by using the method of Pamilo (1990). The method of "neighbor-joining" was additionally applied (Saitou & Nei 1987) which yields trees without origin root, and for this we applied the midpoint rooting method (Farris 1972) to produce trees with direction. The robustness of the genetic dendrograms obtained was assessed by two methods. The first was the Felsenstein's bootstrap test (1985). The second method of assessment was the obtainment of the cophenetic correlation coefficients (Sneath & Sokal 1973), which was applied to all the dendrograms generated, although we only present those with the highest values. The statistical significance of those coefficients was obtained applying an approximate Mantel t test, and using a Monte Carlo simulation with 1,000 permutations.

Spatial autocorrelation analysis - We carried out some autocorrelation analyzes to study the spatial structure of the allozymic markers. Having revealed an extensive genetic heterogeneity for the 13 loci studied in the 23 Colombian stocks of *T.*

cruzi, we searched for a spatial structure of the populations of this organism in Colombia, by using two different analyses: (1) the calculation of two indices of spatial autocorrelation, the index I of Moran, and the c coefficient of Geary (Sokal & Oden 1978a,b). Two different analyses of spatial autocorrelation were carried out with the indices mentioned. The different alleles of each loci were used as allelic frequencies, and the less frequent of them were excluded to break the relationship of lineal dependence of all the alleles of a given locus. Fifty one variables were studied in this case. With this first spatial autocorrelation analysis, two different distance classes were elected. In the first, seven distance classes (DC) were defined, with the number of stock pair comparisons identical for each DC: 1 DC = 0-97 km, 2 DC = 97-166 km, 3 DC = 166-213 km, 4 DC = 213-295 km, 5 DC = 295-351 km, 6 DC = 351-423 km, 7 DC = 423-580 km. The number of stock pair comparisons for each DC was 15. In the second analysis, 8 DC were elected, where the size of the DC was constant independently of the stock pair comparison number: 1 DC = 0-73 km, 2 DC = 73-145 km, 3 DC = 145-218 km, 4 DC = 218-290 km, 5 DC = 290-363 km, 6 DC = 363-435 km, 7 DC = 435-508 km, 8 DC = 508-580 km. Both analyses were carried out to analyze if any change in the spatial parameters could affect the results obtained. On the other hand, the various genotypes for each locus were used as unordered multistate characters. For example, if for *MDH* three genotypes were found (1/1), (2/2) and (3/3) in the 23 different stocks studied, therefore these would represent the states 1, 2 and 3, respectively. If for *PGM* four different genotypes were found, (1/1), (3/4), (5/7) and (5/8), the respective multistate values would be 1, 2, 3 and 4. An analysis of spatial autocorrelation was applied, observing the various genotypes. Thirteen variables were thus defined, as were seven DC, for constant geographical sizes, and disimilar number of comparisons per DC: 1DC = 0-83 km, 2DC = 83-166 km, 3DC = 166-249 km, 4DC = 249-332 km, 5DC =332-415 km, 6DC = 415-497 km, and 7DC = 497-580 km. It is interesting to analyze whether using allelic frequencies, or multistate genotypic forms, similar spatial results are obtained. A contrasting result would indicate the differential importance in the choice of representative values for the alleles and for the genotypes. In addition, for each collection of data, a single autocorrelation coefficient was obtained for each genetic variable studied. In this case, the point pairs were weighted as the inverse square separation distance between the stocks.

The kind of correlograms obtained may suggest the type(s) of evolutionary mechanism(s) leading to a given spatial structure (Sokal & Wartenberg 1983, Sokal et al. 1987, 1989, Sokal & Jacquez 1991, Ruiz-Garcia 1994a, b, Ruiz-Garcia & Jordana 1997, 2000, Ruiz-García & Klein 1997).

Analysis of similarity between correlograms - To determine the similarity between the correlograms, the average Manhattan distance matrices between the coefficients of autocorrelation estimated for variable pairs of correlograms were calculated, both for allele frequencies (7 and 8 DC) and for genotypes. This analysis can determine whether each of the genetic variables studied was subject to the same spatial evolutionary event, or whether they were under pressure from different spatial evolutionary agents. Sokal and Wartenberg (1983) and Sokal et al. (1989) showed, by means of simulation studies, that correlogram pairs generated by the same evolutionary spatial processes have Manhattan distances smaller than 0.1 in the case of Moran's I index, and about 0.15 for the Geary's c coefficient. In order to visualize the spatial relationship between pairs of variables, the algorithm UPGMA was applied to the Manhattan distance matrix between the correlograms corresponding to the variables for each spatial autocorrelation analysis performed.

RESULTS

The 20 new zymodemes detected in the *T. cruzi* stocks of Colombia are different from those reported in this country by Saravia et al. (1987), Tibayrenc and Ayala (1988) and Márquez et al. (1998) (see Table I). Otherwise, some caution has to be taken in account with direct comparisons with already published data since techniques were not performed by the same operators. With this in mind, a detailed comparison for each locus, contrasting our results with those reported by Tibayrenc and Ayala (1988) and Márquez et al. (1998), is as follows:

Gpi: the homozygote (5/5) had been detected in Colombia by Tibayrenc and Ayala (1988). Lately, Márquez et al. (1998) detected the genotypes 8/8; 4/7; 4/4; 3/9 and 9/9. In this study we show four different genotypes, of which three had been reported in other locations of Latin America (3/3; 4/4; 6/6) and the remainder (7/7) is new.

Mdh: the homozygous (2/2) had previously been reported in Colombia and was in fact present in many of the analyzed stocks. The homozygous form (3/3) is however new, both in Colombia and in Latin America.

Pgm: three genotypes unknown in Colombia and in Latin America were detected (3/4; 5/7; 5/8). The homozygotes (1/1; 3/3; 4/4; 5/5) and the heterozygote (2/3) were already known in Colombia.

Idh: two Colombian unknown genotypes were found (2/2; 3/3), being (3/3) also new in Latin

TABLE I

Genetic profiles of the 23 Colombian *Trypanosoma cruzi* and two *Chritidia* sp. stocks by using 13 isoenzymatic loci. The name of the isoenzymatic loci is shown in the text

Stocks	GPI	GOT	MDH	IDH	PGM	GDNAD	GDNADP	ME-1	ME-2	G6PDH	PEP-1	PEP-12	6PGDH
MDID/CO/86/#1	7/7	4/4	2/2	2/2	5/7	Absent	3/3	2/2	2/2	2/2	4/4	1/1	-
MDID/CO/87/407	7/7	4/4	2/2	2/2	5/8	2/2	2/2	2/2	2/2	2/2	4/4	2/2	5/5
MDID/CO/87	6/6	4/4	3/3	3/3	5/8	Absent	3/3	2/2	2/2	3/3	5/5	2/2	6/6
MHOM/CO/87/ S.P. Rodriguez	7/7	4/4	2/2	2/2	5/8	2/2	2/2	2/2	2/2	3/3	4/4	Absent	-
MDID/CO/85/051	7/7	4/4	2/2	2/2	5/8	Absent	3/3	3/3	3/3	-	5/5	-	-
IPAS/CO/94/20	6/6	2/2	2/2	2/2	5/8	Absent	Absent	3/3	3/3	-	5/5	-	-
MHOM/CO/C. Moreno	7/7	4/4	2/2	2/2	5/8	Absent	3/3	3/3	3/3	3/3	4/4	-	-
IRHO/CO/92/92/Munanta	4/4	4/4	2/2	2/2	Absent	Absent	3/3	2/2	2/2	3/3	4/4	1/1	6/6
MHOM/CO/B.M. López	3/3	4/4	2/2	2/2	5/8	Absent	3/3	2/2	5/5	3/3	4/4	2/2	-
IRHO/CO/Ikiakarora	3/3	2/2	2/2	2/2	5/8	Absent	3/3	2/2	2/2	2/2	6/6	2/2	6/6
MDID/CO/87/#3	7/7	4/4	2/2	2/2	5/8	4/4	3/3	2/2	2/2	3/3	2/2	Absent	6/6
MHOM/CO/86/M. Rangel	7/7	7/7	2/2	2/2	5/8	5/5	3/3	3/3	5/5	4/4	5/5	2/2	6/6
MHOM/CO/90/F.Chaparro	7/7	4/4	2/2	2/2	5/8	-	3/3	2/2	2/2	3/3	5/5	2/2	6/6
IRHO/CO/90/Choachi	6/6	4/4	3/3	2/2	3/4	-	3/3	Absent	3/3	3/3	6/6	Absent	6/6
MDID/CO/88/R-55	7/7	4/4	2/2	2/2	5/8	2/2	Absent	2/2	2/2	3/3	5/5	2/2	6/6
MDID/CO/88/R-64	6/6	4/4	3/3	3/3	5/7	Absent	3/3	2/2	2/2	3/3	5/5	2/2	6/6
MDID/CO/86/387	7/7	4/4	2/2	2/2	5/8	2/2	3/3	2/2	2/2	3/3	4/4	2/2	-
MDID/CO/86/R-59	7/7	4/4	2/2	2/2	5/8	3/3	3/3	2/2	2/2	3/3	5/5	2/2	-
MHOM/CO/87/ S.P. Rodriguez2	7/7	4/4	2/2	2/2	5/8	Absent	3/3	2/2	2/2	3/3	4/4	1/1	6/6
MDID/CO/87/5	7/7	4/4	2/2	2/2	5/8	Absent	3/3	2/2	2/2	3/3	4/4	1/1	6/6
MDID/CO/87/445	7/7	4/4	2/2	2/2	5/8	3/3	3/3	3/3	3/3	3/3	4/4	2/2	-
MDID/CO/88/R-56	3/3	4/4	2/2	2/2	5/8	3/3	3/3	2/2	2/2	3/3	5/5	2/2	6/6
IRHO/CO/95/Shubacbarina	3/3	4/4	2/2	2/2	5/8	3/3	3/3	2/2	2/2	3/3	4/4	1/1	6/6
Crithidia 1	2/2	Absent	1/1	2/2	1/1	Absent	Absent	Absent	1/1	1/1	2/2	3/3	1/1

America. The form (1/1) had previously been reported in this country.

Gdh-Nad+: five different genotypes were present; (3/3) had already been reported in Colombia. Genotypes (4/4 and 5/5) are reported for the first time in Latin America. A new probable genotype of null alleles was detected.

Gdh-Nadp+: three genotypes were detected, of which only (2/2) had been detected in Colombia, whereas (3/3) had been found in Latin America. A third genotype probably represents null alleles.

Me-1: three genotypes were found; (2/2) had already been reported in Colombia. Genotypes (2/2) and (3/3) had been found in other parts of Latin America. A compound genotype was detected, probably of null alleles.

Me-2: three genotypes were found (2/2; 3/3; 5/5) all of them unknown in Colombia, where only genotype (4/4) had been reported. In Latin America genotypes (3/3 and 5/5) had been reported, but genotype (2/2) is new.

G6pdh: four diverse genotypes were detected in Colombia (0/0; 2/2; 3/3; 4/4). Only genotypes (5/5, 3/3 and 2/2) had previously been reported in this country, differently from genotypes 0/0 and 4/4, which are new in Colombia. All of them had already been detected in other parts of Latin America, except for that composed of null alleles.

