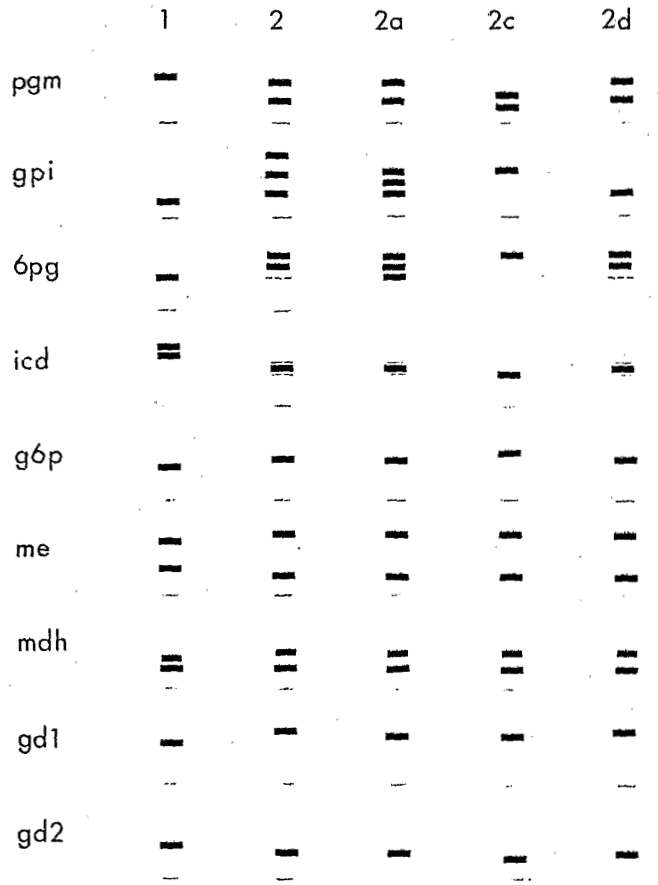

Fig. 2. The main isozyme patterns found in the 212 *Trypanosoma cruzi* stocks. The ME and MDH patterns are determined each by two gene loci.

other patterns shown in Fig. 2 (IS 1, 2, 2a, and 2c) had previously been found in Bolivia, but we did not find IS 1b, 1c, and 2b, which had been observed in other samples (TIBAYRENC *et al.*, 1983).

Lack of genetic recombination

There is no evidence of genetic recombination or mating. Frequently, two different IS are found in the same triatomine bug (see Table I), but the isozyme patterns show juxtaposition of the two IS without genetic recombination. A telling enzyme is GPI: recombination between IS 1 and IS 2c would give a three-banded pattern, because it is a dimer enzyme. Yet the mixture observed show a two-banded pattern (see Fig. 3), in spite of the maximum opportunity of recombination between the two strains in such cases (TIBAYRENC *et al.*, 1985).

It is not possible, of course, to prove for the whole *T. cruzi* taxon that mating never occurs. The present lack of evidence for recombination corroborates what had been previously observed in Bolivia (TIBAYRENC *et al.*, 1981, 1983). We cannot discard the possibility of genetic recombination in other ecosystems; but in the Bolivian domestic transmission cycles, the population structure of *T. cruzi* is basically clonal. If a sexual process occurs at all, it must be exceptional, given that it has never been observed in spite of multiple opportunities.

Spatial patterns

In order to ascertain whether any spatial patterns exist in the distribution of isozymic strains, we have combined 207 previously studied stocks with the 212 stocks now assayed (Table I). We have considered only three isoenzymic strains (1, 2 and 2a) and combined 2 and 2a because they are closely related; 2 and 2a differ by only one allele. Their genetic distance, according to TIBAYRENC & LE RAY (1984) is $D = 0{\cdot}11$ (this measure gives the average number of codon differences per gene between two populations: NEI, 1972), where IS 1 differs from 2 and 2a by 19 alleles out of a possible 22 ($D = 1{\cdot}63$, TIBAYRENC & LE RAY 1984). Isoenzymic strains 2 and 2a also have the same heterozygosity, 4 loci out of 14, while IS 1 is heterozygous at only one locus. The difference is not that large, but it is nevertheless convenient to refer to 2 and 2a as "heterozygous strains." We have not considered IS 2c, because it did not seem appropriate to combine it with IS 2 and 2a given that is less heterozygous than these two (and is not closely related

Table I—Number (and frequency, in parentheses) of stocks of each isozymic strain found in each locality. The samples marked with an asterisk (*) are from a previous study. No mixed stocks appear in the samples from the previous study, because they could not be detected with the techniques then used. In Chiwisivi, some houses were visited more than once, at different times. The locality numbers refer to Fig. 1.

