Parasitol Res (1998) 84: 348–353 

ORIGINAL PAPER

A. da S. Pinto · M. de Lana · B. Bastrenta
C. Barnabé · V. Quesney · S. Noël · M. Tibayrenc

# Compared vectorial transmissibility of pure and mixed clonal genotypes of *Trypanosoma cruzi* in *Triatoma infestans*

Received: 10 March 1997 / Accepted: 21 September 1997

**Abstract** A total of 15 mixtures involving 9 different stocks attributed to the 19/20, 32 and 39 major clonal genotypes of *Trypanosoma cruzi* were used to infect third-instar nymphs of *Triatoma infestans* via an artificial feeding device. Three biological parameters were considered: (1) the percentage of infected insects (%II), (2) the number of flagellates per insect (NFI), and (3) the percentage of trypomastigotes per insect (%DIF). Genetic characterization by both multilocus enzyme electrophoresis (MLEE) and random amplification of polymorphic DNA (RAPD) indicated that in almost all cases (87%), mixtures remained present after completion of the whole cycle in the insect vector. Two lines of comparison were performed: (1) pure clonal genotypes versus corresponding mixed clonal genotypes and (2) the actual behavior of mixed clonal genotypes versus the expected behavior of the theoretical mixture (i.e. the arithmetic mean of the results observed for each of the two clonal genotypes taken separately). Statistical analyses of the variables were made difficult because of the presence of large standard deviations. Nevertheless, in several cases, mixtures differed significantly from pure clonal genotypes, and in one case the actual mixture differed significantly from the theoretical mixture. In some cases, interaction (either potentialization or reciprocal inhibition) could be suspected.

A. da S. Pinto[1] · M. de Lana[2] · B. Bastrenta · C. Barnabé
V. Quesney · S. Noël · M. Tibayrenc (✉)
Centre d'Etudes sur le Polymorphisme des Microorganismes (CEPM), Unité Mixte de Recherche n° 9926 Centre National de la Recherche Scientifique (CNRS)/Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), ORSTOM, BP 5045, F-34032 Montpellier, Cedex 1, France
Fax: +33 4 67 41 62 99

[1] *Permanent address*:
Departamento de Microbiologia,
Instituto de Ciências Biológicas, CP 486,
Universidade Federal de Minas Gerais,
31270-010 Belo Horizonte, MG, Brasil

[2] *Permanent address*:
Departamento de Análises Clínicas,
Faculdade de Farmácia, Rua Costa Sena,
Universidade Federal de Ouro Preto,
35400-000 Ouro Preto, MG, Brasil

## Introduction

*Trypanosoma cruzi*, the agent of Chagas' disease, undergoes an obligatory cycle within the digestive tract of triatomine bugs. In the insect's gut, parasite populations consist mainly of multiplicative epimastigote forms that in the rectum can differentiate into the infective metacyclic trypomastigotes. Among the species of vectors, *Triatoma infestans* remains the most important synantropic vector of Chagas' disease in many South American countries.

It is well established that the species *T. cruzi* is extremely heterogeneous in terms of its general biological behavior (Andrade 1976), growth kinetics (Dvorak et al. 1980), pathogenicity (Miles et al. 1981), isoenzyme patterns (Miles et al. 1980; Andrade et al. 1983; Tibayrenc and Ayala 1988), k-DNA restriction fragment (Morel et al. 1980), random amplification of polymorphic DNA (RAPD; Steindel et al. 1993; Tibayrenc et al. 1993), and ability to multiply and differentiate in the insect vector (Garcia and Dvorak 1982; Schaub 1989; Mello et al. 1996).

Population-genetics analyses have shown that *T. cruzi* presents a typical clonal population structure (Tibayrenc et al. 1986). Among the numerous isolated natural clones, a limited number are overrepresented through vast geographic areas and over long periods. These widespread clonal genotypes have been referred to as major clones (Tibayrenc and Ayala 1988; Tibayrenc and Brenière 1988). Moreover, frequently, isolates are sampled that comprise at least two different clonal genotypes, either in patients or in vectors (Morel et al. 1984; Brenière et al. 1985; Tibayrenc et al. 1985; Bosseno et al. 1996). Considering the incidence of mixed stocks in natural cycles, to better understand the transmission of Chagas' disease and the biological

behavior of *T. cruzi* major clones, the present report presents experimental results dealing with the compared transmissibility by *T. infestans* of pure and mixed clonal genotypes of *T. cruzi*.