Pep-1: four different genotypes were detected (2/2; 4/4; 5/5; 6/6). Genotypes (1/1; 5/5; 4/4; 2/2) had previously been reported in Colombia. Genotype 6/6 reported here is new. All of them had already been reported in other places of Latin America. Curiously, Márquez et al. (1998) detected an important number of heterozygote genotypes for this locus (2/7; 2/5; 1/3; 2/6; 1/7; 2/3) in Colombia, while we did not detect any.

Pep-2: genotypes (1/1; 2/2) were detected, as well as a null form. Of these, genotype (1/1) had previously been detected in Colombia.

6pgdh: genotype (4/4) had been reported in the previous study by Tibayrenc and Ayala (1988). In contrast, we report new genotypes (0/0; 5/5; 6/6) in Colombia, although previously reported in Latin America, except for the null genotype.

Hardy-Weinberg equilibrium - The Hardy-Weinberg equilibrium analysis showed the inexistence of this equilibrium at all levels. In this case, by using the f of Robertson and Hill (1984), an excess of homozygosis was found for the total population ($f = 0.1910$; $\chi^2 = 5.471$; 1 df; $P < 0.05$; see Table II), although applying the F of Wright, a non significant excess of heterozygosis was obtained ($F = -0.3158$; $\chi^2 = 14.958$, 21 df; $P = 0.825$). Márquez et al. (1998) found an overall observed heterozygosity, which was not statistically different from the Hardy-Weinberg proportions. However, the overall and the individual absence of Hardy-Weinberg equilibrium of this magnitude in our data is typical of a clonal structure.

Levels of individual diversity and hetero-geneity in the T. cruzi stocks surveyed in Colombia - In this study the proportion of different zy-modemes for Colombia is noteworthy higher (87 %) than the values obtained by previous authors. However, the mean heterozygosity (H) found in this work (around $H = 0.04$) is substantially lower (ten times lesser) than the value reported by Márquez et al. (1998) ($H = 0.40$). Our value is more similar to that reported by Tibayrenc and Ayala (1988) ($H = 0.06$). In addition, we found only one locus (*PGM*) in heterozygote condition out of the 13 loci analyzed (7.7 %). On the contrary, Márquez et al. (1998) found heterozygous genotypes for the loci *Alat*, *Mpi*, *Pep*, *Gpi*, *Acon*,

TABLE II

Hardy-Weinberg equilibrium tests using the Robertson & Hill's f statistics of the 13 isoenzymatic loci analyzed in 23 Colombian *Trypanosoma cruzi* stocks

Locus	Robertson & Hill	Variance	χ^2	d.f.	p
GPI	1.000	0.01	108.507	1	<0.01
GOT	1.000	0.0133	81.380	1	<0.01
MDH	1.000	0.0200	54.253	1	<0.01
IDH	1.000	0.0400	27.127	1	<0.01
PGM	0.1910	0.0067	5.471	1	0.0193
GDNAD	1.000	0.0100	108.507	1	<0.01
GDNADP	1.000	0.0200	54.253	1	<0.01
ME-1	1.000	0.0200	54.253	1	<0.01
ME-2	1.000	0.0133	81.380	1	<0.01
G6PDH	1.000	0.0100	108.507	1	<0.01
PEP-1	1.000	0.0133	81.380	1	<0.01
PEP-12	1.000	0.0133	81.380	1	<0.01
6PGDH	1.000	0.0133	81.380	1	<0.01

d.f.: degree of freedom; p: probability

Icd, Ldh, Asat and *Pgm* (in lower magnitude for this locus than the value reported here) out of the13 loci analyzed (9/13 = 69. 2 %).

Our estimate of genetic heterogeneity among the *T. cruzi* stocks analyzed in Colombia is one of the highest reported for whatever organism in the world (G_{ST} = 0.93) and it is strongly significant. For 33 Colombian clonets, Márquez et al. (1998) obtained a F_{ST} range of 0.33-0.67, also highly significant. Therefore, there is agreement between both works in this aspect.

Genetic distances - The genetic distances found between pairs of Colombian stocks of *T. cruzi* are enormous in many cases, suggesting an extensive degree of genetic diferentiation between pairs of stocks. Tibayrenc et al. (1986b) found for Latin America values of Nei's distances ranging from 0.017 to 2.015, with a mean value of 0.757 ± 0.478. Although the geographical span analyzed in Colombia is considerably smaller, the values found ranged from 0.0-0.147 to 1.833, with mean values according to those for all Latin America by Tibayrenc and Ayala (1988) (Table III). This means that the enormous divergence between the stocks studied in Colombia is of the same order of magnitude than that found by Tibayrenc and Ayala (1988) for 121 stocks covering all Latin America, including populations of USA. The results obtained applying the distance of Nei were also found using the other genetic distances calculated. The *Chritidia* stocks differed significantly from those of *T. cruzi* with Nei's distances of 2.545. Remarkably, the values between pairs of stocks of *T. cruzi* and *Chrithidia* were in some cases smaller than those between certain pairs of stocks of *T. cruzi*.

The most prominent dendrograms were observed when applying the algorithm WPGMA, and the distances of Prevosti and Cavalli-Sforza & Edwards. Both dendrograms (Fig. 1) clearly separated *Crithidia* from all *T. cruzi* stocks. The first *T. cruzi* stock which diverged was that of Chile (MN) representing the Z2 zymodeme of domestic cycle. Otherwise, the Bolivian stock (SO34) was more related to the Colombian stocks, showing that these stocks basically belong to the Z1 zymodemes. Inside of the Colombian array, those of Amalfi (Antioquia) and some of the Cundinamarca department (central Colombia) were the first which diverged. However, there is not a clearly defined number of clusters within the departments. These dendrograms highly differed from the Single analysis with the Nei's distance because of grouping together stocks 5 (Arboledas, North Santander) and 7 (Zetaquirá, Boyacá) with the cluster where are stocks 1 (El Zulia, North-Santander) and 8 (Guateque, Boyacá). The dendrogram generated with the method of neighbor-joining and the distance of Prevosti leads to a similar result, display-

TABLE III

Nei's genetic distance matrix among 23 Colombian *Trypanosoma cruzi* stocks analyzed

	ZUL-NS	DUR-NS1	RIC-CUN1	SAB-CAS	ARB-NS	AM-ANT	ZET-BOY	GUA-BOY	PA-CUN	CA-NS	MA-TOL	CHA-CES	TI-NS	CH-CUN	RI-CUN2	RI-CUN3	DU-NS2	RI-CUN4	SA-CAS2	RI-CUND5	DU-NS3	RI-CUND6	TI-NS2
ZUL-NS	0.0000																						
DUR-NS1	0.4155	0.0000																					
RIC-CUN1	0.8675	1.0216	0.0000																				
SAB-CASA	0.4155	0.2744	1.0216	0.0000																			
ARB-NS	0.5447	1.0216	1.0216	0.6539	0.0000																		
AM-ANT	1.0788	0.6094	1.2730	1.0216	0.2744	0.0000																	
ZET-BOY	0.4155	0.8210	1.0216	0.3857	0.1744	0.5108	0.0000																
GUA-BOY	0.3481	0.7536	0.5994	0.5994	0.9359	1.4467	0.5994	0.0000															
PA-CUN	0.4155	0.6539	0.6539	0.5108	0.6339	1.0216	0.3857	0.4659	0.0000														
CA-NS	0.5447	0.6539	0.6539	1.0216	1.0216	1.0216	1.0216	0.5994	0.5108	0.0000													
MA-TOL	0.5447	0.6539	0.6539	0.3857	0.6539	1.2730	0.5108	0.4659	0.6539	0.6539	0.0000												
CHA-CES	1.0877	1.0216	1.0216	1.2730	0.6539	1.0216	0.8210	1.1590	0.8210	0.8210	0.8210	0.0000											
TI-NS	0.4155	0.5108	0.2744	0.5108	0.5108	1.0216	0.5108	0.3481	0.3857	0.3857	0.2744	0.5108	0.0000										
CH-CUN	1.1394	1.8326	0.5798	1.1394	0.5798	0.7340	0.4463	0.7536	0.9163	0.9163	0.7340	1.1394	0.7340	0.0000									
RI-CUN2	0.6932	0.3857	0.5108	0.3857	0.8210	1.0216	0.8210	0.5994	0.6539	0.6539	0.3857	0.6539	0.1744	1.1394	0.0000								
RI-CUN3	0.8210	1.0788	0.0202	1.0788	1.0788	1.3471	1.0788	0.5994	0.6932	0.6932	0.6932	1.0788	0.3011	0.5798	0.5447	0.0000							
DU-NS2	0.4155	0.3857	0.5108	0.2744	0.8210	1.6094	0.5108	0.5994	0.3857	0.8210	0.5108	1.0216	0.3857	0.9163	0.3857	0.5447	0.0000						
RI-CUN4	0.5447	0.6539	0.3857	0.5108	0.6539	1.2730	0.6539	0.7536	0.5108	0.8210	0.5108	0.8210	0.2744	0.9163	0.3857	0.4155	0.1744	0.0000					
SA-CASA2	0.1984	0.5108	0.5108	0.3857	0.6539	1.2730	0.3857	0.1474	0.3857	0.5108	0.2744	0.8210	0.1744	0.7340	0.3857	0.5447	0.3857	0.5108	0.0000				
RI-CUND5	0.1984	0.5108	0.5108	0.3857	0.6539	1.2730	0.3857	0.1474	0.3857	0.5108	0.2744	0.8210	0.1744	0.7340	0.3857	0.5447	0.3857	0.5108	0.0000	0.0000			
DU-NS3	0.5447	0.6539	1.0216	0.5108	0.3857	0.8210	0.1744	0.7536	0.3857	1.0216	0.6539	0.6539	0.5108	0.7340	0.6539	1.0788	0.3857	0.3857	0.5108	0.5108	0.0000		
RI-CUND6	0.6932	0.6539	0.3857	0.6539	0.8210	1.2730	0.8210	0.4659	0.3857	0.3857	0.3857	0.6539	0.1744	0.9163	0.2744	0.4155	0.5108	0.2744	0.3857	0.3857	0.5108	0.0000	
TI-NS2	0.4155	0.6539	0.6539	0.5108	1.0216	1.6094	0.6539	0.2427	0.3857	0.5108	0.3857	1.0216	0.3857	0.9163	0.5108	0.6932	0.5108	0.5108	0.1744	0.1744	0.5108	0.1744	0.0000

ing it more clearly than the other methods the group comprised by stocks 5, 6, 7 and 12.

Spatial autocorrelation analysis - The interpretations based on the results derived from the spatial autocorrelation analysis, due to the use of genotypic multistates, or the use of the allelic frequencies, are remarkably different.