Locality	Isozymic strains						Mixed stocks	Date of collecting	Number suburbs or villages	Number of houses
	IS 1	IS 2	IS 2a	IS 2c	IS 2d	Total				
1 Yungas 1	6 (0·43)	8 (0·57)	0	0	0	14	0	Nov. 1981, Apr. & July 1983	6	8
Yungas 2*	4 (0·57)	3 (0·43)	0	0	0	7	—	July 1981	3	3
Total Yungas*	10 (0·48)	11 (0·52)	0	0	0	21	—		9	11

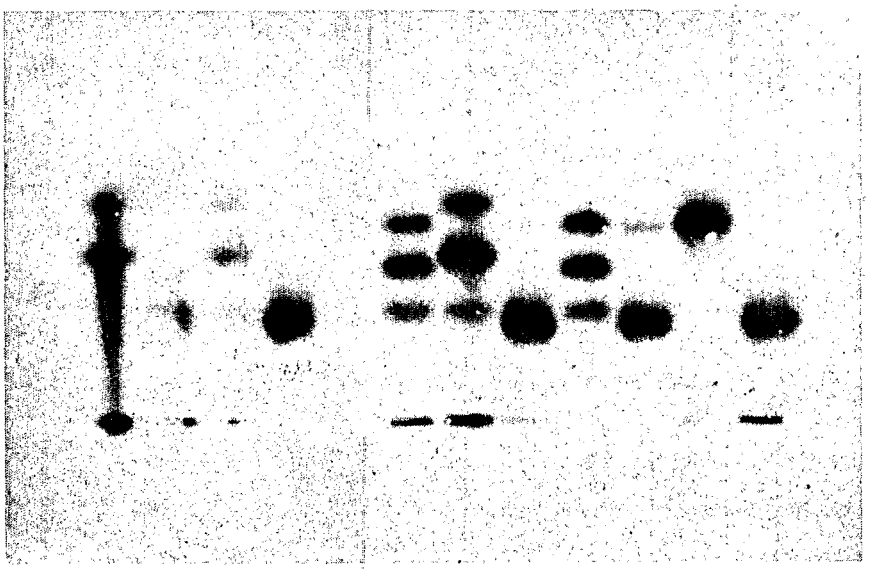

Fig. 3. Isozyme patterns for GPI. The products of four alleles are shown; the bands encoded by alleles *1* and *4* migrate the fastest and the slowest, respectively. Samples are numbered from left to right. Slots 1, 3, and 7: heterozygous genotype *1/3* (three-banded), IS 2. Slot 2: homozygous genotype *3/3*, IS 2d. Slots 4 and 8: homozygous genotype *4/4*, IS 1. Slots 6 and 9: heterozygous genotype *2/3* (three-banded), IS 2a. Slots 10 and 12, mixed stocks with genotypes *2/2* and *4/4* (two-banded patterns; IS 1 and 2c). Slot 11: Homozygous genotype *2/2* (IS 2c).

to them, $D = 0{\cdot}42$). When IS 2 and 2a are compared to IS 1, there is significant heterogeneity across localities ($\chi^2 = 142$, with 8 degrees of freedom, $P<0{\cdot}001$).

We have correlated the frequency of IS 2 and 2a with various parameters: latitude, longitude, altitude, and data for climatic factors obtained from the Bolivian Meteorological Institute (Table II). We have only geographical data for the Chiwisivi site, and Yungas has been excluded because this area encompasses several collection sites with only a small sample from each site. The spatial variation in the frequency of IS 2 and 2a is negatively correlated with altitude ($r = -0{\cdot}76$, with 6 degrees of freedom, $P<0{\cdot}05$) and longitude ($r = -0{\cdot}88$, with 6 degrees of freedom, $P<0{\cdot}01$); no correlation was apparent with latitude. The altitudinal and longitudinal trends may not be independent from one another, given that the localities sampled increase in altitude towards the tops of the Cordillera Real as their longitude increases from east to west. The altitudinal trend is consistent with the intermedite frequency of heterozygous strains at Yungas, where the sites range from 1200 to 1800 m.

The climatic variables are highly intercorrelated. Because only a small number of sites were sampled we decided to correlate the frequency of heterozygous strains with a weighted average to the climatic factors. A weighted average that accounts for most of the variation in the climatic variables was obtained by a This component is significantly positively correlated with the frequency of the heterozygous strains ($r = 0{\cdot}81$, with 5 degrees of freedom, $P< 0{\cdot}05$). These findings suggest a climatic association for the isozyme patterns, although more extensive sampling would be required in order to confirm association; it would be, for example, interesting to sample populations from the same altitude and similar climates but with different longitudes.

Temporal variation.

We have three samples from Chiwisivi: March 1981, June 1981 and October 1982. IS 1 is the most common strain by far in all three samples; the only other strain found is IS 2. The very low frequency of IS 2 makes it impossible to attempt any meaningful analysis to ascertain whether the relative frequency of the two strains changes with time.

Conclusions

The extensive sampling of more than 400 stocks (previous and present data) of *T. cruzi* lead to the following conclusions concerning the domestic transmission cycles of Chagas' disease in Bolivia: (i) population structure is basically clonal, without any indication of genetic recombination between the isozymic strains; (ii) different isozymic strains are found sympatrically, often in the same house and in the same triatomine bug; (iii) there is no vector

e ordered according
ed at different sites

Average humidity (%)
39·6
51·9
—
50·2
54·0
65·1
69·1
73·5

al. (1977, 1981; MILES, 1983), found that heterozygous strains are rare or absent in the Amazon Basin, which is at low altitude and has a climate comparable to that of low-altitude populations in Bolivia.

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