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Trypanosoma cruzi Populations: More Clonal than Sexual

M. Tibayrenc and F.J. Ayala

The ancient question of trypanosome sexuality has recently been reactivated in view of important observations in the African species Trypanosoma brucie, *in which Mendelian sexuality has been proposed as a working hypothesis on the basis of indirect isozyme evidence[1]. Subsequent experiments have confirmed that recombination can occur in* T. brucei *under defined experimental conditions and suggest that this parasite undergoes meiosis[2–4]. In this article, Michel Tibayrenc and Francisco Ayala discuss the intraspecific variability of another species,* Trypanosoma cruzi *– causative agent of american trypanosomiasis or Chagas disease. They interpret the variation revealed by extensive isozyme analysis and restriction endonuclease analysis of kinetoplast DNA, to suggest that* T. cruzi *is diploid, genetically very polymorphic, and has a clonal structure that manifests a lack of (or very restricted) sexuality.*

Apparently heterozygous isozyme patterns are common in *T. cruzi* (in 8 loci out of 15), suggesting it to be a diploid organism[5–8]. This is consistent with a variety of results based on isozyme and DNA studies, that support the notion that diploidy is the general condition in *Leishmania* and *Trypanosoma*[1,9,10]. However, sexual recombination is either totally absent, or at least severely restricted, in the natural populations of *T. cruzi* studied by us[6–8,11–14]. The main sources of evidence are as follows:

(1) Some zymodemes show 'fixed heterozygosity' at several loci (i.e. heterozygosis that remains constant generation after generation) which is incompatible with Mendelian segregation[6–8,11].

(2) At any given locus, many theoretically possible genotypes (i.e. diploid combinations of the alleles present in the population) are absent, despite the frequent occurrence of potential parental genotypes in close sympatry (in the same house, same human host, same insect vector), and hence with ample opportunity for mating[7,11–15]. This absence of most possible genotypes is statistically corroborated by large departures from Hardy-Weinberg equilibrium in the populations of the parasite (i.e. the populations are not at all panmictic)[13], whereas at the same geographical scale their insect vector populations are in equilibrium – they appear to mate randomly (i.e. tend to panmixia)[16].

(3) When two or more loci are jointly considered, certain genotypes are systematically associated in a strong linkage disequilibrium[8]. The statistical values of this linkage disequilibrium are close to the maximum possible (Q. Zhang, M. Tibayrenc and F.J. Ayala, unpublished), higher than the values observed in wild barley (*Hordeum spontaneum*) which is a predominantly self-fertilizing species (selfing rate above 99%) (Q. Zhang, unpublished).

All these results suggest, for the samples examined (which represent a large ecogeographical range and various hosts), a clonal structure in *T. cruzi* populations. According to this idea, the entities called 'zymodemes' (formerly recognized using a phenetic interpretation of the zymograms[17–19]) are simply natural clones of the parasite which can be identified by means of isozyme techniques. In the same way, 'schizodemes'[20] are natural clones identified by restriction endonuclease variability in kinetoplast DNA.

It is worth comparing our results with those obtained in *Escheria coli* using similar methods. Natural populations of this bacterium show a predominantly clonal structure, although genetic recombination is readily obtained under experimental conditions[21]. A clonal structure implies that observations based on any two sets of independent genetic characteristics should infer similar genetic relationships among a group of natural isolates. This prediction was confirmed in *E. coli* by comparing isozyme and biotyping data[22]. Similarly, we have recently observed in *T. cruzi* a highly significant statistical correlation between isozyme and kinetoplast DNA patterns[23], which favours the hypothesis of a clonal structure in *T. cruzi* populations. Our results do not rule out completely the possibility of mating in *T. cruzi*, but rather show that recombination is at least severely restricted.

Taxonomic Clustering

The genetic distance (average number of codon differences per gene between two populations[24]) between the *T. cruzi* zymodemes (based on 15 isozyme loci) are often very high[6,8,13], which reflects the extensive isozyme polymorphism of the parasite. We have used a Wagner network* to illustrate the phylogenetic relationships among the zymodemes. Fig. 1 shows that the 43 zymodemes observed in *T. cruzi* cannot be clustered

*Wagner networking[25,26] is a method for depicting the phylogenetic relationships among a given set of populations. Each character is coded as either 1 or 0 (presence/absence) in each stock. Computer programs are available for ready application of this method.

