TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1986), 80, 442-447

Additional data on *Trypanosoma cruzi* isozymic strains encountered in Bolivian domestic transmission cycles

M. TIBAYRENC[1,2], A. HOFFMANN[2], O. POCH[3], L. ECHALAR[4], F. LE PONT[1], J. L. LEMESRE[5], P. DESJEUX[6] AND F. J. AYALA[2]

[1]*ORSTOM, IBBA, Embajada de Francia, Casilla 824, La Paz, Bolivia;* [2]*Department of Genetics, University of California, Davis, California 95616, USA;* [3]*Université Louis Pasteur, 67000 Strasbourg, France;* [4]*Universidad Mayor de San Andrès, La Paz, Bolivia;* [5]*CIBP, Institut Pasteur, 15 rue Camille Guérin, 59019 Lille, France;* [6]*IBBA & Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France*

Abstract

We have collected in Bolivia 212 stocks of *Trypanosoma cruzi* from domestic transmission cycles and have assayed for nine enzyme systems (11 gene loci). Only a few different isozyme profiles exist, without recombination between them, a situation also encountered in previous Bolivian samples. The 212 stocks, combined with 207 stocks previously studied, have been analysed to uncover any spatial patterns. The frequency of heterozygous strains (2 and 2a) decreases westwards and with increasing altitude. Given that longitude and altitude are correlated with each other, it is not possible to decide which of these two geographic variables is the relevant one, or if both are. These associations might be due to climatic factors. Studies by other authors have shown, however, that heterozygous strains are rare or absent in the Amazon Basin, which is at low altitude.

Introduction

MILES and his collaborators (1977, 1981; MILES, 1983) have conducted extensive isozyme studies of strains of *Trypanosoma cruzi*, the agent of Chagas' disease, collected from the Amazon Basin, mostly in silvatic transmission cycles. For comparison purposes, we have studied the strains collected in Bolivian transmission cycles (TIBAYRENC & DESJEUX, 1983; TIBAYRENC *et al.*, 1983). We present here the final results of this study.

Materials and Methods

The stocks are all isolated from the main domestic vector of Chagas' disease, *Triatoma infestans*, using a previously described method (TIBAYRENC *et al.*, 1982). The collecting localities are shown in Fig. 1. The stocks were grown in LIT monophasic culture medium. They were harvested by centrifugation, lysed with hypotonic enzyme stabilizer (GODFREY & KILGOUR, 1976) and stored at −70°C until used. A single culture tube provided sufficient material to assay all nine enzymes.

The enzymes assayed are: glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), glucose phosphate isomerase (GPI, E.C.5.3.1.9), glutamate dehydrogenase $NADP^+$ (GDP $NADP^+$, E.C.1.4.1.2), glutamate dehydrogenase NAD^+ (GDP NAD^+, E.C.1.2.1.2), isocitrate dehydrogenase (ICD, E.C.1.1.1.42), malate dehydrogenase (MDH, E.C.1.1.1.37), malate dehydrogenase (oxaloacetate decarboxylating) $NADP^+$ or malic enzyme (ME, E.C.1.1.1.40), phosphoglucomutase (PGM, E.C.2.7.5.1), and 6 phosphogluconate dehydrogenase (6PG, E.C.1.1.1.44). These nine enzymes yield 11 genetic loci; two loci are present for each MDH and ME.

Electrophoresis was done on HELENA cellulose acetate plates. Most of the recipes are from LANHAM *et al.* (1981), as modified by TIBAYRENC & LE RAY (1984). We used two reference strains in each plate: Tehuentepec and Tulahuen, respectively isozyme strains 1e and 2a according to the classification of TIBAYRENC & LE RAY (1984).

Results and Discussion

Enzyme profiles

As in previous studies (TIBAYRENC *et al.*, 1983), there were very few different isozyme profiles: each profile is called an "isozymic strain" (IS), without prejudging the taxonomical or medical significance. Fig. 2 depicts the patterns of the five isozyme strains among the 212 stocks studied; for a genetic interpretation, see TIBAYRENC & LE RAY (1984). Table I gives the number of stocks exhibiting each particular profile. IS 2d, present only in two stocks from Camiri, had not been observed in any previous collection; it differs from IS 2 at the *Gpi* locus, where IS 2d is homozygous but IS 2 is heterozygous (see Fig. 3). The

