The presence in Bolivia of two distinct zymodemes of *Trypanosoma cruzi*, circulating sympatrically in a domestic transmission cycle

MICHEL TIBAYRENC AND PHILIPPE DESJEUX
ORSTOM—Institut Pasteur, IBBA, Embajada de Francia, Casilla 824, La Paz, Bolivia

Summary

The enzyme profiles of 109 Bolivian stocks of *Trypanosoma cruzi* were determined by cellulose acetate electrophoresis using the four enzymes: malate dehydrogenase (oxaloacetate decarboxylating) ($NADP^+$) (E.C.1.1.1.40, ME), phosphogluconate dehydrogenase (E.C.1.1.1.44, 6PGDH), phosphoglucomutase (E.C.2.7.5.1, PGM) and glucosephosphate isomerase (E.C.5.3.1.9, GPI). As previously, two principal zymodemes were found sympatrically. Both were isolated from man, one appeared to be more frequent at high altitude, the other appeared to be more frequent at low altitude. These results agree with our previous hypothesis on the genetics of *T. cruzi* (diploidy, lack of actual sexuality).

Introduction

Previous studies of Bolivian *Trypanosoma cruzi* stocks have confirmed the enzymic variability of this parasite that was first demonstrated in Brazil by MILES *et al.* (1977, 1978, 1980), and allowed us to suggest a genetic interpretation of flagellate zymograms (TIBAYRENC *et al.*, 1981a and b). Here, based on the examination of 109 Bolivian stocks, we confirm the presence of two principal Bolivian zymodemes, and reveal some interesting aspects of their geographical distribution. The paper that follows (TIBAYRENC & MILES, 1983) compares the enzyme profiles of Bolivian and Brazilian zymodemes and discusses their relationships.

Materials and Methods

Trypanosoma cruzi stocks were collected in Bolivia from four geographical localities, which are briefly described below:

Chiwisivi—a high river valley, at about 2,700 m and 60 km south of the capital La Paz. The climate is temperate and very dry.

The Yungas—valleys at an intermediate altitude of approximately 1,700 m, 100 km north-east of La Paz. The climate is subtropical, far more humid than at Chiwisivi, and there is a lush vegetation.

Santa Cruz, more specifically the suburban village of Cotoca—at an altitude of 400 m, 500 km south-east of La Paz. The climate and vegetation are also subtropical.

Camiri—a city, also at 400 m, 300 km south of Santa Cruz but with a drier climate than in Santa Cruz.

The origins of the stocks examined are summarized in Table I. Most of the stocks were isolated directly from domestic or peridomestic triatomine faeces using a simple method, described elsewhere (Tibayrenc & Desjeux, 1982). The stocks were grown in GLSH liquid medium (LE RAY, 1974) and passaged weekly. One culture tube, containing about 10 ml, gave sufficient material for enzyme electrophoresis—large volume cultures were not necessary for the examination of four enzymes, because of the sensitivity of cellulose acetate electrophoresis (CAE). Stocks were harvested routinely once only, except when specifically checking the stability of enzyme profiles. The packed cells were mixed with an equal volume of enzyme stabilizers (MILES *et al.*, 1977), stood on ice for 30 min and stored at −70°C in a Revco deep freeze. Whole lysates were electrophoresed without centrifugation.

The four enzymes used to examine the Bolivian *T. cruzi* stocks were as follows: malate dehydrogenase (oxaloacetate decarboxylating) ($NADP^+$) E.C.1.1.1.40,

Table I—Origins of the *Trypanosoma cruzi* stocks examined

	No. of houses	Group I	Group II	Group III (?)
Chiwisivi	12	64	2	0
Yungas	9	5	4	0
Santa Cruz (Cotoca)	6	2	19	0
Camiri[1]	4	3	1	0
CENETROP				
Human	6	3	1	1[2]
Triatoma	4	4	0	0

[1]including stock Tachiperenda (see text)
[2]stock 19-79

All stocks isolated from *Triatoma infestans*, except for the human stocks from CENETROP, and one stock isolated from *Triatoma sordida* at CENETROP.

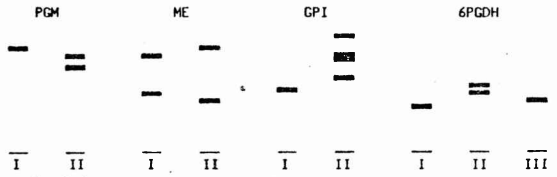

Fig. 1. Enzyme profiles of the Bolivian zymodemes for enzymes PGM, ME, GPI and 6PGDH.

ME), phosphogluconate dehydrogenase (E.C.1.1.1.44, 6PGDH), phosphoglucomutase (E.C.2.7.5.1, PGM), and glucosephosphate isomerase (E.C.5.3.1.9, GPI). The CAE equipment of Helena Laboratories (Beaumont, Texas) was employed throughout. For the enzymes ME. 6PGDH and GPI, we adopted the methods of KREUTZER & CHRISTENSEN (1980), but for the development of ME substituted a Tris-malic acid buffer, pH 7·0 (Tris 6g/l, malic acid 30g/l, adjusted with NaOH 4N) and, for 6PGDH and GPI used the tank buffer No. 3 of SHAW & PRASAD (1970). For the enzyme PGM we used the method of KREUTZER & CHRISTENSEN (1980) without modification.