## Materials and methods

### Parasites

Nine stocks were selected from a standardized set of 21 stocks used in our laboratory for all biology studies dealing with *Trypanosoma cruzi* (Revollo 1995; Laurent et al. 1997). This set of 21 stocks is representative of 3 major clonal lineages of *T. cruzi* numbered 19/20, 32, and 39 according to the coding system used by Tibayrenc et al. (1986). Major clones 19, 20, 32, and 39, characterized by 15 enzyme loci (Tibayrenc et al. 1986), revealed limited additional variability with the use of more discriminative markers (Tibayrenc et al. 1993), as expected. They should be regarded as families of closely related clones rather than as actual clones (Tibayrenc and Ayala 1988). Moreover, the 19/20 group, formerly distinguished into natural clones 19 and 20 on the basis of 15 isoenzyme loci (Tibayrenc et al. 1986), are less clearly separated by more discriminative methods (Tibayrenc et al. 1993). In the present study they were plotted together into a unique group of closely related clonal genotypes designated 19/20. The 3 classes of clonal genotypes, namely 19/20, 32, and 39, illustrate different phylogenetic relationships; 32 and 39 are more closely related to each other, whereas 19/20 is more distantly related to both 32 and 39 (see Fig. 1).

**Fig. 1** A UPGMA (unweighted pair-group method with arithmetic averages) dendrogram (Sneath and Sokal 1973) depicting the phylogenetic relationships among the 9 *Trypanosoma cruzi* stocks under study as assayed by 22 isoenzyme loci (Tibayrenc et al. 1993). The *top cluster* corresponds to the clonal genotype 19/20, the *middle cluster* corresponds to the clonal genotype 39, and the *bottom cluster* corresponds to the clonal genotype 32 (genotype numbering after Tibayrenc and Ayala 1988). The scale indicates genetic distances estimated with the index of Jaccard (1908)

All stocks were fully characterized by both multilocus enzyme electrophoresis for 22 different genetic loci and RAPD (Tibayrenc et al. 1993). They were cloned at the laboratory, and actual cloning was verified under the microscope. Information on the laboratory code, host, and geographic origin of the nine stocks under survey is given in Table 1. The nine stocks studied were analyzed for their transmissibility in *Triatoma infestans* (Lana et al., unpublished data). According to their transmission behavior in *T. infestans*, they entered the composition of 15 different mixtures designed to represent various behavioral situations. For each genotype category, either 19/20 or 32 or 39, we selected three stocks, a "fast" one (easily transmitted through *T. infestans*), a "slow" one, and a "medium" one according to the results recorded by Lana et al. (unpublished data). For each case of mixed genotypes (19/20 + 32, 19/20 + 39, 32 + 39), 5 different classes were designed, namely, medium/medium, fast/fast, slow/slow, slow/fast, and fast/slow, resulting in a total of 15 different mixtures (see Table 2). This design aims at eliminating a "stock effect" such that representative results are reached for mixtures of clonal genotypes rather than mixtures of particular stocks.

### Experimental conditions

Experiments were undertaken with third-instar nymphs of *T. infestans* reared under laboratory conditions, i.e., at about 26–27 °C and 65–70% relative humidity, and allowed to feed on chickens every 3 weeks. The insects originated from an outbred colony consisting of individuals from Chile, Uruguay, and Brazil. The insects were exposed to infection using middle-log-phase culture forms from LIT medium at 28 °C in an artificial xenodiagnosis device through latex membranes. In all, 8 ml of parasite suspension in citrated mouse blood at a final concentration of $5.0 \times 10^5$ cells/ml was used. The system was maintained at 37 °C and continuously homogenized with a magnetic stirring bar. Only engorged insects were considered.