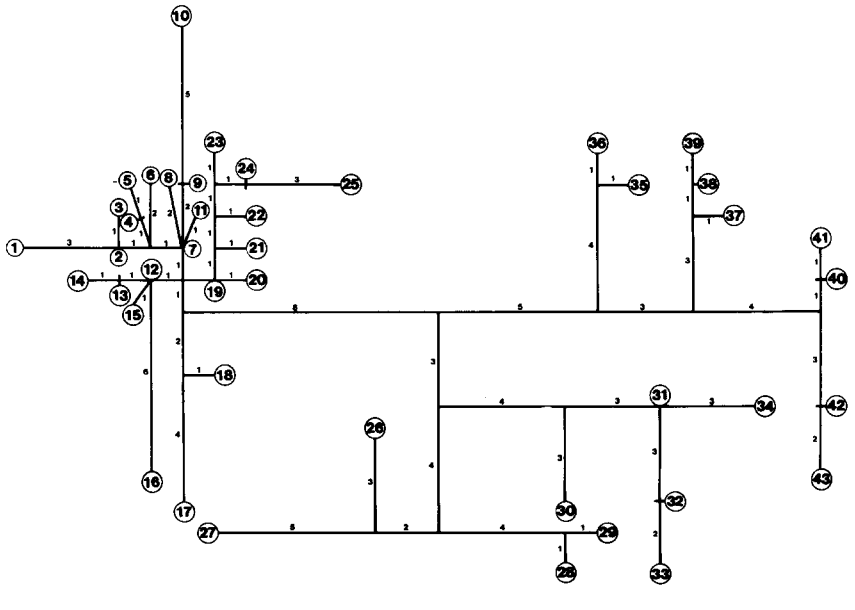

Fig. 1. Minimum-length Wagner network showing the inferred phylogenetic relationships among 43 T. cruzi zymodemes. The numbers identifying the zymodemes (each surrounded by a circle) are given at the terminal points of branches. The numbers along the branches are the patristic, or evolutionary, distances for each segment (from Ref. 8).

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into a few, strictly-delimited, subgroups (or 'principal zymodemes'[6,19,27]) that could represent natural taxa. The only apparent natural grouping, although still extremely heterogeneous, encompasses the zymodemes numbered 1 to 25 in Fig. 1. Additional groupings seem largely arbitrary.

It seems likely that clonal evolution in *T. cruzi* is ancient and that numerous clones have been evolving independently for a long time. The large biochemical heterogeneity of the parasite – shown for example, by the large genetic distance values – would be a direct consequence of the long separate evolution of the natural clones. The correlation observed between isozyme and schizodeme patterns[23] strongly suggests that both types of variability are related to evolutionary time (representing a sort of 'molecular clock'), which reinforces their usefulness for determining phylogenetic relationships. The present distribution of *T. cruzi* genotypes would be the combined result of absent (or severely limited) recombination, chance extinction of lineages, various distribution and historical factors, and possible selective differences among clones.

Since *T. cruzi* zymodemes do not fall into a limited set of well-separated clusters, it seems likely that examination of a larger number of enzyme loci (or other genetic markers) and a more diversified sample of stocks would probably identify additional clones. This makes it unwise to assign any number or label to the zymodemes or schizodemes that would pretend to be definitive, and rejects a typological approach to the infraspecific variability of *T. cruzi*. New isolates should be characterized in comparison with a representative set of reference laboratory stocks, using a sufficient range of markers.

The clonal structure of *T. cruzi* may have important medical implications. Even if some recombination may occur in the evolutionary scale, the natural clones clearly retain their characteristics over large geographical areas and long periods of time (indeed, in our sample, several clones were identified without any change over many years in various countries). The clones thus behave largely as independent genetic entities, (or 'agamospecies') the medical characteristics of which should be studied separately. The central question is whether the high clonal diversity can account for all or part of the medical and biological variability of *T. cruzi*[8]. This question can only be answered by studying the medical and biological characteristics of a large, representative sample of natural clones, which has not yet been done.

A genetic analysis of the zymograms and a populational approach reveal the genetic structure and multiplication system of *T. cruzi*, make it possible to replace descriptive concepts (such as zymodeme and schizodeme) by an explanatory one (natural clone), and to propose a general model of *T. cruzi* infraspecific variability suitable for applied studies. The question of mating in *T. cruzi* remains open, even though recombination in natural conditions seems to be rare or absent. Nevertheless, success in obtaining mating in the laboratory would be extremely valuable for undertaking experiments about the biology of *T. cruzi*.