Genotypic multistates: by using Moran's I index, only *G6pdh* out of the 13 variables studied was significant (p = 0.005) with a clear circular clinal structure, showing a 1DC significantly positive, 4DC significantly negative, and the 5DC significantly positive again when seven DC were used. *Got* follows a similar spatial pattern, but it did not reach the level of statistical significance. Two other markers, *Gdh-Nadp+* and *6pgdh*, also stood close to the point of statistical significance, and displayed a strong decline in genetic similarity at 4DC and 3DC, respectively (Table IV). The average correlogram is clearly not significant. With the Geary's c coefficient, the same trends are observed more clearly. G6PDH showed a significant circular cline, as with the Moran's I index. In contrast to this, G*dh-Nad+* presented a significant case of dif-

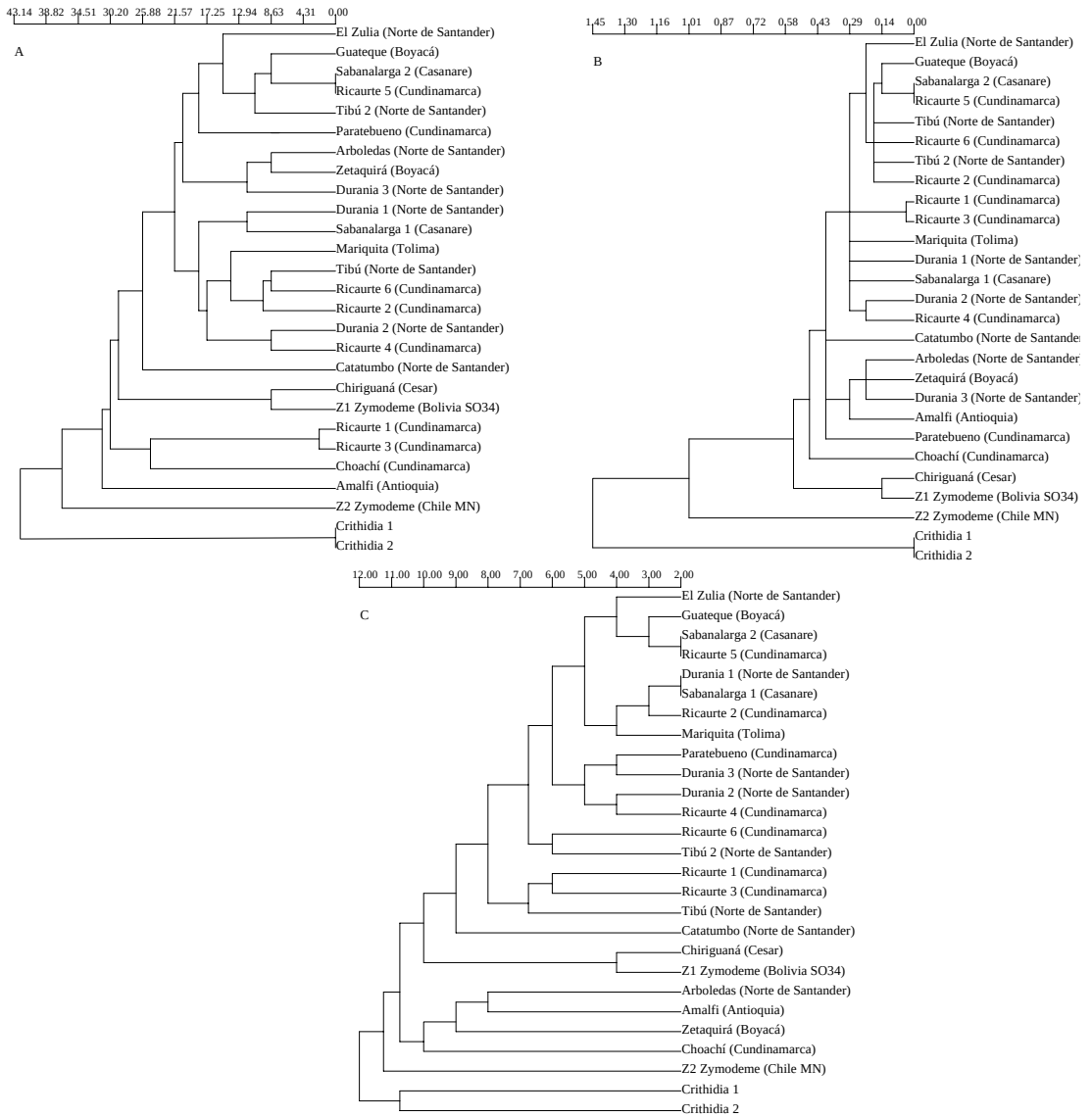

Fig. 1: dendrograms showing the genetic relationships among 23 Colombian *Trypanosoma cruzi* and two reference (Z2 Bolivian zymodeme and Z1 Chilean zymodeme) stocks by using 13 isoenzymatic loci. Two *Chritidia* stocks were used as outgroups. A: WPGMA algorithm with the Cavalli-Sforza & Edwards's chord distance; B: single algorithm with the Nei's genetic distance; C: neighbor-joining algorithm with the Prevosti's distance

ferentiation at long distance (Sokal et al. 1989), with an extremely high value at 7DC (c = 3.70; p <0.0001). *Gdh-Nadp+* also displayed a significant circular cline. The remaining variables did not display any kind of spatial structure. In general, the application of genotypic multistates did not reveal the presence of much significant spatial structure. This is reflected in the following results: both the percent of significant coefficients of autocorrelation, as well as the percent of significant global correlograms, did not differ from the 5% of type I error for any of the two indices applied (Moran and Geary: 8.79%; 8/91; $\chi^2 = 1.05$, 1 df, NS; Moran: 7.69%; 1/13; NS; Geary: 23%; 3/13; NS, respectively). Neither the 13 variables analyzed showed significant values for a single autocorrelation coefficient without defining distance classes (Table V).

The analysis of similarity for the correlograms derived from the matrices of distances of Manhattan for the Moran's I index is based on the percent of distances between the correlograms of pairs of variables with values smaller than 0.1. This percentage was 1.3%, which did not even reach the error type I of 5%. By applying genotypic

TABLE IV

Spatial autocorrelation correlograms using Moran's I and Geary's c coefficient of the 13 isoenzimatic loci analyzed in this study with multistate unordered genotypes, and the average correlogram for seven equal distance classes for 23 Colombian *Trypanosoma cruzi* stocks

Moran I index

	Distance classes							
	83	166	249	332	415	497	580	CgramProb
Loci								
GPI	-0.18	-0.24	0.12	0.00	-0.25	0.09	-0.12	1.000
GOT	0.02	-0.15	0.19	-0.35*a*	-0.35	0.17	0.47	0.181
MDH	-0.03	0.26[a]	-0.19	-0.23	-0.02	-0.13	-0.18	0.259
IDH	-0.02	0.19	-0.09	-0.00	-0.06	-0.38	-0.54	0.506
PGM	-0.04	-0.12	0.03	-0.22	-0.09	0.02	0.11	1.000
GDNAD	0.00	-0.14	-0.20	0.07	-0.08	0.09	-0.51	0.752
GDNADP	-0.05	0.04	-0.06	-0.42*b*	-0.03	0.19	0.16	0.066
ME-1	-0.06	-0.35	0.15	0.12	-0.30	0.17	-0.69	0.376
ME-2	-0.08	-0.01	-0.36	0.14	-0.29	-0.09	0.60*a*	0.269
G6PDH	0.36[a]	0.12	-0.08	-0.55*b*	-0.40	0.15	0.76*a*	0.005
PEP-1	-0.03	-0.11	0.10	-0.08	-0.15	-0.23	0.00	1.000
PEP-1.2	-0.18	-0.20	-0.03	0.15	-0.07	-0.26	0.06	0.519
6PGDH	0.01	0.15	-0.53*b*	0.09	-0.01	-0.21	0.14	0.068
Average	-0.02	-0.04	-0.07	-0.10	-0.16	-0.03	0.02	

Geary's c

	Distance classes							
	83	166	249	332	415	497	580	CgramProb
Loci								
GPI	1.04	1.25	1.37	0.67	1.26	0.46	0.65	0.443
GOT	0.17	1.22	1.73[a]	1.19	0.89	0.34	0.88	0.313
MDH	0.37	1.26	1.63	0.76	0.60	1.10	1.43	0.486
IDH	0.62	1.19	0.85	0.55	0.76	1.86*a*	2.42	0.290
PGM	1.34	1.38	0.81	1.20	0.90	0.53	0.24	1.000
GDNAD	0.28	0.84	1.57	0.38	0.82	1.33	3.70*b*	0.023
GDNADP	0.43	0.81	1.55	2.05*b*	0.52	0.14*a*	0.18	0.042
ME-1	0.75	1.36	1.03	1.00	0.99	0.47	1.71	0.439
ME-2	0.89	0.76	1.74*b*	0.49	1.17	0.89	1.29	0.055
G6PDH	0.33	0.55	1.09	2.11*b*	1.04	0.47	0.29	0.009
PEP-1	0.70	1.31	1.14	0.66	1.28	1.07	0.60	0.944
PEP-1.2	0.89	1.23	1.29	0.81	0.89	0.96	0.71	0.754
6PGDH	0.98	0.74	1.33*a*	0.90	0.95	1.16	0.85	0.318
Average	0.67	1.07	1.32	0.98	0.93	0.83	1.15	

Cgramprob: global correlogram probability; *a*: P<0.05; *b*:P<0.01; distance class in km

multistates, it was not possible to reveal any degree of similarity in the spatial distribution of the 13 variables analyzed (Fig. 2A).

Allelic frequencies- The use of the allelic frequencies, in contrast, revealed a significant spatial structure of various kinds for the 51 variables analyzed for the 7 and 8 DC defined. The alleles that showed significant global correlograms with the Moran's I index and with 7 DC were 12 (12/51 = 23. 529 % of significant overall correlograms) and there was 10.9 % of significant individual autocorrelation coefficients (39/357), both percentages significant (Table VI): *Got 7*, *Gdnad 5*, *G6pdh 4* and *Pep1 5* with a clear genetic differentiation at long distance; *Gdh-Nadp+0* presents a typical symmetrical circular cline with 4DC being significantly negative, and 1DC and 7DC the most positive of all seen; *Me1-0*, *Me2-1*, *G6pdh-1*, *Pep2-3* and *6pgdh 1* with a significant global structure of regional genetic patch from 166 to 213 km; *G6pdh 2* has the structure of a regional patch, of a considerably larger size than the other alleles with this structure (1DC significantly positive, and 6DC significantly negative) and *G6pdh 3* with high genetic similarity in the first 213 km and strong genetic differences from 351 km. In addition, other nine alleles showed correlograms very near to the significance border (9/51 = 10.92%) *Gpi 2*, *Got 0*, *Mdh 1*, *6pgdh 0* and *Pgm 1*, with a near global structure of regional genetic patch from 166 to 213 km. The high similarity between the spatial patterns for the frequencies of those alleles at different loci may be an important evidence of a strong linkage disequilibrium between groups of alleles of different loci. *Gnadp 3* showed a correlogram typical of a circular cline, *Pep2 2* and *Pep2 0* presented negative values for the first distance class but significant ones for the second class distance (97-166 km). With 8 DC defined the results were similar. In fact, the spatial genetic structure is larger and highly significant, as well. For the Moran I index, 31.4% (16/51) of the overall correlograms and 10.5% (43/408) of the individual autocorrelation coefficients were respectively significant. The alleles which showed significant correlograms were *Gpi 2*, *Gpi 3*, *Got 0*, *Got 7*, *Mdh 1*, *Pgm 1*, *Gdnad 5*, *Gnadp 0*, *Me1 0*, *Me2 1*, *G6pdh 2*, *G6pdh 3*, *G6pdh 1*, *G6pdh 4 Pep2 3 and 6Pgdh 1*. The vast majority was significant with 7 DC defined, or was significant borderline, as well.