Results and Discussion

Table I gives the electrophoretic results for all the stocks examined. With the exception of one human stock from CENETROP (19-79), only two zymodemes, or combinations of patterns, were found (Fig. 1). Except in the case of stock 19-79, ME, 6PGDH, PGM and GPI pattern 1 always occurred together as did ME, 6PGDH, PGM and GPI pattern 2. In stock 19-79 PGM and GPI pattern 1 were associated with ME pattern 2 and a 6PGDH pattern that was not seen in the other stocks. 25 of the stocks were examined twice, at an interval of one month, to check the stability of their enzyme profiles; no changes in patterns were observed, except that on one occasion, the stock Tachiperenda appeared to show a mixture of both PGM patterns.

An hypothesis on *Trypanosoma cruzi* genetics was proposed previously, namely diploidy (TIBAYRENC *et al.*, 1981a) and a lack of actual sexuality (TIBAYRENC *et al.*, 1981b). The present results with a greater number of stocks confirm the lack of genetic exchange between strains 1 and 2, and the constant heterozygous state in strain 2 for enzymes 6PGDH, PGM, and GPI (checked for 27 stocks), which is scarcely compatible with sexual recombination within this strain.

The existence of two principal enzymic strains (or zymodemes) in Bolivia, with the exception of stock 19-79, which requires further study, has been confirmed. It is interesting to compare the geographical distribution of the two Bolivian zymodemes between Chiwisivi and Cotoca (Table I). In Chiwisivi, from 12 different households, 64 stocks with enzyme profile 1 were recorded and two with enzyme profile 2. In contrast, from six houses in Cotoca we recorded only two stocks with enzyme profile 1 but 19 with enzyme profile 2. In the Yungas, amongst nine stocks we found five with enzyme profile 1 and four with enzyme profile 2. In Cotoca, all the triatomine bugs were collected inside houses. In Chiwisivi and the Yungas most were collected from peridomestic guinea-pig houses, but, as all of the guinea-pig houses shared a common wall with the houses themselves, this distinction is not necessarily meaningful. It is not possible, on the basis of the present results, to decide if the distribution of the zymodemes constitutes really a cline related to geographical distance or climate; further studies are required from other localities at similar altitudes. Both of the principal zymodemes were found in man (amongst stocks isolated at CENETROP, Santa Cruz). Furthermore, in the Yungas and in Cotoca, both zymodemes were recorded from the same house.

In conclusion, these observations have confirmed our previous genetic interpretation (diploidy and lack of actual sexuality in *T. cruzi*) and the presence of at least two principal zymodemes in Bolivia. Both zymodemes occur in man and they are sympatric, with different geographical frequencies: the first zymodeme appears to be more common at high altitude, the second zymodeme appears to be more common at low altitude. Bolivia, with the different levels of altitude, gives excellent conditions for comparative ecological studies. We are beginning further studies to investigate relationships between geographical localities and zymodemes, and to study relationships between clinical features and zymodemes.

Acknowledgements

We thank Dr. Carlos La Fuente, Dr. Benjamin Ribeira and Dr. Christian Darras who have given nine stocks from CENETROP.

References

Kreutzer, R. D. & Christensen, H. A. (1980). Characterization of *Leishmania* spp. by isozyme electrophoresis. *American Journal of Tropical Medicine and Hygiene*, **29**, 199-208.

Le Ray, R. (1974). *Les structures antigéniques de* Trypanosoma brucei brucei *(Protozoa: Kinetoplastida). Analyse immunoélectrophoretique et étude comparative.* Thèse Sciences, Université catholique de Louvain.

Miles, M. A., Toye, P. J., Oswald, S. C. & Godfrey, D. G. (1977). The identification by isoenzyme patterns of two distinct strain-groups of *Trypanosoma cruzi*, circulating independently in a rural area of Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 217-225.

Miles, M. A., Souza, A., Povoa, M., Shaw, J. J., Lainson, R. & Toye, P. J. (1978). Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonous patients with Chagas' disease in Amazonian Brazil. *Nature*, **272**, 819-821.

Miles, M. A., Lanham, S. M., de Souza, A. A. & Povoa, M. (1980). Further enzymic characters of *Trypanosoma cruzi* and their evaluation for strain identification. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 221-236.

Shaw, C. R. & Prasad, R. (1970). Starch gel electrophoresis: a compilation of recipes. *Biochemical Genetics*, **4**, 297-320.

Tibayrenc, M., Cariou, M. L. & Solignac, M. (1981a). Interprétation génétique des zymogrammes de Flagellés des Genres *Trypanosoma* et *Leishmania*. *Comptes Rendus de l'Académie des Sciences Paris*, **292**, 623-625.

Tibayrenc, M., Cariou, M. L., Solignac, M. & Carlier, Y. (1981b). Arguments génétiques contre l'existence d'une sexualité actuelle chez *Trypanosoma cruzi*. Implications taxonomiques. *Comptes Rendus de l'Académie des Sciences Paris*, **293**, 207-209.

Tibayrenc, M. & Desjeux, P. (1982). Une méthode simple pour isoler directement des souches de *Trypanosoma cruzi* á partir du tube digestif de *Triatoma infestans*. *Cahiers ORSTOM, Sér. Entomologie médicale et Parasitologie* (in press).

Tibayrenc, M. & Miles, M. A. (1983). A genetic comparison between Brazilian and Bolivian zymodemes of *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 77, 76-83.

Accepted for publication 9th June, 1982.