For parasite quantification, 30 days later the whole digestive tube was removed and gently ground in 0.6-ml Eppendorf tubes containing 10 µl of phosphate-buffered saline (PBS, pH 7.2). The suspension was then homogenized and used to prepare fresh slide smears covered with $22 \times 22$ lids. Preparations were scored

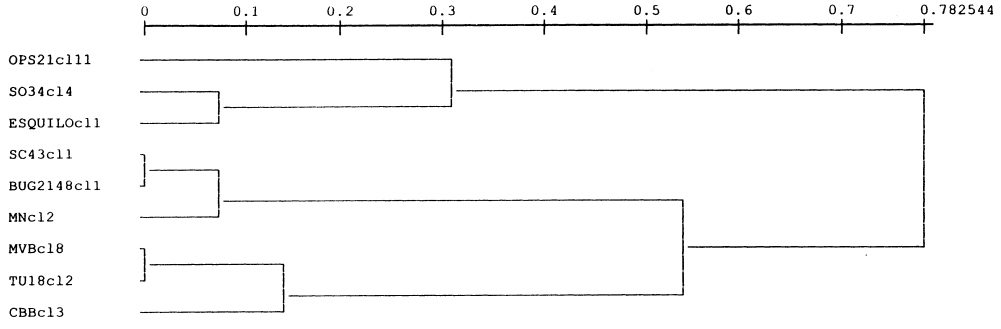


**Table 1** Laboratory code, hosts, and geographic origin of the 9 *Trypanosoma cruzi* stocks used in the present study. Clonal genotypes are numbered according to Tibayrenc and Ayala (1988)

| Name | Country | Place | Host | Clonal genotype |
|---|---|---|---|---|
| Esquilo cl1 | Brazil | Sao Paulo | *Sciurus aestuans ingramini* | 19/20 |
| SO34cl4 | Bolivia | Toropalka (Potosi) | *Triatoma infestans* | 19/20 |
| OPS21 cl11 | Venezuela | Cojedes | Humans | 19/20 |
| CBB cl3 | Chile | Tulahén | Humans | 32 |
| MVB cl8 | Chile | Santiago | Humans, chronic form | 32 |
| Tu18 cl2 | Bolivia | Tupiza | *T. infestans* | 32 |
| SC43 cl1 | Bolivia | Santa Cruz | *T. infestans* | 39 |
| Bug2148 cl1 | Brazil | Rio Grande do Sul | *T. infestans* | 39 |
| MN cl2 | Chile | IV Region | Humans, chronic form | 39 |

**Table 2** Mixtures of stocks representative of the 19/20, 32, and 39 *T. cruzi* clonal genotypes used to infect third-instar nymphs of *Triatoma infestans* (*Medium*, *Fast*, *Slow* Behavior in monoclonal infection as determined considering the variables %II, NPI, and %DIF)

| Genotype mixture | Stock mixture | | | | |
|---|---|---|---|---|---|
| | Medium + medium | Fast + fast | Slow + slow | Slow + fast | Fast + slow |
| 19/20 + 32 | Esquilo cl1 + CBB cl3 | SO34 cl1 + MVB cl8 | OPS21 cl11 + TU18 cl2 | OPS21 cl11 + MVB cl8 | SO34 cl4 + TU18 cl2 |
| 19/20 + 39 | Esquilo cl1 + MN cl2 | SO34 cl1 + SC43 cl1 | OPS21 cl11 + Bug2148 cl1 | OPS21 cl11 + SC43 cl1 | SO34 cl4 + Bug2148 cl1 |
| 39 + 49 | CBB cl3 + MN cl2 | MVB cl8 + SC43 cl1 | TU18 cl2 + Bug2148 cl1 | TU18 cl2 + SC43 cl1 | MVB cl8 + Bug2148 cl1 |