Applying the Geary's c coefficient, the situation was similar. With 7 DC defined, 27.4% of significant overall correlograms (14/51) and 15.4% of significant individual autocorrelation coefficients (55/357): *Got 7*, *Gdnad 5*, *G6pdh 4* and *Pep1 5* showed significant differentiation at long distance; *Gdnadp 3*, *Gdnadp 0* and *G6pdh 0* yielded circular clines; *Me1 0*, *Me2 1*, *G6pdh 1*, *Pep1 2 Pep2 3 and 6pgdh 1* showed significant regional patches; *G6pdh 3* showed striking genetic similarity until 213 km and a strong disimilarity between the stocks at 351 km, and *G6pdh 2* showed a monotonic clinal divergence until 423 km, with the last DC not significant. Additionally, 13.7% of other correlograms were borderline significant (7/51; *Gp1 2*, *Got 0*, *Mdh 2*, *Mdh 1*, *Pgm 5*, *Pgm 1 and G6pdh 0*). With 8 DC, 33.3% (17/51) of overall correlograms and 12.5% (51/408) of individual autocorrelation coefficients were significant, respectively. The alleles showing significant correlograms were *Gp1 2*, *Got 0*, *Got 2*, *Got 7*, *Mdh 1*, *Pgm 1*, *Gdnad 5*, *Gdnadp 0*, *Me1 0*, *Me2 1*, *6pgdh 3*, *G6pdh 1*, *G6pdh 4*, *Pep1 5*, *Pep1 2*, *Pep2 3* and *6Pgdh 1*. The spatial trends found were very similar

TABLE V

Single autocorrelation coefficients for each one of the 13 isoenzymatic loci analyzed in 23 Colombian *Trypanosoma cruzi* stocks considering unordered multistate genotypes. The point pairs were weighted as the inverse square separation distance between the stocks

Coefficients	Moran's I Index		Geary's c	
Locus	I	Global probability	c	Global probability
GPI	0.09	0.328	0.72	0.289
GOT	0.03	0.381	0.32	0.142
MDH	0.03	0.372	0.34	0.151
IDH	0.11	0.277	0.38	0.181
PGM	-0.02	0.432	0.90	0.436
GDNAD	-0.15	0.403	0.55	0.243
GDNADP	-0.21	0.337	0.74	0.322
ME-1	-0.16	0.398	0.93	0.445
ME-2	-0.27	0.291	0.94	0.452
G6PDH	0.35	0.114	0.80	0.344
PEP-1	-0.08	0.485	0.67	0.242
PEP-1.2	-0.41	0.182	1.22	0.291
6PGDH	-0.14	0.426	1.00	0.496

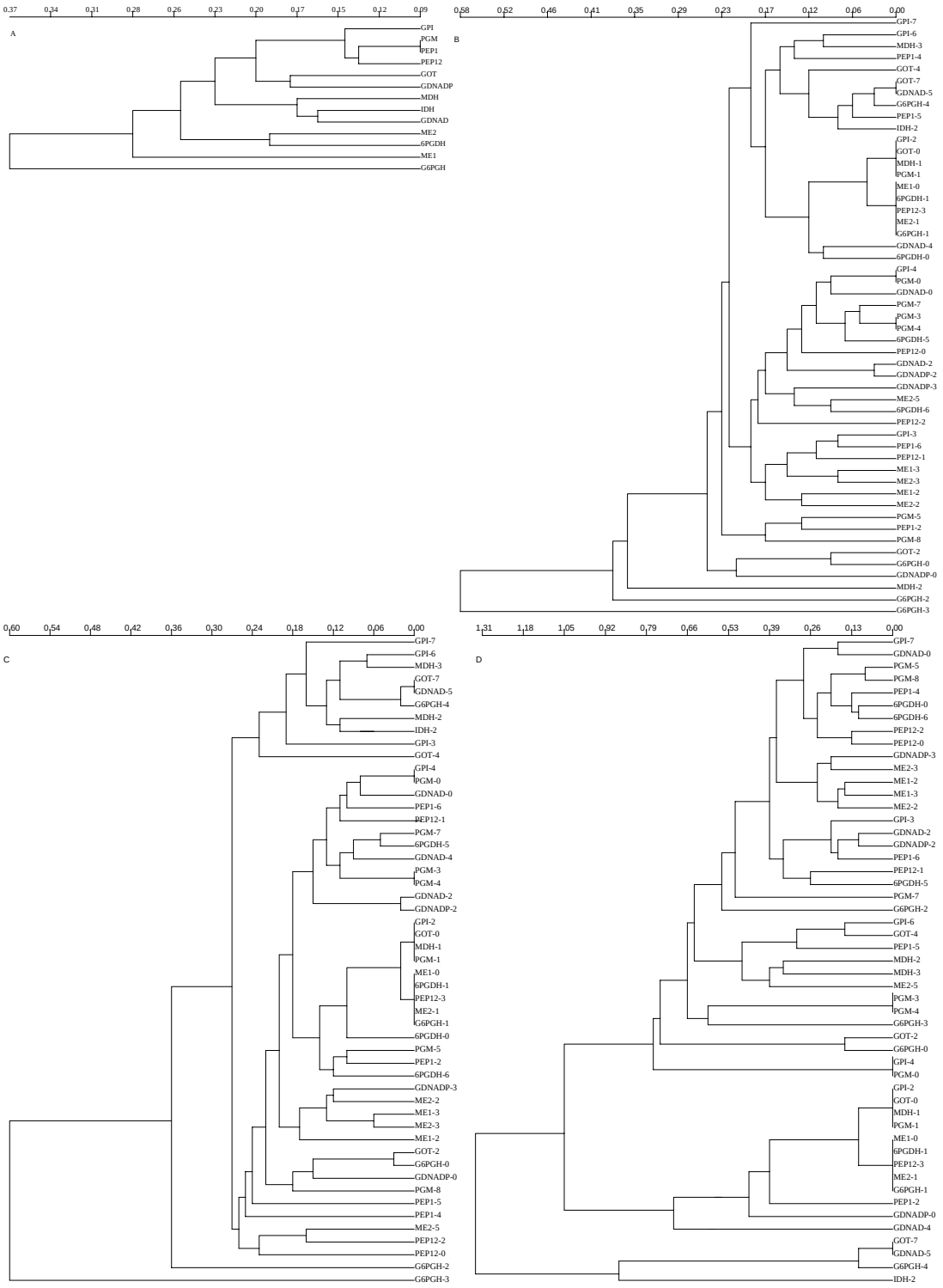

Fig. 2-A: UPGMA analysis of the spatial autocorrelation coefficients with the Moran's I index of the 13 isoenzymatic loci analyzed in this study, considering genotypic unordered multistates having defined seven distance classes with constant geographical sizes; B: UPGMA analysis of the spatial autocorrelation coefficients with the Moran's I index of the 51 alleles analyzed in this study, considering allele frequencies, having defined six distance classes with a relatively equal number of point pairs per distance class; C: UPGMA analysis of the spatial autocorrelation coefficients with the Moran's I index of the 51 alleles analyzed in this study, considering allele frequencies, having defined seven distance classes with a relatively equal number of point pairs per distance class; D: UPGMA analysis of the spatial autocorrelation coefficients with the Moran's I index of the 51 alleles analyzed in this study, considering allele frequencies, having defined eight distance classes with constant geographical sizes.

TABLE VI

Spatial autocorrelation correlograms using Moran's I index and Geary's c coefficient of the 51 allele frequencies from 13 isoenzymatic loci analyzed in this study (A) for seven unequal distance classes and (B) for eight constant distance classes for 23 Colombian *Trypanosoma cruzi* stocks. Distance classes in kilometers

(A)
Moran I index

	Distance classes							
Dist. Class	97	166	213	295	351	423	582	CgramProb
Loci								
GPI-7	0.05	-0.20	0.07	0.23	-0.34	0.04	-0.36	0.506
GPI-6	0.08	0.08	-0.17	0.17	-0.17	-0.08	-0.42[a]	0.273
GPI-4	-0.21	-0.14	0.07	-0.00	-0.14	-0.07	-0.00	0.501
GPI-2	0.07	-0.21	-0.29[a]	-0.21	-0.00	0.07	0.07	0.098
GPI-3	-0.38	0.08	-0.23	-0.00	-0.23	0.35[a]	-0.08	0.102
GOT-4	0.09	-0.18	0.05	-0.32	0.14	0.09	-0.36	0.512
GOT-0	0.07	-0.21	-0.29[a]	-0.21	-0.00	0.07	0.07	0.098
GOT-2	-0.08	0.08	-0.08	-0.04	-0.38	-0.08	0.08	0.365
GOT-7	0.07	-0.00	-0.14	-0.00	-0.07	0.07	-0.43[b]	0.043
MDH-2	0.08	-0.25	-0.08	0.08	0.17	-0.08	-0.42[a]	0.273
MDH-3	-0.00	0.19	-0.15	-0.08	0.15	-0.15	-0.46[a]	0.120
MDH-1	0.07	-0.21	-0.29[a]	-0.21	-0.00	0.07	0.07	0.098
IDH-2	0.07	-0.14	-0.14	-0.07	0.07	0.07	-0.36[a]	0.159
PGM-5	0.17	-0.25	0.17	-0.25	-0.08	-0.25	0.00	0.919
PGM-7	-0.14	-0.00	-0.00	-0.00	-0.07	-0.29[a]	-0.00	0.185
PGM-8	-0.11	-0.39	0.41[a]	-0.27	-0.18	0.05	0.00	0.117
PGM-1	0.07	-0.21	-0.29[a]	-0.21	-0.00	0.07	0.07	0.098
PGM-0	-0.21	-0.14	0.07	-0.00	-0.14	-0.07	-0.00	0.501
PGM-3	-0.07	-0.21	-0.00	-0.00	0.07	-0.21	-0.07	0.398
PGM-4	-0.07	-0.21	-0.00	-0.00	0.07	-0.21	-0.07	0.398
GDNAD-0	-0.18	-0.27	0.05	0.00	-0.11	-0.02	0.05	1.000
GDNAD-2	-0.31	-0.08	-0.15	-0.00	0.19	-0.15	-0.00	0.599
GDNAD-4	0.07	-0.14	-0.21	-0.00	0.07	-0.21	-0.07	0.501
GDNAD-5	0.07	-0.00	-0.14	-0.00	-0.07	0.07	-0.43[b]	0.043
GDNADP-3	-0.18	0.00	0.11	-0.57[b]	0.14	-0.09	0.09	0.062
GDNADP-2	-0.31	-0.00	-0.15	-0.08	0.27[a]	-0.15	-0.08	0.270
GDNADP-0	0.15	-0.00	-0.04	-0.69[b]	-0.15	0.08	0.15	0.003
ME-1-2	-0.33	0.11	-0.33	-0.00	-0.28	0.22	0.11	0.753
ME-1-0	0.07	-0.07	-0.43[b]	-0.21	0.07	-0.00	0.07	0.018
ME-1-3	-0.40	-0.00	-0.20	0.30[a]	-0.30	-0.00	0.10	0.283
ME-2-2	-0.21	0.04	-0.32	-0.14	0.05	-0.09	0.18	0.788
ME-2-1	0.07	-0.07	-0.43[b]	-0.21	0.07	-0.00	0.07	0.018
ME-2-3	-0.45[a]	0.07	-0.09	0.36[a]	-0.55[a]	0.07	0.09	0.117
ME-2-5	-0.15	-0.00	-0.15	-0.00	-0.08	-0.15	0.04	1.000
G6PDH-2	0.75[b]	-0.00	-0.17	-0.17	-0.33	-0.58[b]	-0.00	0.000
G6PDH-3	0.98[b]	0.00	0.50[b]	-0.14	-0.86[b]	-0.61[a]	0.38	0.000
G6PDH-1	0.07	-0.07	-0.43[a]	-0.21	0.07	-0.00	0.07	0.018
G6PDH-0	-0.08	0.08	-0.15	-0.04	-0.46[a]	-0.00	0.15	0.150
G6PDH-4	0.07	-0.00	-0.07	-0.07	-0.00	-0.00	-0.43[b]	0.043
PEP-1-4	0.39[a]	-0.44	-0.22	-0.06	-0.06	0.11	-0.22	0.149
PEP-1-5	0.10	0.10	-0.30	-0.10	0.10	0.20	-0.60[b]	0.033
PEP-1-2	0.15	-0.31	-0.12	-0.15	-0.00	-0.08	-0.00	0.781
PEP-1-6	-0.31	-0.15	-0.08	-0.00	-0.15	0.27[a]	-0.08	0.283
PEP-2-1	-0.38	-0.15	0.08	-0.08	-0.15	0.19	-0.00	0.357
PEP-2-2	-0.28	0.44[a]	-0.17	0.06	-0.44	-0.11	0.00	0.095
PEP-2-0	-0.39	0.44[a]	-0.11	0.11	-0.28	-0.17	-0.11	0.095
PEP-2-3	0.07	-0.07	-0.43[b]	-0.21	0.07	-0.00	0.07	0.018
6PGDH-0	-0.00	-0.11	-0.56[a]	0.11	0.17	-0.11	-0.00	0.089
6PGDH-5	-0.14	-0.00	-0.00	-0.14	-0.07	-0.14	-0.00	1.000
6PGDH-6	0.04	-0.02	-0.23	-0.13	0.04	-0.34	0.14	0.913
6PGDH-1	0.07	-0.07	-0.43[b]	-0.21	0.07	-0.00	0.07	0.018