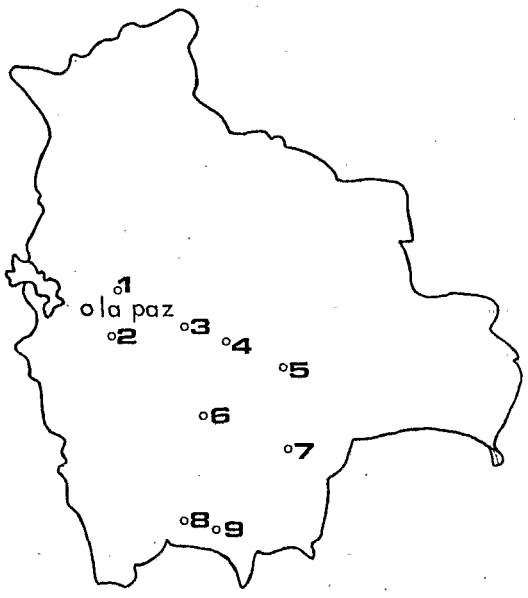

Fig. 1. Map of Bolivia showing the collecting sites. 1, Yungas; 2, Chiwisivi; 3, Cochabamba; 4, Comarapa; 5, Santa Cruz; 6, Sucre; 7, Camiri; 8, Tupiza; 9, Tarija.

[2]Address for reprint requests.

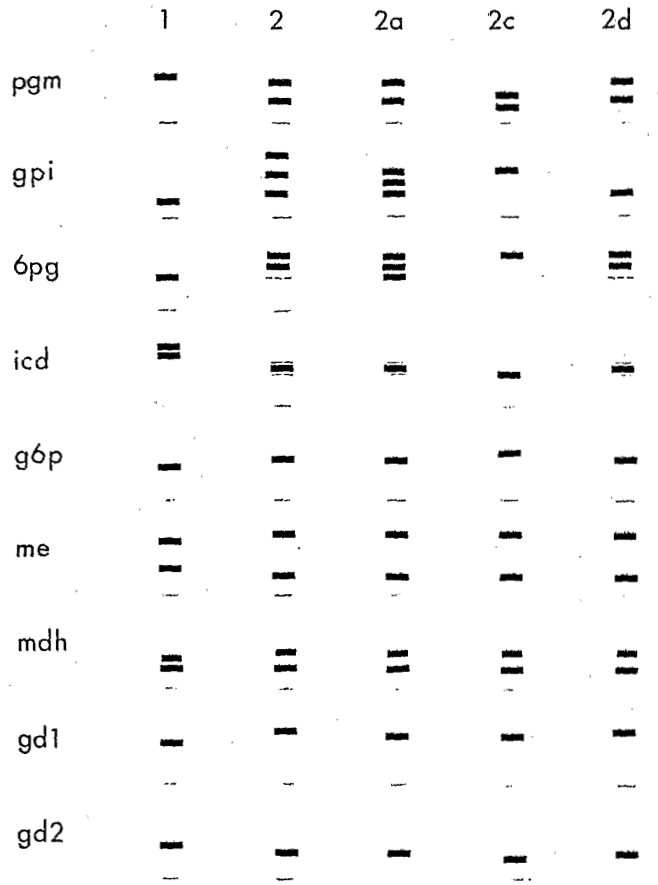

Fig. 2. The main isozyme patterns found in the 212 *Trypanosoma cruzi* stocks. The ME and MDH patterns are determined each by two gene loci.

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
2 Chiwisivi	33 (1)	0	0	0	0	33	0	June 1981	1	2
Chiwisivi	63 (0·99)	1 (0·01)	0	0	0	64	1	Oct 1982	1	4
Chiwisivi*	45 (0·98)	1 (0·02)	0	0	0	46	—	March 1981	2	11
Total Chiwisivi	141 (0·99)	2 (0·01)	0	0	0	143	—		2	13
3 Cochabamba	8 (0·57)	5 (0·36)	1 (0·07)	0	0	14	0	Nov 1982	2	5
4 Comarapa	9 (0·43)	8 (0·38)	0	4 (0·19)	0	21	7	Nov 1982	4	9
5 Santa Cruz*	3 (0·13)	20 (0·87)	0	0	0	23	—	May 1981	1	4
6 Sucre	23 (0·49)	16 (0·34)	0	8 (0·17)	0	47	9	Nov 1982	7	17
7 Camiri	4 (0·24)	6 (0·35)	7 (0·41)	0	2	19	3	Nov 1982	3	6
8 Tupiza*	45 (0·45)	31 (0·31)	14 (0·15)	9 (0·09)	0	99	—	Dec 1981	16	42
9 Tarija*	8 (0·25)	20 (0·63)	4 (0·12)	0	0	32	—	Dec 1981	4	12
TOTAL:	251 (0·60)	119 (0·29)	26 (0·06)	21 (0·05)	2 (0·005)	419	—	—	48	119