microscopically for the presence of flagellates, total numbers of flagellates, and percentages of metacyclic trypomastigotes. In all, 200 fields for each preparation were examined. Additionally, the entire intestinal tracts of two insects from each stock were aseptically removed and introduced into 15-ml Falcon tubes containing 3.0 ml of LIT medium supplemented with gentamicin at 50 µg/ml. The tubes were kept at 28 °C and examined weekly for the presence of flagellates. Isolates were checked for identification of the clones by their isoenzyme profiles and RAPD patterns. Cellulose-acetate electrophoresis was performed under conditions described elsewhere (Ben Abderrazak et al. 1993). Five enzyme systems were assayed: phosphoglucose isomerase (E.C. 5.3.1.9, PGI), phosphoglucomutase (E.C. 5.4.2.2, PGM), isocitrate dehydrogenase (E.C.1.1.1.42, IDH), 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6PGD), and glutamate oxalate transaminase (E.C.2.6.1.1, GOT). For RAPD analysis, primers A7 (GAAACGGGTG) and A10 (GTGATCGCAG; kit A, Operon Technologies, Alameda, Calif.) were used under conditions described elsewhere (Williams et al. 1990; Tibayrenc et al. 1993).

### Data analysis

Three variable were considered: (1) the percentage of infected insects (%II), (2) the number of flagellates per insect (NFI), and (3) the percentage of metacyclic trypomastigotes per insect (%DIF). Each of the 15 mixtures was exposed to 6 insects and a total of 90 insects were examined. The reduced deviation test (SAS program, version 5-06) was used to compare the parameters between the three categories of mixtures of clonal genotypes (19/20 + 39, 19/20 + 32, 39 + 32). Within each of the three categories of mixtures, for the three parameters under study, the value taken into account was the arithmetic mean between the following five classes: medium/medium, fast/fast, slow/slow, slow/fast, and fast/slow (see above). Comparisons of percentages were used in the case of the %II parameter, whereas comparisons of means were used in the case of the two other parameters.

Two different categories of comparison were performed: (1) a given mixture with the corresponding pure clonal genotypes according to the results obtained by Lana et al. (unpublished data) and (2) a given mixture with the corresponding theoretical mixture, i.e., its expected result in the case of an equal effect of the two corresponding clonal genotypes (arithmetic mean of the values recorded for the two corresponding clonal genotypes, taken separately).

## Results

### Persistence of mixed genotypes

The combined results of MLEE and RAPD analyses showed that in most cases (87.0%), genotype mixtures remained present after completion of the whole cycle in the insect vector. MLEE and RAPD allowed the detection of 71.11% and 60.0% of the total mixtures, respectively. Mixed-infection percentages recorded for two given clonal genotypes remaining after completion of the cycle were 19/20 + 32 = 73.33%, 19/20 + 39 = 93.33%, and 32 + 39 = 93.33% when MLEE and RAPD results were combined. In mixtures of the 32 and 39 genotypes the latter appeared very frequently as weak bands only (see Fig. 2), which suggests that this genotype was in the minority in the mixture at that step, although no quantification was attempted in the present study. Finally, both MLEE and RAPD identified cases in which only one of the

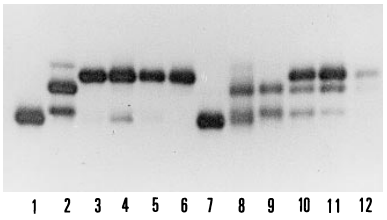


**Fig. 2** Isoenzyme profiles revealed with the enzyme system glucose phosphate isomerase (GPI) in parasites obtained from third-instar nymphs of *Triatoma infestans* exposed to mixed *T. cruzi* populations attributed to the 19/20, 32, and 39 clonal genotypes (Tibayrenc and Ayala 1988) (*Lane 1* Genotype 19/20 monoclonal infection, *lane 2* genotype 39 monoclonal infection, *lane 3* genotype 32 monoclonal infection, *lanes 4–6* genotypes 19/20 + 32 mixed infection, *lanes 7–9* genotypes 19/20 + 39 mixed infection, *lanes 10–12* genotypes 32 + 39 mixed infection)

genotypes was detectable after completion of the cycle. In these conditions the genetic group 19/20 was most frequently encountered, with 57.13% of the cases having only one detectable genotype after completion of the cycle, followed by the 32 and 39 genotypes at 28.57% and 14.29%, respectively. Figures 2 and 3 show typical electrophoresis profiles obtained by MLEE and RAPD and revealing the persistence of mixed genotypes after completion of the cycle.

Behavior of the mixtures in *Triatoma infestans*

Table 3 summarizes the values obtained for the pure clonal genotypes, the actual mixtures, and the theoretical mixtures. Table 4 gives the statistical data that proved to be significant. All other tests gave nonsignificant results.