continue...

Geary's c

	Distance classes							
Dist. Class	97	166	213	295	351	423	582	CgramProb
Loci								
GPI-7	0.88	1.13	0.88	0.75	1.25	0.88	1.25	0.708
GPI-6	0.39	0.97	0.97	1.36	0.97	0.78	1.56	0.372
GPI-4	2.00	1.50	0.00	0.50	1.50	1.00	0.50	0.501
GPI-2	0.00	2.00	2.50[a]	2.00	0.50	0.00	0.00	0.098
GPI-3	1.88[a]	0.27[a]	1.35	0.54	1.35	0.81	0.81	0.262
GOT-4	0.48[a]	0.95	1.11	1.75[a]	0.95	0.48[a]	1.27	0.115
GOT-0	0.00	2.00	2.50[a]	2.00	0.50	0.00	0.00	0.098
GOT-2	0.81	0.27[a]	0.81	2.15[a]	1.88[a]	0.81	0.27	0.233
GOT-7	0.00	0.50	1.50	0.50	1.00	0.00	3.50[b]	0.043
MDH-2	0.39	1.75[a]	1.36	0.97	0.19[a]	0.78	1.56	0.094
MDH-3	0.54	1.35	1.08	0.81	0.00[a]	1.08	2.15[a]	0.136
MDH-1	0.00	2.00	2.50[a]	2.00	0.50	0.00	0.00	0.098
IDH-2	0.00	1.50	1.50	1.00	0.00	0.00	3.00[a]	0.159
PGM-5	0.78	1.75[a]	0.78	1.17	0.78	1.17	0.58	0.094
PGM-7	1.50	0.50	0.50	0.50	1.00	2.50[a]	0.50	0.185
PGM-8	1.11	1.59[a]	0.48[a]	1.11	0.95	1.11	0.64	0.136
PGM-1	0.00	2.00	2.50[a]	2.00	0.50	0.00	0.00	0.098
PGM-0	2.00	1.50	0.00	0.50	1.50	1.00	0.50	0.501
PGM-3	1.00	2.00	0.50	0.50	0.00	2.00	1.00	0.398
PGM-4	1.00	2.00	0.50	0.50	0.00	2.00	1.00	0.398
GDNAD-0	0.95	1.11	1.11	0.64	1.11	0.95	1.11	1.000
GDNAD-2	1.62	0.81	1.08	0.54	1.35	1.08	0.54	0.754
GDNAD-4	0.00	1.50	2.00	0.50	0.00	2.00	1.00	0.501
GDNAD-5	0.00	0.50	1.50	0.50	1.00	0.00	3.50[b]	0.043
GDNADP-3	0.95	0.64	1.27	1.91[b]	0.95	0.80	0.48	0.025
GDNADP-2	1.62	0.54	1.08	0.81	1.08	1.08	0.81	0.794
GDNADP-0	0.00[a]	0.54	2.15[a]	2.96[b]	1.08	0.27[a]	0.00	0.003
ME-1-2	1.17	0.78	1.30	1.17	1.17	0.65	0.78	0.459
ME-1-0	0.00	1.00	3.50[b]	2.00	0.00	0.50	0.00	0.018
ME-1-3	1.26	0.84	0.98	0.98	1.26	0.84	0.84	1.000
ME-2-2	1.13	0.88	1.25	1.13	0.88	1.00	0.75	0.708
ME-2-1	0.00	1.00	3.50[b]	2.00	0.00	0.50	0.00	0.018
ME-2-3	1.43	0.80	0.80	1.11	1.59[a]	0.80	0.48	0.178
ME-2-5	1.08	0.54	1.08	0.54	0.81	1.08	1.88	0.582
G6PDH-2	0.58	0.58	0.97	0.97	1.36	1.94[b]	0.58	0.019
G6PDH-3	0.00[b]	0.88	0.50[b]	1.13	1.75[b]	1.50[a]	1.25	0.000
G6PDH-1	0.00	1.00	3.50[b]	2.00	0.00	0.50	0.00	0.018
G6PDH-0	0.81	0.27	1.08	2.15[a]	2.15[b]	0.54	0.00	0.060
G6PDH-4	0.00	0.50	1.00	1.00	0.50	0.50	3.50[b]	0.043
PEP-1-4	0.78	1.30	1.04	0.91	1.04	0.91	1.04	0.706
PEP-1-5	0.56[a]	0.84	1.12	1.26	0.84	0.56[a]	1.82[b]	0.011
PEP-1-2	0.00[a]	1.62	2.42[b]	1.08	0.54	0.81	0.54	0.048
PEP-1-6	1.62	1.08	0.81	0.54	1.08	1.08	0.81	0.794
PEP-2-1	1.88[a]	1.08	0.27	0.81	1.08	0.35	0.54	0.288
PEP-2-2	1.17	0.52[a]	1.04	0.78	1.30	1.04	1.17	0.133
PEP-2-0	1.30	0.52[a]	1.04	0.91	1.30	1.04	0.91	0.133
PEP-2-3	0.00	1.00	3.50[b]	2.00	0.00	0.50	0.00	0.018
6PGDH-0	1.04	0.91	1.43[a]	0.91	0.91	1.04	0.78	0.213
6PGDH-5	1.50	0.50	0.50	1.50	1.00	1.50	0.50	1.000
6PGDH-6	0.88	1.00	1.13	1.00	0.88	1.25	0.88	0.922
6PGDH-1	0.00	1.00	3.50[b]	2.00	0.00	0.50	0.00	0.018