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References

1 Tait, A. (1980) *Nature* 287, 536–538
2 Jenni, L. *et al.* (1986) *Nature* 322, 173–175
3 Zampetti-Bosseler, F. *et al.* (1986) *Proc. Natl Acad. Sci. USA* 83, 6063–6064
4 Paindavoine, P. *et al.* (1986) *EMBO J.* 5, 3631–3636
5 Tibayrenc, M. *et al.* (1981) *C.R. Acad. Sci. Paris* 292, 623–625
6 Tibayrenc, M. and Miles, M.A. (1983) *Trans. R. Soc. Trop. Med. Hyg.* 77, 76–83
7 Tibayrenc, M. *et al. (1985) Genetica* 67, 223–230
8 Tibayrenc, M. *et al.* (1986) *Proc. Natl Acad. Sci. USA* 83, 115–119
9 Gibson, W.C. and Miles, M.A. (1986) *EMBO J.* 5, 1299–1305
10 Gibson, W.C. *et al.* (1985) *Mol. Biochem. Parasitol.* 16, 231–242
11 Tibayrenc, M. *et al.* (1981) *C.R. Acad. Sci. Paris* 293, 207–209
12 Tibayrenc, M. and Desjeux, P. (1983) *Trans. R. Soc. Trop. Med. Hyg.* 77, 73–75
13 Tibayrenc, M. *et al.* (1984) *Trans. R. Soc. Trop. Med. Hyg.* 78, 519–525
14 Tibayrenc, M. (1985) *Trans. R. Soc. Trop. Med. Hyg.* 79, 882–883
15 Brénière, S.F. *et al.* (1985) *C.R. Acad. Sci. Paris* 300, 555–558
16 Dujardin, J.P. *et al.* (1987) *J. Med. Entomol.* 24, 40–45
17 Toyé, P.J. (1974) *Trans. R. Soc. Trop. Med. Hyg.* 68, p. 147
18 Miles, M.A. *et al.* (1977) *Trans. R. Soc. Trop. Med. Hyg.* 71, 217–225
19 Ready, P.D. and Miles, M.A. (1979) *Trans. R. Soc. Trop. Med. Hyg.* 74, 238–242
20 Morel, C. *et al.* (1980) *Proc. Natl Acad. Sci. USA* 77, 6810–6814
21 Ochman, H. and Selander, R.K. (1984) *Proc. Natl Acad. Sci. USA* 81, 198–201
22 Miller, R.D. and Hartl, D.L. (1986) *Evolution* 40, 1–12
23 Tibayrenc, M. and Ayala, F.J. (1987) *C.R. Acad. Sci. Paris* 304, 89–93
24 Nei, M. (1972) *Amer. Natural.* 106, 283–292
25 Farris, J.S. (1970) *Syst. Zool.* 19, 83–92
26 Felsenstein, J. (1978) *Syst. Zool.* 27, 401–410
27 Gibson, W.C. and Miles, M.A. (1985) *Brit. Med. Bull.* 41, 231–242

Malaria: an Intra-erythrocytic Neoplasm?

Ya Zhang

Drug resistance is a serious problem in malaria, and prospects for new drugs are not optimistic. In 1963, the US Army began a huge programme to develop new antimalarials; they screened over 235 000 compounds, but very few were sufficiently active and safe for use in humans[1]. Part of the problem is that not enough is known about the biochemical properties of malaria parasites, especially the metabolic differences between them and their host cells which could offer targets for specific chemotherapy.

An important characteristic of malaria infection is the rapid growth of the parasite population, and changes in host metabolism that result from this. A similar effect occurs in many cancers. In this article, Ya Zhang argues that malaria parasites also have metabolic similarities with tumour cells, and suggests that careful comparison of these two could provide insight for new drug development.

Unlike many infectious agents such as bacteria, neither malaria parasites nor cancer cells excrete specific toxic factors to injure their host cells. Instead, they cause disease by rapid growth – competing with host cells for energy and nutritional substrates. During intraerythrocytic schizogony, plasmodia multiply rapidly to produce 10–25 merozoites per cycle (24–48 h). This suggests that the syntheses of proteins, RNA, and DNA, as well as energy consumption of the parasites, must increase 10–25 fold during that period[2]. The growth rate of malaria parasites is much more rapid than that of the most malignant tumour cells, and so the course of malaria is acute while cancer is chronic.

Glucose and Energy Metabolism

Early studies indicated that most tumour cells, when metabolizing glucose under aerobic or anaerobic conditions, produce lactate at a higher rate than do normal tissues[3]. In cancer tissues exhibiting aerobic glycolysis, the quantity of

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