## Discussion

Persistence in many cases of the mixed genotypes after completion of the whole cycle is a relevant observation that confirms data previously obtained using in vitro cultures (François 1995) and is consistent with data from the field (Morel et al. 1984; Brenière et al. 1985; Tibayrenc et al. 1985; Bosseno et al. 1996). Experiments need to be designed to survey the possible persistence of mixtures in the long run, after the completion of many cycles. Nevertheless, it should be emphasized that clonal genotype 32, whose transmissibility appeared to be weaker than that of the 19/20 genotype when these were considered separately (see Table 3), was obviously present in many cases of mixtures after completion of the cycle (see Figs. 2, 3). Although an acute quantification was not performed in the present study, MLEE

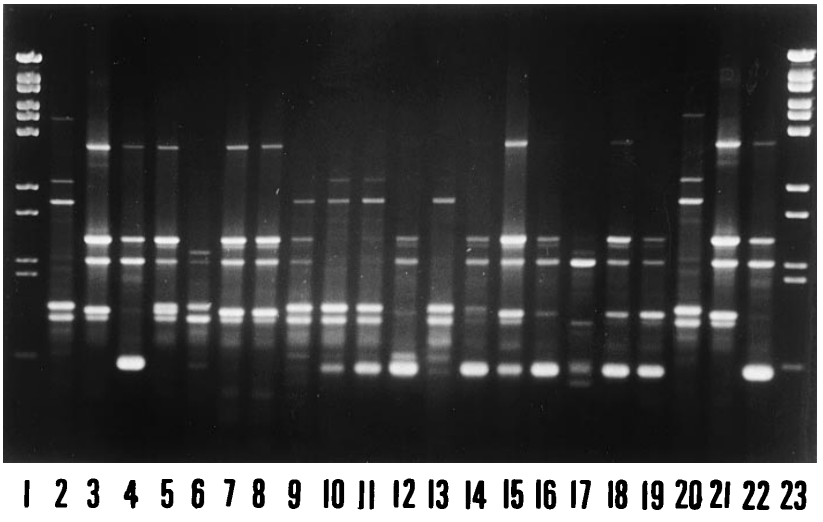


**Fig. 3** RAPD using the A10 primer in parasites obtained from third-instar nymphs of *T. infestans* exposed to mixed *T. cruzi* populations attributed to the 19/20, 32, and 39 clonal genotypes (Tibayrenc and Ayala 1988) (*Lanes 1*, *23* Scale ladders, *lanes 2*, *20* genotype 19/20 monoclonal infection, *lanes 3*, *21* genotype 32 monoclonal infection, *lanes 4*, *22* genotype 39 monoclonal infection, *lanes 5–9* genotypes 19/20 + 32 mixed infection, *lanes 10–14* genotypes 19/20 + 39 mixed infection, *lanes 15–19* genotypes 32 + 39 mixed infection)

**Table 3** Numerical results obtained for 3 biological parameters (NFI, DIF, and %II; see Materials and methods) with the 9 *T. cruzi* stocks attributed to the 3 groups of clonal genotypes (19/20, 32, and 39), taken separately and in mixture. *Data in parentheses* correspond to the theoretical mixtures, i.e., the average of the values obtained for the two clonal genotypes involved in the actual mixture, taken separately (see Materials and methods)

| | NFI | DIF | %II |
|---|---|---|---|
| 19/20 | 119.92 ± 241.30 | 16.04 ± 20.98 | 0.91 |
| 39 | 44.56 ± 127.24 | 5.64 ± 11.89 | 0.70 |
| 32 | 29.89 ± 70.64 | 0.48 ± 1.66 | 0.56 |
| 19/20 + 39 | 184.40 ± 344.75 (80.83 ± 194.03) | 11.10 ± 19.89 (10.64 ± 17.63) | 0.90 (0.78) |
| 19/20 + 32 | 147.33 ± 199.91 (74.91 ± 182.95) | 12.20 ± 14.95 (8.26 ± 16.77) | 0.87 (0.73) |
| 39 + 32 | 12.02 ± 24.12 (37.50 ± 103.91) | 1.71 ± 4.73 (3.15 ± 9.00) | 0.62 (0.60) |