(B)
Moran I index

	Distance classes								
Dist. Class	73	145	218	290	363	435	508	580	CgramProb
Loci									
GPI-7	0.07	-0.16	0.02	0.18	-0.18	-0.02	-0.04	-1.00[a]	0.178
GPI-6	0.14	0.07	-0.13	-0.48[a]	0.31[a]	-0.27	-0.25	-0.69	0.103
GPI-4	-0.22	-0.01	-0.09	-0.02	-0.12	-0.02	-0.04	0.07	0.967
GPI-2	0.07	-0.01	-0.36[b]	-0.29	0.02	0.07	0.07	0.07	0.019
GPI-3	-0.37	-0.09	-0.13	0.06	-0.21	0.49[b]	-0.08	-0.13	0.045
GOT-4	0.12	-0.03	-0.08	-0.15	-0.04	0.14	-0.32	-0.66	0.751
GOT-0	0.07	-0.01	-0.36[b]	-0.29	0.02	0.07	0.07	0.07	0.019
GOT-2	-0.06	-0.01	-0.02	0.20	-0.42[a]	-0.04	0.04	0.15	0.086
GOT-7	0.07	-0.01	-0.09	-0.02	-0.03	0.07	-0.36[b]	-0.73[b]	0.021
MDH-2	0.14	-0.02	-0.25	0.25	0.08	-0.27	-0.25	-0.69	0.605
MDH-3	0.05	0.20	-0.13	0.06	0.05	-0.33	-0.31	-0.71	0.407
MDH-1	0.07	-0.01	-0.36[b]	-0.29	0.02	0.07	0.07	0.07	0.019
IDH-2	0.07	-0.08	-0.14	-0.02	0.02	-0.02	-0.25	-0.46[b]	0.389
PGM-5	-0.20	0.07	0.12	-0.27	-0.09	-0.27	0.00	-0.06	1.000
PGM-7	-0.12	-0.08	0.02	-0.02	-0.12	-0.20	-0.04	0.07	0.883
PGM-8	-0.38	-0.15	0.24[a]	-0.32	-0.02	-0.12	-0.05	0.02	0.325
PGM-1	0.07	-0.01	-0.36[b]	-0.29	0.02	0.07	0.07	0.07	0.019
PGM-0	-0.22	-0.01	-0.09	-0.02	-0.12	-0.02	-0.04	0.07	0.967
PGM-3	-0.03	-0.31[a]	0.02	0.07	0.02	-0.29[a]	-0.04	-0.20	0.104
PGM-4	-0.03	-0.31[a]	0.02	0.07	0.02	-0.29[a]	-0.04	-0.20	0.104
GDNAD-0	-0.38	-0.12	-0.01	-0.09	-0.15	0.22	0.30	-0.66	0.751
GDNAD-2	-0.48[a]	-0.01	-0.13	-0.04	0.13	-0.13	-0.08	0.15	0.313
GDNAD-4	0.07	-0.08	-0.20	-0.02	-0.03	-0.11	-0.14	0.07	0.864
GDNAD-5	0.07	-0.01	-0.09	-0.02	-0.03	0.07	0.36[b]	-0.73[b]	0.021
GDNADP-3	-0.32	0.11	-0.12	-0.49	-0.08	0.07	0.36	0.09	0.451
GDNADP-2	-0.42	0.01	-0.11	-0.08	0.07	-0.09	0.15	-0.08	0.426
GDNADP-0	0.15	0.08	-0.19	-0.77[b]	-0.13	0.15	0.15	0.15	0.012
ME-1-2	-0.31	0.15	-0.20	-0.25	-0.00	0.13	-0.03	-0.17	1.000
ME-1-0	0.07	0.00	-0.42[b]	-0.14	0.02	0.07	0.07	0.07	0.007
ME-1-3	-0.38	-0.06	-0.05	0.20	-0.18	0.07	0.00	-0.10	0.938
ME-2-2	-0.35	0.22	-0.25	-0.36	0.18	-0.04	-0.33	0.23	0.788
ME-2-1	0.07	0.00	-0.42[b]	-0.14	0.02	0.07	0.07	0.07	0.007
ME-2-3	-0.43	0.00	-0.02	0.40[a]	-0.32	0.05	-0.09	0.09	0.298
ME-2-5	-0.13	-0.06	-0.16	0.15	-0.13	-0.01	-0.62[a]	0.73[b]	0.079
G6PDH-2	0.56[b]	0.25	-0.15	-0.13	-0.44[a]	-0.46[a]	0.04	0.25	0.033
G6PDH-3	0.99[b]	0.19	0.50[b]	-0.57[a]	-0.79[b]	-0.46	-0.38	-0.63	0.000
G6PDH-1	0.07	0.00	-0.42[b]	-0.14	0.02	0.07	0.07	0.07	0.007
G6PDH-0	-0.13	0.08	0.18[a]	-0.65[b]	-0.31	-0.01	0.15	0.15	0.054
G6PDH-4	0.07	0.00	-0.12	0.07	-0.04	0.07	-0.46[b]	-0.79[b]	0.008
PEP-1-4	0.39[a]	-0.22	-0.24	-0.08	0.17	-0.23	-0.44	-0.00	0.298
PEP-1-5	0.12	0.03	0.02	-0.55[a]	0.13	-0.14	-1.00[b]	0.20	0.068
PEP-1-2	0.15	-0.13	-0.24	-0.08	-0.08	-0.01	-0.04	0.15	1.000
PEP-1-6	-0.23	-0.28	-0.06	0.04	-0.08	0.20	-0.04	-0.08	0.749
PEP-2-1	-0.42	-0.13	-0.00	-0.08	0.18	-0.18	-0.23	0.15	0.426
PEP-2-2	-0.10	0.15	0.06	-0.17	-0.29	-0.11	-0.58	0.50	0.601
PEP-2-0	-0.38	0.20	0.06	0.08	-0.21	-0.23	-0.17	-0.00	0.936
PEP-2-3	0.07	0.00	-0.42[b]	-0.14	0.02	0.07	0.07	0.07	0.007
6PGDH-0	-0.10	-0.01	0.36[a]	0.08	0.17	-0.11	-0.17	-0.00	0.340
6PGDH-5	-0.20	0.00	-0.03	-0.14	-0.14	-0.08	0.07	0.07	1.000
6PGDH-6	-0.04	0.17	-0.31	-0.06	-0.04	-0.25	0.07	0.29	0.629
6PGDH-1	0.07	0.00	-0.42[b]	-0.14	0.02	0.07	0.07	0.07	0.007

Geary's c

	Distance classes								
Dist. Class	73	145	218	290	363	435	508	580	CgramProb
Loci									
GPI-7	0.85	1.07	0.94	0.78	1.11	0.94	0.94	1.88[a]	0.179
GPI-6	0.27	1.04	0.88	1.70	0.66	1.22	1.17	2.19	0.409
GPI-4	2.05	0.54	1.13	0.63	1.36	0.63	0.75	0.00	0.967
GPI-2	0.00	0.54	3.00[b]	2.50	0.34	0.00	0.00	0.00	0.019
GPI-3	1.84	0.87	1.01	0.34	1.28	0.67	0.81	1.01	0.626
GOT-4	0.43	0.68	1.19	1.59	1.08	0.40[a]	1.19	1.79	0.253
GOT-0	0.00	0.54	3.00[b]	2.50	0.34	0.00	0.00	0.00	0.019
GOT-2	0.73	0.58	0.61	1.68	2.02[b]	0.67	0.40	0.00	0.031
GOT-7	0.00	0.54	1.13	0.63	0.68	0.00	3.00[b]	5.63[b]	0.021
MDH-2	0.27	1.25	1.60[a]	0.73	0.40[a]	1.22	1.17	2.19	0.160
MDH-3	0.37	1.44	1.01	0.34	0.37[a]	1.68	1.62	3.03[a]	0.245
MDH-1	0.00	0.54	3.00[b]	2.50	0.34	0.00	0.00	0.00	0.019
IDH-2	0.00	1.07	1.50	0.63	0.34	0.63	2.25	3.75[a]	0.389
PGM-5	1.06	1.04	1.17	1.22	0.80	1.22	0.58	0.73	1.000
PGM-7	1.36	1.07	0.38	0.63	1.36	1.88	0.75	0.00	0.883
PGM-8	1.30	1.19	0.84	1.19	0.87	1.19	0.72	0.60	1.000
PGM-1	0.00	0.54	3.00[b]	2.50	0.34	0.00	0.00	0.00	0.019
PGM-0	2.05	0.54	1.13	0.63	1.36	0.63	0.75	0.00	0.967
PGM-3	0.68	2.68[a]	0.38	0.00	0.34	2.50[a]	0.75	1.88	0.104
PGM-4	0.68	2.68[a]	0.38	0.00	0.34	2.50[a]	0.75	1.88	0.104
GDNAD-0	1.30	0.85	1.07	0.80	1.08	0.60	0.95	1.79	0.855
GDNAD-2	2.20[a]	0.58	1.01	0.67	1.10	1.01	0.81	0.00	0.166
GDNAD-4	0.00	1.07	1.88	0.63	0.68	1.25	1.50	0.00	0.864
GDNAD-5	0.00	0.54	1.13	0.63	0.68	0.00	3.00[b]	5.63[b]	0.021
GDNADP-3	1.19	0.45[a]	1.41	1.91[a]	1.19	0.51	0.00[a]	0.48	0.129
GDNADP-2	2.02[a]	0.50	0.92	0.81	1.41	0.87	0.00	0.81	0.310
GDNADP-0	0.00[a]	0.25[a]	2.20[b]	3.23[b]	1.01	0.00[a]	0.00	0.00	0.011
ME-1-2	1.13	0.73	1.24	1.36	0.88	0.69	0.97	1.17	0.734
ME-1-0	0.00	0.47	3.41[b]	1.50	0.38	0.00	0.00	0.00	0.007
ME-1-3	1.23	0.92	0.95	1.05	1.05	0.75	1.05	1.26	1.000
ME-2-2	1.25	0.70	1.19	1.31	0.75	0.94	1.25	0.75	0.659
ME-2-1	0.00	0.47	3.41[b]	1.50	0.38	0.00	0.00	0.00	0.007
ME-2-3	1.39	0.89	0.87	1.19	1.19	0.85	0.80	0.48	1.000
ME-2-5	1.01	0.76	1.10	0.00	1.01	0.58	2.69[b]	2.42	0.067
G6PDH-2	0.73	0.55	0.93	0.88	1.60[a]	1.67[a]	0.49	0.00	0.174
G6PDH-3	0.00[b]	0.70	0.51[b]	1.50[a]	1.69[b]	1.34	1.25	1.50	0.000
G6PDH-1	0.00	0.47	3.41[b]	1.50	0.38	0.00	0.00	0.00	0.007
G6PDH-0	1.01	0.25[a]	0.92	2.83[b]	1.62	0.58	0.00	0.00	0.055
G6PDH-4	0.00	0.47	1.36	0.00	0.75	0.00	3.75[b]	6.00[b]	0.008
PEP-1-4	0.81	1.09	1.06	0.97	0.88	1.11	1.30	0.78	1.000
PEP-1-5	0.53	0.92	0.86	1.68[a]	0.74	1.05	2.10[b]	1.26	0.035
PEP-1-2	0.00[a]	1.01	2.39[b]	0.81	0.81	0.58	0.67	0.00	0.020
PEP-1-6	1.35	1.51	0.73	0.40	0.81	1.44	0.67	0.81	0.924
PEP-2-1	2.02[a]	1.01	0.55	0.81	1.01	1.15	1.35	0.00	0.310
PEP-2-2	0.97	0.85	0.80	0.97	1.17	1.11	1.62	0.78	0.434
PEP-2-0	1.30	0.73	0.88	0.97	1.17	1.11	0.97	0.78	0.906
PEP-2-3	0.00	0.47	3.41[b]	1.50	0.38	0.00	0.00	0.00	0.007
6PGDH-0	1.13	0.85	1.24	0.97	0.88	0.97	0.97	0.78	0.734
6PGDH-5	1.88	0.47	0.68	1.50	1.50	1.07	0.00	0.00	1.000
6PGDH-6	0.94	0.82	1.19	0.94	0.94	1.21	0.94	0.75	0.880
6PGDH-1	0.00	0.47	3.41[b]	1.50	0.38	0.00	0.00	0.00	0.007

CgramProb: global correlogram probability; *a*: $P < 0.05$; *b*: $P < 0.01$

to those obtained with the Moran's I index and with 7 DC. On the contrary, when only 6 DC were considered, with this coefficient, some spatial significant structures changed. *Pgm 5* and *Pep-1 5* showed significant global correlograms as regional patches, and differentiation at long distance, respectively. Furthermore, with 6 DC, 13 alleles showed significant global correlograms, and 19 alleles showed a tendency to spatial arrangements of no statistical significance.

There seems to be a slightly better chance to reveal spatial structures, or trends, with the use of the coefficient c of Geary than with the I of Moran. For instance, with 6 DC some of the alleles which were not detected with the Moran's I index were *Got 4* (high similarity among neighbor stocks), *Got 7* (differentiation at long distance), *Got 0* (regional patch), *Got 2* (circular cline), *Pgm 8* (regional patch) and *Gdh-Nadp+ 3* (circular cline). It is interesting to note that each allele of the *Got* locus yielded a different spatial trend. The arrays of alleles that presented strong linkage in their spatial structures were basically the same by using the Moran's I index and the Geary's c coefficient, and were the same independently of 6, 7 or 8 DC chosen. When no distance classes were considered, the individual autocorrelation coefficient for each allele consisted in 105 pair comparisons. For the Moran's I Index, the alleles *Gdnad 2* (P = 0.048), *Gdnadp 2* (P = 0.05), *G6pdh 3* (P = 0.01) and *6Pgdh 5* (P = 0.008) were significant. This means that these were the alleles which displayed more significant spatial structures. For the Geary's c coefficient, the alleles *Gdnad 2* (P = 0.014), *Gdnadp 2* (P = 0.014), *G6pdh 3* (P = 0.013) and *6Pgdh 5* (P = 0.008) were also significant (Table VII). Altogether, it can be stated that the amount of spatial structure found in Colombia for *T. cruzi* is noteworthy significant. Some of the overall results support this view. The percentage of significant coefficients of autocorrelation was superior to the error type I of 5%, both for the Moran's I and for the c of Geary for 6 DC (17.3%; 53/306; $\chi^2 = 23.4$; 1 df; P <0.001), for 7 DC (Moran: 10.9%; Geary: 15.4%) and for 8 DC (Moran: 10.5%; Geary: 12.5%). A percent value above the error type I of 5% was also found for both indices related with the number of significant global correlograms for 6 DC (Moran: 23.5%; 12/51; $\chi^2 = 7.2$; 1 df; P <0.01; Geary: 25.5%; 13/51; $\chi^2 = 8.3$; 1 df; P <0.01), for 7 DC (Moran: 23.5%; Geary: 27.4%) and for 8 DC (Moran: 31.4%; Geary: 33.3%). Therefore, there is not doubt about the striking spatial structure among the Colombian *T. cruzi* stocks, when the allele frequencies are employed.