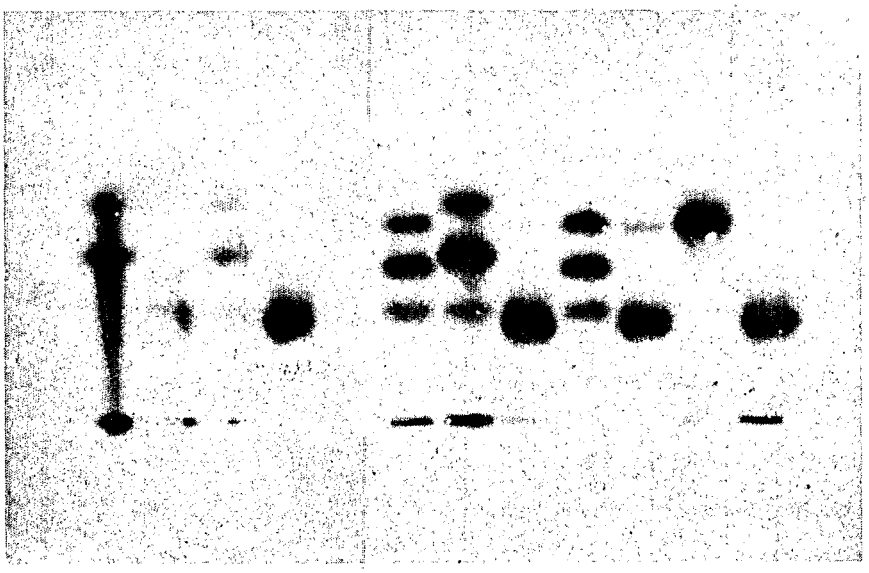

Fig. 3. Isozyme patterns for GPI. The products of four alleles are shown; the bands encoded by alleles *1* and *4* migrate the fastest and the slowest, respectively. Samples are numbered from left to right. Slots 1, 3, and 7: heterozygous genotype *1/3* (three-banded), IS 2. Slot 2: homozygous genotype *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 principal components analysis. The first component accounted for 84% of the variation, and provided weights of 0·91, 0·98, 0·78, 0·97 and 0·94 for precipitation, average, maximum and minimum temperatures, and average humidity, respectively. 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 specificity; all isozymic strains are transmitted by the same vector, *Triatoma infestans*. (iv) The heterozygous strains are more frequent eastward and at lower altitude, which is possibly related to climatic factors. It should, nevertheless, be pointed out that MILES *et*

Table II—Geographic and climatic data used for the statistical correlations. Climatic data are averages over three years. The localities are ordered according to altitude; the locality numbers refer to Fig. 1. Locality 1 (Yungas) is excluded because it is made up of several small samples collected at different sites

	Altitude (m)	Latitude S.	Longitude W.	Average precipitation per month (mm)	Average temperature (° Celsius)	Maximum temperature (° Celsius)	Minimum temperature (° Celsius)	Average humidity (%)
8 Tupiza	2952	21°26′	65°43′	31·2	14·5	28·6	− 0·6	39·6
6 Sucre	2750	19°01′	65°17′	64·0	15·1	27·3	4·1	51·9
2 Chiwisivi	2600	16°58′	67°54′	—	—	—	—	—
3 Cochabamba	2548	17°27′	66°06′	43·4	17·4	30·2	3·6	50·2
9 Tarija	1875	21°32′	64°43′	53·0	18·7	33·2	4·1	54·0
4 Comarapa	1814	17°54′	64°30′	49·2	18·1	28·7	7·2	65·1
7 Camiri	810	20°03′	63°34′	85·0	22·8	35·7	9·5	69·1
5 Santa Cruz	414	17°45′	63°10′	130·9	23·7	33·2	13·4	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.

Acknowledgements

We are indebted to Professor Dominique Le Ray (Tropical Medicine Institute in Antwerp), who kindly provided the laboratory reference strains from Tehuentepec and Tulahuen. This study was carried out with support from the French Technical Cooperation Program and from the Ministère de l'Industrie et de la Recherche; extension of grant number PVD/81/L-1423.

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Accepted for publication 11th April, 1985.