**Table 4** Results of the statistically significant tests for the biological parameters NFI and DIF (see Materials and methods) [*39 + 32 obs vs 39 + 32 calc NFI* Comparison (*vs* versus) of the mean data recorded for the parameter NIF between actual (*obs*) and theoretical (*calc*) mixtures of the clonal genotypes 39 and 32]

| Comparison | Level of significance |
|---|---|
| 39 + 32 obs vs 39 + 32 calc NFI | $P < 0.01$ |
| 32 vs 32 + 39 obs NIF | $P < 0.03$ |
| 39 vs 32 + 39 obs NIF | $P < 0.02$ |
| 32 vs 19/20 + 32 obs NIF | $P < 0.01$ |
| 39 vs 19/20 + 32 obs NIF | $P < 0.03$ |
| 32 vs 19/20 + 32 obs DIF | $P < 0.0001$ |
| 39 vs 19/20 + 39 obs DIF | $P < 0.03$ |
| 32 vs 32 + 39 obs DIF | $P < 0.03$ |
| 39 vs 32 + 39 obs DIF | $P < 0.01$ |

results do suggest that the proportion of parasites attributed to the 32 genotype in these persistent mixtures was noteworthy (see Fig. 2).

When the NFI, DIF, and %II parameters were considered, statistical comparisons were rendered difficult because of considerable standard deviations. Nevertheless, some highly significant results were obtained (see Table 4). They concern only the NFI and DIF parameters, since no significant result was observed for the %II parameter.

Considering the crude results (Table 3), ''unexpected'' results for the NFI parameter were apparent; that is, the mixtures were either ''faster'' than the ''fastest'' clonal genotype considered separately (the case for the 19/20 + 39 and the 19/20 + 32 mixtures), or ''slower'' than the ''slowest'' genotype considered discretely (the case for the 39 + 32 mixture). These results suggest an interaction between the genotypes involved in the mixture. Nevertheless, only the last case showed statistically significant departures from both the theoretical mixture and the 32 genotype (the ''slowest'' one) considered separately (see Table 4). This suggests a reciprocal inhibition of the two genotypes involved. In the case of the DIF parameter, when significant results were observed, they were ''expected''; that is, they were (1) intermediary between the results recorded for each of the two corresponding clonal genotypes taken separately or (2) not statistically different from the corresponding theoretical mixtures.

**Acknowledgements** This work was supported by the WHO Special Program for Research and Training in Tropical Diseases (number 910268), by EEC STD3 grant TS3* CT92-0155, and by a Groupement de Recherche grant from the CNRS/French Army. A. Pinto and M. De Lana were supported by a fellowship from CNPq, Brazil.

## References

Andrade SG (1976) Tentative for grouping different *Trypanosoma cruzi* strains in some types. Rev Inst Med Trop Sao Paulo 18: 140–141

Andrade V, Brodskyn C, Andrade SG (1983) Correlation between isoenzyme patterns and biological behaviour of different strains of *Trypanosoma cruzi*. Trans R Soc Trop Med Hyg 77: 796–799

Ben Abderrazak S, Guerrini F, Mathieu-Daudé F, Truc P, Neubauer K, Lewicka K, Barnabé C, Tibayrenc M (1993) Isozyme electrophoresis for parasite characterization. In: Hyde JE (ed) Protocols in molecular parasitology. (Methods in molecular biology series, vol 21) Humana, Totowa, New Jersey, pp 361–382

Bosseno MF, Telleria J, Vargas F, Yaksic N, Noireau F, Morin A, Brenière SF (1996) *Trypanosoma cruzi*: distribution of two widespread clonal genotypes in Bolivian *Triatoma infestans* vectors shows a high frequency of mixed infections. Exp Parasitol 83: 275–282

Brenière SF, Tibayrenc M, Antezana G, Pabon J, Carrasco R, Selaès H, Desjeux P (1985) Résultats préliminaires en faveur d'une relation faible ou inexistante entre les formes cliniques de la maladie de Chagas et les souches isoenzymatiques de *Trypanosoma cruzi*. C R Acad Sci [III] 300: 555–558