It is of upmost importance to determine whether it is better to obtain the spatial autocorrelation by using the allelic frequencies or the genotypic multistates. We find the use of the allelic frequencies much more informative than that of genotypic states, since for the latter we have assigned the value of each state randomly, while ignoring the type of order and evolutionary transition that might have existed between the different genotypes. In contrast, with the results obtained when using the genotypic multistates, the analysis of correlogram similarity based on the allelic frequencies showed significant percent values of Manhattan distances. For instance, for the Moran's I Index, a percent of Manhattan distances smaller than 0.1 of 8.8% (113/1275) was obtained for 6 DC and 8.4% (107/1275) for 7 DC, being both significantly superior to the error type I of 5% ($\chi^2 = 14.6$; 1 df; P < 0.001 and $\chi^2 = 11.7$; 1 df; P < 0.01). There is, therefore, a significant fraction of variables with the same patterns of spatial arrangement. For 6 DC, and with the Moran's I coefficient, there was the presence of ten clusters of highly spatial related alleles (Fig. 2b): (1) *Gpi 6* and *Mdh 3*; (2) *Got 4, Got 7, Gdh-Nad+ 5, G6pdh 4, Pep-1 5* and *Idh 2*. This array was comprised by the variables that have spatial structure of differentiation at long distance. Except in a few cases, it was observed that most spatial clusters contained alleles from different loci, a manifestation of linkage disequilibrium; (3) *Gpi 2, Got 0, Mdh 1, Pgm 1, Me-1 0, Me-2 1, 6pgdh 1, Pep-2 3, Gdh-Nad+ 4* and *6pgdh 0*. These were the alleles which typically showed correlograms describing regional patches: (4) *Gpi 4, Pgm 0, Gdh-Nad+ 0, Pgm 7, Pgm 3, Pgm 4, 6pgdh 5*, and *Pep-2 0*; (5) *Gdh-Nad+ 2* and *Gdh-Nadp+ 2*; (6) *Me-2* and *6pgdh 6*; (7) *Gpi 3, Pep-1 6, Pep-2 1, Me-1 3* and *Me-2 3*; (8) *Me-1 2* and *Me-2 2*; (9) *Pgm 5* and *Pep-1 2* ; and (10) *Got 2* and *G6pdh 0*.

When 7 DC were used, eight clusters of highly spatial-related alleles were conformed with the Moran's I Index and nine with the Geary´s c coefficient. Some of these allele-spatial related clusters were slightly different among them (Fig. 2C,D).

In addition, we studied the similarity among the various distance classes to understand the contribution of each one to the spatial behaviour of the genotypes and allele frequencies studied (Fig. 3). For the analysis with 7DC, the most divergent distance class, simultaneously for genotypes and for allele frequencies, was the 7 DC. Therefore, this is the distance class with the most differentiated spatial behaviour, which is in agreement with certain isolation-by-distance structure.

DISCUSSION

Some of the results of the population genetics analysis presented here support the point of view of a clonal structure of the Colombian *T. cruzi* stocks proposed by Tibayrenc (1996) and Tibayrenc and Ayala (1988, 1991).

TABLE VII

Single autocorrelation coefficients for each one of the 51 isoenzymatic alleles analyzed in 23 Colombian *Trypanosoma cruzi* stocks considering the allele frequencies. The point pairs were weighted as the inverse square separation distance between the stocks

	Moran's I Index		Geary's c Coefficients	
Locus	I	Global probability	c	Global probability
GPI-7	0.26	0.189	0.65	0.160
GPI-6	0.16	0.249	0.32	0.101
GPI-4	-0.11	0.399	1.26	0.399
GPI-2	0.03	0.247	0.31	0.247
GPI-3	-0.14	0.412	1.04	0.475
GOT-4	0.19	0.235	0.36	0.077
GOT-0	0.03	0.247	0.31	0.247
GOT-2	0.04	0.357	0.43	0.420
GOT-7	0.05	0.200	0.16	0.200
MDH-2	0.14	0.270	0.38	0.122
MDH-3	0.11	0.277	0.38	0.181
MDH-1	0.03	0.247	0.31	0.247
IDH-2	0.03	0.234	0.27	0.234
PGM-5	0.05	0.363	0.69	0.202
PGM-7	-0.03	0.379	0.69	0.379
PGM-8	-0.01	0.428	0.77	0.304
PGM-1	0.03	0.247	0.31	0.247
PGM-0	-0.11	0.399	1.26	0.399
PGM-3	-0.01	0.340	0.59	0.340
PGM-4	-0.01	0.340	0.59	0.340
GDNAD-0	-0.52	0.105	1.50	0.099
GDNAD-2	-0.55	0.058	2.50[a]	0.014
GDNAD-4	0.03	0.232	0.27	0.232
GDNAD-5	0.05	0.200	0.16	0.200
GDNADP-3	-0.53	0.117	1.61	0.104
GDNADP-2	-0.58	0.060	2.60[a]	0.014
GDNADP-0	0.11	0.287	0.19	0.133
ME-1-2	-0.44	0.177	1.32	0.207
ME-1-0	0.04	0.243	0.25	0.243
ME-1-3	-0.49	0.146	1.41	0.167
ME-2-2	-0.51	0.137	1.41	0.141
ME-2-1	0.04	0.243	0.25	0.243
ME-2-3	-0.51	0.128	1.58	0.115
ME-2-5	-0.04	0.467	0.70	0.340
G6PDH-2	-0.09	0.483	1.49	0.197
G6PDH-3	0.86[a]	0.010	0.16[a]	0.013
G6PDH-1	0.04	0.243	0.25	0.243
G6PDH-0	-0.40	0.161	1.96	0.093
G6PDH-4	0.05	0.210	0.13	0.210
PEP-1-4	-0.10	0.473	1.17	0.334
PEP-1-5	-0.29	0.287	1.15	0.362
PEP-1-2	0.09	0.309	0.26	0.154
PEP-1-6	-0.05	0.469	0.71	0.345
PEP-2-1	-0.13	0.425	1.02	0.489
PEP-2-2	-0.46	0.167	1.37	0.172
PEP-2-0	-0.52	0.133	1.45	0.128
PEP-2-3	0.04	0.243	0.25	0.243
6PGDH-0	-0.42	0.188	1.41	0.146
6PGDH-5	-0.44[b]	0.008	3.61[b]	0.008
6PGDH-6	0.29	0.182	0.60	0.146
6PGDH-1	0.04	0.243	0.25	0.243

a: $P < 0.05$; *b*: $P < 0.01$

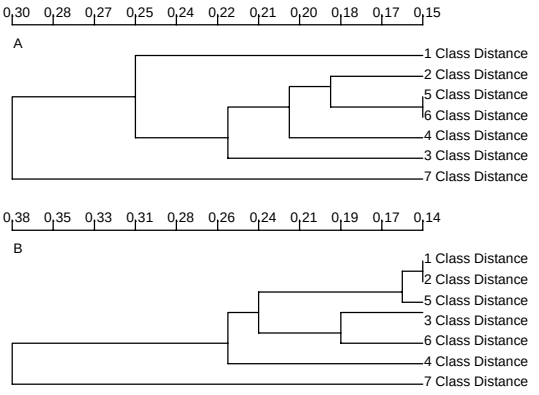

Fig. 3: UPGMA analysis of the contribution of each one of the diverse seven distance classes considered in the spatial structure found among 23 Colombian *Trypanosoma cruzi* stocks; A: with 13 genotypic unordered states; B: with 51 allele frequencies

Clonal population structure

Hardy-Weinberg equilibrium - We found total absence of Hardy-Weinberg equilibrium in the stocks of the Colombian *T. cruzi*. This is a clear evidence of clonal structure. When the absence of equilibrium Hardy-Weinberg of this parasite is seen in the context of the genetic structure of its vectors and hosts an additional result is obtained, which indirectly supports the view of the clonal structure. Dujardin et al. (1987) studied the genetic structure of the vector *T. infestans* in Bolivia and demonstrated that it is in Hardy-Weinberg equilibrium. Tibayrenc et al. (1991) found in Hardy-Weinberg equilibrium the populations of humans exposed to various species of *Trypanosoma*, having studied several markers in more than 1,600 people in South America, Europe, Africa and French Polynesia. Previous studies of human populations in Colombia, by other authors and ourselves, have found these Colombian human populations to be in Hardy-Weinberg equilibrium for most markers used (Castillo & Ruiz-García 2000, for HLA DQa, LDLR, GYPA, HBGG, D7S8 and Gc; Jaramillo-Correa et al. 2000, for APO-E and ACE; Duran & Ruiz-García 2000 for VWA and TH01). That is to say, apparently the genetic structure of human populations did not condition the absence of Hardy-Weinberg equilibrium in the Colombian *T. cruzi* stocks. Perhaps, if some vector, or host, were not in H-W equilibrium, it could either reinforce the clonal structure, or hide the presence of a panmictic structure to a certain degree. There are indications within our data to support this view. The three stocks, with less genetic diversity (a value of zero), were obtained from *R. prolixus*, whereas the rest of the stocks, with some degree of identical genetic diversity were from humans and from *D. marsupialis*.

Tibayrenc and Ayala (1991) have put forward the view that the excess of homozygotes is not explained by the Wahlund effect. We also believe that the Wahlund effect is not a reasonable candidate to explain the evolution of the current genetic profiles found in *Trypanosoma*, although we do not share their explanation for it. The Wahlund effect could in fact be taking place in certain points of the distribution of a species, but not necessarily at every point within its range of distribution; this conjunction could however be the product of a clonal structure, unless the structure of the species is comprised by microdemes with high reproductive isolation (Ehrlich & Raven 1969), and the sampling procedure provided was not fine enough at the microgeographical level. The presence of loci fixed in heterozygosity is not sufficient to state that this might not be constituted by Wahlund effect. Negative Wahlund effect due to migration and in-breading among individuals from different genetic pools (Ruiz-García & Alvarez 2000), patterns with phylopatric females and migratory males (Chesser 1991a,b), overlaping of different generations with cross breading (Ennos 1985, Lopez-Alonso & Pascual-Reguera 1989) can generate patterns of excess of heterozygotes. This is not parsimonic enough, and is the reason why the Wahlund effect does not seem to be the cause for the observed patterns, is the possibility that simultaneous positive and negative Wahlund effect might be taking place, at such magnitude, for different loci of the same stocks.

