studies from Senegal have demonstrated antigenic stability of asymptomatic infections in the absence of transmission, and that clinical disease is associated with a sharp rise in parasite densities and acquisition of new antigenic types. Furthermore, polymerase chain reaction (PCR)-based studies are emerging from several laboratories reporting that symptomatic infections appear less polyclonal than asymptomatic ones (I. Felger, Abstract). As very dominant variants interfere with the detection by PCR of less abundant ones, this finding is consistent with a disease-related swamping of a polyclonal, asymptomatic infection by a single or few variants, presumably those causing the clinical episode (A. Farnet, Abstract).

The parasite density of asymptomatic infection appears to differ between areas of different endemicity. Thus, parasite densities appear to fluctuate around 400-500 parasites $\mu l^{-1}$ in Tanzania (high endemicity), well below 500 parasites $\mu l^{-1}$ in The Gambia (medium endemicity), and lingering around the level of detection by PCR in Eastern Sudan (low endemicity). If such a relationship is indeed genuine, it will fit well with our model, assuming each antigenic variant to be kept very close to the point of eradication by variant-specific immunity. The overall parasite density must therefore depend on the number of concurrent variants, and it seems reasonable that this number should correlate with transmission intensity. Finally, the absence of seasonality in the prevalence of clinical disease in areas of very intense but seasonal transmission may be explained in terms of the model, as inhabitants of such an area can be expected to have sufficient immunity against a large repertoire of variants at all times.

In conclusion, we feel that it is misguided to discount variant-specific responses as important elements in protective immunity to malaria. At the same time, the available evidence clearly leaves room for concurrent ADCC-type immune responses.

Acknowledgements
David Amot, Odile Mercereau-Puijalon and Thor Theander are thanked for helpful comments. LH is a Weimann senior research fellow.

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Trine Staalsoe and Lars Hvidt are at the Centre for Medical Parasitology, RHiMA Centre M7641, Copenhagen University Hospital (Rigshospitalet), Tagersvej 20, 2200 Copenhagen N, Denmark. Tel: +45 35 45 73 77, fax: +45 35 45 76 44, e-mail: tscmp@rh.dk

Note: See Letters, this issue

Trypanosoma cruzi: How Many Relevant Phylogenetic Subdivisions are There?

S. Brisse, C. Barnabé and M. Tibayrenc

Trypanosoma cruzi, the agent of Chagas disease, has been the 'pet' model of pioneering studies on genetic characterization of pathogens. Isoenzyme analysis has been widely used for $T. cruzi$ strain characterization, and has permitted the identification of three principal zymodemes, subdivided into a few lesser ones (zymodeme is a collection of strains that share the same isoenzyme profile).

Evolutionary genetic approaches made it possible subsequently to explore the origin of $T. cruzi$ genetic polymorphism. $Trypanosoma cruzi$ was shown to exhibit a typical clonal population structure: genetic exchange is either absent or rare, which leads to the propagation of clones that are stable in space and time. Clonality has several implications that are relevant in terms of applied research:

1. Because many genetic characters are linked (transmitted together as a whole, like 'blocks of genes'), knowing the genotype of one gene in a given strain makes it possible to predict the genotypes of many other genes. This permits indirect marking and identification of a strain with only one genetic character (for example, a molecular probe or a polymerase chain reaction diagnostic).
2. Clones are transmitted unchanged (except for mutations), akin to 'genetic photocopiers', so they can be used as stable markers for follow-up of the epidemics. (3) Since gene exchange is either rare or absent, the clones diverge more and more by accumulation of mutations, which might be particularly relevant when considering those genes that govern medically important characters (pathogenicity, resistance to drugs).

Based on both isoenzyme and random amplified polymorphic DNA (RAPD) polymorphism, we have proposed recently$^5$ that $T. cruzi$ natural clones can be ranked into two main phylogenetic subdivisions or clades. Clade 1 includes the formerly described zymodeme 1, whereas Clade 2 includes zymodemes 2 and 3 (Ref. 1). It is important to note that each of these two main clades is genetically very heterogeneous.$^6$