Dvorak JA, Hartman DL, Miles AM (1980) *Trypanosoma cruzi*: correlation of growth kinetics to zymodeme type in clones derived from various sources. J Protozool 27: 472–474

François C (1995) Mélanges expérimentaux de clones naturels de *Trypanosoma cruzi*: cinétique et détection par des marqueurs moléculaires Dissertation, University of Montpellier II

Garcia ES, Dvorak JA (1982) Growth and development of two *Trypanosoma cruzi* clones in the arthropod *Dipetalogaster maximus*. Am J Trop Med Hyg 31: 259–262

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaudoise Sci Nat 44: 223–270

Laurent JP, Barnabé C, Quesney V, Noël S, Tibayrenc M (1997) Impact of clonal evolution on the biological diversity of *Trypanosoma cruzi*. Parasitology 114: 213–218

Mello CB, Azambuja P, Garcia ES, Ratcliffe NA (1996) Differential in vitro and in vivo behavior of three strains of *Trypanosoma cruzi* in the gut and hemolymph of *Rhodnius prolixus*. Exp Parasitol 82: 112–121

Miles MA, Lanham SM, De Sousa AA, Povoa M (1980) Further enzymatic characters of *Trypanosoma cruzi* and their evaluation for strain identification. Trans R Soc Trop Med Hyg 74: 221–237

Miles MA, Povoa MM, Prata A, Cedillos RA, Sousa AA, Macedo V (1981) Do radically dissimilar *Trypanosoma cruzi* strains (zymodemes) cause Venezuelan and Brazilian forms of Chagas' disease? Lancet II: 1338–1340

Morel CM, Chiari E, Camargo EP, Mattei DM, Romanha AJ, Simpson L (1980) Strains and clones of *Trypanosoma cruzi* can be characterized by pattern of restriction products of kinetoplast DNA. Proc Natl Acad Sci USA 77: 6810–6814

Morel CM, Deane MP, Gonçalves AM (1984) The complexity of *Trypanosoma cruzi* populations revealed by schizodeme analysis. Parasitol Today 2: 97–101

Revollo S (1995) Impact de l'évolution clonale de *Trypanosoma cruzi*, agent de la maladie de Chagas, sur certains proprités biologiques médicalement importantes du parasite. PhD dissertation, Université de Montpellier

Schaub GA (1989) *Trypanosoma cruzi*: quantitative studies of development of two strains in small intestine and rectum of the vector *Triatoma infestans*. Exp Parasitol 68: 260–273

Sneath PHA, Sokal RR (1973) Numerical taxonomy. In: Kennedy D, Park RB (eds) The principle and practice of numerical classification. Freeman, San Francisco, p 537

Steindel M, Dias Neto E, Menezes CLP de, Romanha AJ, Simpson AJG (1993) Random amplified polymorphic DNA analysis of *Trypanosoma cruzi* strains. Mol Biochem Parasitol 60: 71–80

Tibayrenc M, Ayala FJ (1988) Isoenzyme variability in *Trypanosoma cruzi*, the agent of Chagas' disease: genetical, taxonomical, and epidemiological significance. Evolution 42: 277–292

Tibayrenc M, Brenière SF (1988) *Trypanosoma cruzi*: major clones rather than principal zymodemes. Mem Inst Oswaldo Cruz 83: 249–255

Tibayrenc M, Cariou ML, Solignac M, Dédet JP, Poch O, Desjeux P (1985) New electrophoretic evidence of genetic variation and diploidy in *Trypanosoma cruzi*, the causative agent of Chagas' disease. Genetica 67: 223–230

Tibayrenc M, Ward P, Moya A, Ayala FJ (1986) Natural populations of *Trypanosoma cruzi* the agent of Chagas' disease, have a complex multiclonal structure. Proc Natl Acad Sci USA 83: 115–119

Tibayrenc M, Neubauer K, Barnabé C, Guerrini F, Skarecky D, Ayala FJ (1993) Genetic characterization of six parasitic protozoa: parity between random-primer DNA typing and multilocus enzyme electrophoresis. Proc Natl Acad Sci USA 90: 1335–1339

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535