Linkage disequilibrium - The existence of strong linkage disequilibrium seems to be an essential feature of the clonal structure. Tibayrenc et al. (1986a,b) applied the method of Ohta (1982) to detect linkage disequilibrium in subdivided populations and found high levels of disequilibrium for the stocks they analyzed, even higher than those found in self-fertilizing species such as *Hordeum spontaneum*. We have not analyzed here the linkage disequilibrium in particular, but have found a significant percentage of variables with identical correlograms, and are therefore affected by the same type of evolutionary events. The high degree of spatial structure similarity in many of the alleles analyzed for different loci could be due to the presence of a significant linkage disequilibrium. Ruiz-García and Klein (1997) demonstrated that the absence of gametic disequilibrium in populations of cats was associated to the non existence of similar correlograms between the loci analyzed. Our results are in agreement with those of Márquez et al. (1998) and Lewicka et al. (1995), who encountered considerable linkage disequilibrium to analyze 24 *T. cruzi* stocks in French Guiana. However, it is impossible to rule out the possibility of some re-

sidual sexuality, especially in the sylvatic cycles. Bogliolo et al. (1996) and Carrasco et al. (1996) have reported some possible cases of hibridization. The last authors analyzed 36 Amazonian *T. cruzi* stocks, and, apparently, found the *Pgm* locus in Hardy-Weinberg equilibrium, and some RAPD profiles seemed parentally homozygotes and its hybrids heterozygotes. As we show in Fig. 4, the Colombian *T. cruzi* are strongly related with the Bolivian SO34 *T. cruzi*, which represented the Z1 zymodeme of sylvatic origin, and not with some Chilean MN stocks representing the Z2 zymodeme of domestic origin. This result agrees with those obtained in the present study with 23 new Colombian *T. cruzi* stocks, and also with that reported by Márquez et al. (1998) who found that the major part of the Colombian stocks they studied were closely related to the Z1 zymodeme of sylvatic origin. This means that sylvatic zymodemes are the most frequent in domestic conditions in Colombia, and the probability of some genetic recombinant events could be more frequent due to this sylvatic origin than in the domestic zymodemes (Z2, Z3), which prevail in other Latin American countries (Chile, Argentina, part of Bolivia, etc.). In addition, this dendrogram puts forward that the 23 *T. cruzi* stocks surveyed here were not especially related to other forms of *Trypanosoma*, such as *T. rangeli* or *T. cruzi marenkellei*. This last taxa was represented by *Trypanosoma* sp. from Antioquia and conformed a different cluster.

Genetic heterogeneity - The mean genetic heterogeneity found in this study (G_{ST} = 0.934) is one of the highest values ever reported for any organism, the values for this statistic being commonly lower than 0.1. This is another symptomatic event of clonal structure. To illustrate this, the values found in various animal species are as follows: cats in Spain, Balearic Islands and Italy (G_{ST} = 0.03, 0.02 and 0.03, respectively, Ruiz-Garcia 1993, 1994a, Randi & Ragni 1991), Yanomamas (G_{ST} = 0.069, Weitkamp et al. 1972), *Drosophila pseudoobscura* (G_{ST} = 0.008 Alvarez et al. 2000), brown trout (G_{ST} = 0.041, Chakraborty et al. 1982), *Canis latrans* (G_{ST} = 0.08, Hamilton & Kennedy 1986). In mammals, two of the highest values reported for this statistic have been for *Mus musculus* in Europe (G_{ST} = 0.465, Britton-Davidian 1990), and *Dipodomys ordii* (G_{ST} = 0.674, Johnson & Selander 1971). Some of the values reported for plants have been *H. spontaneum* (G_{ST} = 0.25, Baum et al. 1997; G_{ST} = 0.284, Nevo et al. 1986), and *Triticum turgidum* (G_{ST} = 0.37, Nevo et al. 1988). None of these values get near that obtained for *Trypanosoma*. As observed in the study of Tibayrenc and Ayala (1988), the range of the genetic distances found is enormous, and some of the values are extremely high (more than 1.8 for the Nei's distance). This is another evidence of clonal structure. For example, Ayala (1975) found in *Drosophila*, for this distance, mean values of 0.581 for sinmorphic species, and 1.056 for alomorphic species. Some of the values we found are higher, though it does not mean that we have stocks from different species. The continuum values for these distances between pairs of stocks suggest that we face a species of exceedingly high heterogeneity due to its clonal structure. The only values which were not continuous were those between the two stocks of *Chrithidi*a sp. and the remaining stocks of *T. cruzi*, portraying the difference between the two genera. This continuity of values reflecting an enormous heterogeneity, does not allow typological aproximations to be made in order to describe the genetic variability of *T. cruzi*. Attempts made by Miles et al. (1977), and Ready and Miles (1980), resulted obsolete as the number of markers and geographical areas analyzed increase. We here entirely agree with the conceptual definition of the clonet, put forward by Tibayrenc and Ayala (1991), above the typical definitions of population or species as the evolutionary unit for this organism.

Spatial autocorrelation analysis - However, two typical clonal characteristics are not apparently observed in the present data, neither in the data from Márquez et al. (1998), with the exeption, maybe, of their clonet 17. We did not found an over-representation of some identical multi-genotype zymodemes in vast areas of Colombia with obvious different enviromental characteristics. Our new Colombian zymodemes are not geographically randomly distributed. As we previously commented, an elevated fraction of the correlograms obtained showed the classic regional patch structure. However, lack of repeated genotypes can be seen in clonal populations when clonal diversity is significant as in the present case, since every stock corresponds to a different genotype.

Otherwise, another clonal feature is not clearly present in our data. There is not a persistence of the same multi-genotypes zymodemes through time in some populations analyzed in different years. It is clearly supported by the dendrograms shown in Fig. 1, where the zymodemes of Ricaurte (Cundinamarca), Tibu and Durania (North of Santander), and Sabanalarga (Casanare) (three different areas of distribution of *T. cruzi* in Colombia) sampled with some year intervals were not clustered together. In a parallel study, we have investigated some zymodemes of other Colombian stocks of *T. cruzi*, *T. rangeli* and some stocks classified as *Trypanosoma* sp. sampled in different areas, at different times (such as *Trypanosoma* sp. in Antioquia, *Trypanosoma* sp. in Ubaqué, *Trypanosoma* sp. in

Arboledas). Fig. 4 shows that the stocks sampled in a same locality through years were not clustered jointly. This is apparently in contradiction with all the characteristics of the clonal population structure discovered in other Latin America areas.

The extraordinary genetic diversity showed at the isoenzymatic level in America by Tibayrenc and collaborators for *T. cruzi*, together with the 20 new zymodemes found in Colombia alone and presented in this study, could also suggest the possibility of extensive genetic variability of those loci involved in the growth regulation of *T. cruzi*, its development in specific vectors, as well as those governing diverse pathogenic properties of the organism, which could in turn explain the variety of forms in which Chagas disease manifests itself. This could be so, particularly if we accept the clonal structure, given the possible correlation between groups of variables. A thorough procedure of sampling of populations within the range of distribution of the parasite is therefore of enormous importance to accurately understand the genetic characteristics of *T. cruzi* in different areas of the American continent.

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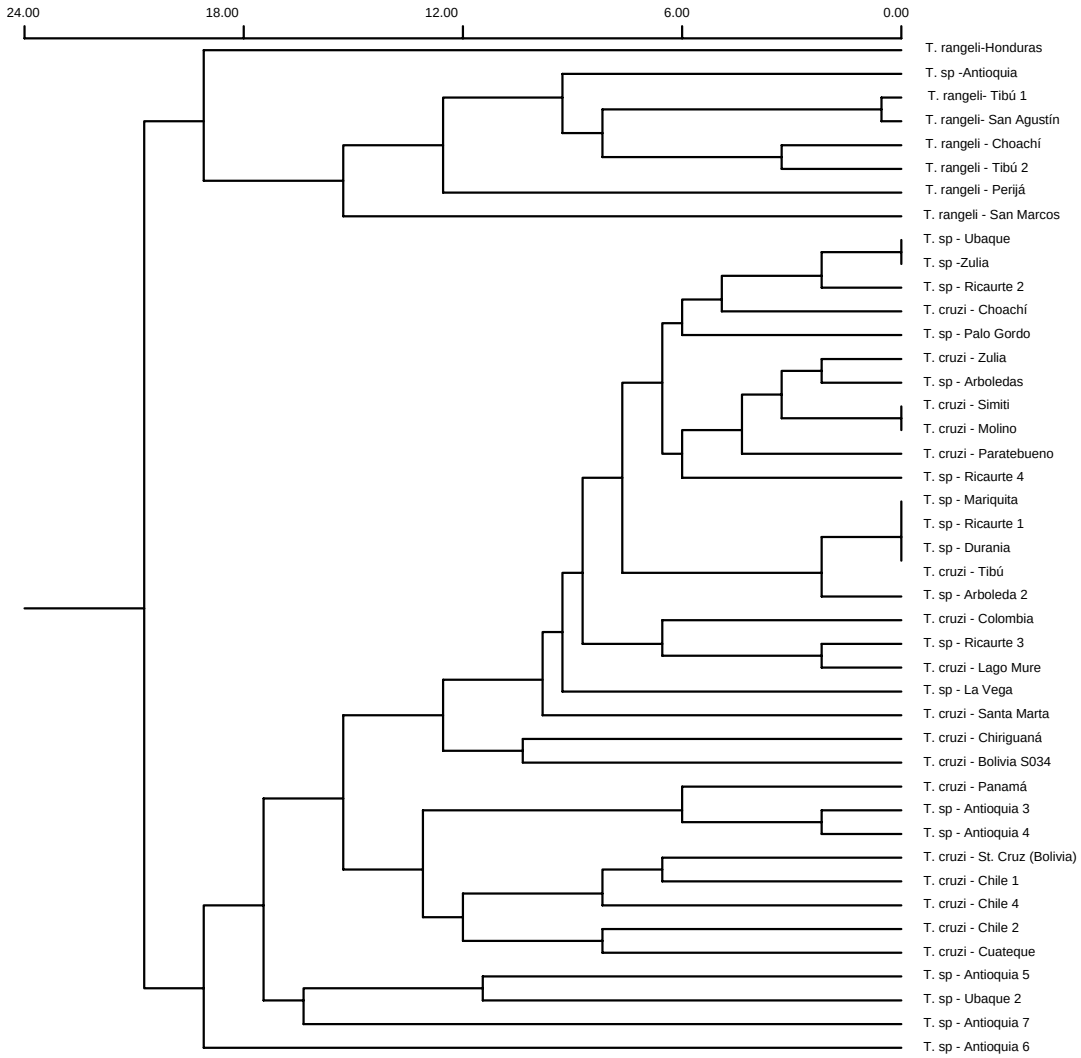

Fig. 4: UPGMA analysis by using the Rogers's genetic distance of 41 Colombian stocks of *Trypanosoma rangeli*, *T. cruzi* and *Trypanosoma* spp. This analysis shows the nonexistence of constant genetic profiles through time and the clear differentiation between *T. rangeli* and *T. cruzi*.

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