Souto et al.$^8$ recently made a somewhat different proposal. By using a limited number of molecular markers, they identified two major phylogenetic lineages within $T. cruzi$, which correspond to the ones we previously described. This verification is possible since their work and ours include many common
Subdivision 1 from Souto et al.\textsuperscript{6} corresponds to our Clade 2 (Ref. 5). Nevertheless, Souto et al.\textsuperscript{6} perceive the two main subdivisions as 'clear-cut', separated by vast phylogenetic gaps. This result appears to us to be due to the fact that the few genetic characters used by the authors\textsuperscript{5} have a relatively slow molecular clock, and can be equated to some extent to 'synapomorphic characters' (in the cladistic jargon, a derived character that is shared by all members of a given clade, and only by them). A very comparable picture would be obtained if we retained only those isoenzyme and RAPD characters that appear to be synapomorphic (clade-specific) in our own results\textsuperscript{6,5}. However, we feel that such an approach would be misleading, for it does not take into account the actual phylogenetic diversity of the agent of Chagas disease. Recent results obtained in our laboratory do confirm that the two major clades of \textit{T. cruzi} are extremely heterogeneous. Isoenzyme analysis of >400 strains with 22 loci (C. Barnabé, unpublished) revealed a total of 144 and 118 different genotypes within Clades 1 and 2, respectively. Moreover, the average and maximum genetic distances within each clade are not much lower than those recorded in the whole species. This phylogenetic heterogeneity of both Clades 1 and 2 is fully confirmed by extensive RAPD analysis involving the screening of 120 different primers (S. Brisse, unpublished). Moreover, this RAPD study revealed another informative result: Clade 2 is strongly structured into five lower phylogenetic subdivisions, each identifiable by robust RAPD synapomorphies. These results, together with previous data\textsuperscript{2-4,5}, strongly support the view that Clades 1 and 2 are not operational units for applied studies. Apart from their considerable phylogenetic diversity, the ecological range of the strains attributed to each of the clades is impressive. Both Clades 1 and 2 include strains isolated from either sylvatic or domestic cycles. For example, Clade 1 is identified both in Amazonian wild mammals (mainly \textit{Diekalhiph sp.} and rodents), and in domestic \textit{Triatoma} infestans from Andean countries\textsuperscript{1}. In Chagas disease, strains attributed to either Clade 1 or Clade 2 can be isolated from both acute and chronic forms\textsuperscript{2}. It is possible that there is some statistical specificity of each of the clades in terms of epidemiology and pathogenicity. Nevertheless, from this point of view, the predictive value of the attribution of a given strain to one of the clades is probably very low, as also noted by Souto et al.\textsuperscript{6}. For epidemiology and studies dealing with pathogenicity or resistance to drugs, the approach developed presently in our laboratory is to take as targets either the lower clades of \textit{T. cruzi} or the natural clones, with special emphasis on those clones that are more frequently recorded (major clones).\textsuperscript{2} The biological and epidemiological specificity of either lower clades or natural clones is probably much sharper than that of Clades 1 and 2. The results of Souto et al.\textsuperscript{6} have indeed contributed to our basic knowledge of \textit{T. cruzi} molecular evolution by strongly confirming the existence of two main clades within this species, using innovative molecular tools. Souto et al.\textsuperscript{6} proposed also that one of the genotypes identified by them is the product of genetic exchange. Although alternative hypotheses can be considered, we do agree that hybridization is the most parsimonious one. We are aware that the model of clonal evolution proposed for \textit{T. cruzi}\textsuperscript{2} and other parasitic protozoa\textsuperscript{3} does not exclude the possibility of limited genetic exchange. The hypothesis of hybridization elegantly supported by the molecular analysis of Souto et al.\textsuperscript{6} is consistent with the proposals of other authors\textsuperscript{7}, and with RAPD results obtained in our laboratory (S. Brisse, unpublished).

\section*{References}
1 Miles, M.A. et al. (1978) Nature 272, 819-821

Sylvian Brisse, Christian Barnabé and Michel Tibayrenc are at the Centre d'Etudes sur le Polymorphisme des Microorganismes (CEPM), UMR CNRS/ORSTOM 9926, ORSTOM, BP 5045, 34032 Montpellier Cedex 1, France.

\section*{Note: See Letters, this issue.}

\section*{Websites of Interest}

\textbf{African Malaria Vaccine Testing Network} This now has a lively website at \url{http://www.amvtn.org}. It has a newsletter for up-to-date information about molecular biology products, lab instrumentation, and supplies. See \url{http://www.biosupplynet.com}, which also has an interactive online companion to the book.

\textbf{Cryptosporidiosis} Information from the CDC about prevalence in the USA can be found at \url{http://www.cdc.gov/ncidid/diseases/cryptosources.htm} and about Cryptosporidium research at Kansas State University at \url{http://www.ksu.edu/parasitology/}.

\textbf{GrantsNet} Young scientists looking for sources of support for research and advanced training in the biomedical sciences should look at \url{http://www.grantsnet.org}, a website sponsored by the Howard Hughes Medical Institute and the American Association for the Advancement of Science.

\textbf{Leishmania PCR primers} Jeffrey Shaw (shaw@tba.com.br) is preparing a list of PCR primers (ldNA or ndNA) used for diagnosing leishmaniasis for the International Leishmania Network's website (\url{http://www.bdb.org.br/bdb/leishnet}). He wants sequences, with relevant literature citation.

\textbf{Parasites and Parasitical Resources} An informative website exists at \url{http://www.biosci.ohio-state.edu/~l79/parasites.html}. It has links to other sites such as \url{http://www.scienceguide.com}.

\textbf{Malaria abstracts} Abstracts added to the database of the library of the Environmental Health Project of the US Agency for International Development, Arlington Virginia each week can be found at \url{http://www.access.digex.net/~ehp under malaria information}. Dan Campbell (CAMPBE8LDDB@cdcm.com) is the Librarian.

\textbf{1998 Source Book} (available free to researchers) provides up-to-date information about molecular biology products, lab instrumentation, and supplies. See \url{http://www.biosupplynet.com}, which also has an interactive online companion to the book.
Mating patterns of Plasmodium

- Life history evolution in nematodes
- Ribosomal DNA plasmids of Entamoeba
- Vector biology and transmission potential