

## Clonality in *Leishmania*

In their reply<sup>1</sup> to my recent comments<sup>2</sup>, Pagès and colleagues dispute the clonality in *Leishmania*<sup>3</sup> on account of a lack of resolution of multilocus isoenzyme electrophoresis (MIE) by comparison with pulse field gel electrophoresis (PFGE). It must not be forgotten that 90% of modern population genetics has been based on isoenzymes for the past 20 years. The use of this technique as a tool for population genetics and evolutionary studies is hence quite well documented, which is definitely not the case for PFGE; although this last method is a promising technique to explore the structure of the genomes, the evolutionary pattern of the polymorphisms evidenced by it are still poorly understood.

When considering the question under study (the reproductive system of a given species and its population structure), this means that the compartment of the null hypothesis (random genetic exchange) is well ascertained when isoenzymes are used, while it is a black box in the case of PFGE. When a species is sexual (man, fruitflies), MIE does show this. When a species is clonal in a population genetics sense (most species of bacteria, parthenogenetic organisms), this is again clearly shown by MIE, as confirmed by an impressive amount of data. Comparing what is comparable (according to MIE results), *Leishmania* definitely departs from the null hypothesis expectations and shows patterns that are extremely similar to the organisms known as clonal.

As recalled many times by us<sup>4</sup>, the clonal model by no means states that the lineages

As an example, we have previously seen 43 different 'clones' or zymodemes in *Trypanosoma cruzi* by using 15 isoenzyme loci<sup>4</sup>. We have recently shown, with 22 loci, additional variability within each of the previously identified clones (C. Bamabé, unpublished). The only means to determine the actual number of clones present in a given species would be to survey its entire genome<sup>4</sup>. Now when *L. infantum* is surveyed by 15 variable isoenzyme loci<sup>5</sup>, a considerable linkage disequilibrium is recorded, and a widespread zymodeme (that is to say; a homogeneous MIE genotype) is observed, which is MON1. Whatever be the additional variability shown by PFGE within MON1 (Ref. 1), the isoenzyme results are incompatible with the hypothesis that *L. infantum* is a sexual species, and far more parsimoniously explained by the clonal model<sup>3</sup>. It does not mean that MON1 is an actual clone, but simply that it has been generated by predominantly clonal propagation. We have proposed the term 'clonet'<sup>6</sup> to designate, in a clonal species, all the isolates that appear to be genetically identical on the basis of a particular set of markers: MON1, if not a clone, is a clonet.

Such a classical demonstration of circumstantial evidence of clonality (wide geographical propagation of a given multilocus genotype) would be missed by focusing only on studies performed in sympatric conditions. Allopatric and sympatric studies are complementary in population genetics. The last ones have not 'ruled out' the hypothesis of clonality in *Plasmodium falciparum*<sup>1</sup>; until now, they had shown no departure from panmictic expectations, but this is different. Failure to

such sympatric conditions, high linkage disequilibrium have been recently seen in *P. falciparum* (B. Abderrazak et al., unpublished), although it should be emphasized that the evidence for clonality in this last species remains less clear than for *Trypanosoma cruzi* and... *Leishmania*. Pagès et al. do not accept the general model of broad-sense clonality for *Leishmania*, and prefer to focus on the particular patterns of given epidemiological situations. I do not deny that particular cases are informative. But a central goal of science is to build general models. One cannot afford a specific population genetics for each epidemiological focus of *Leishmania*. The question addressed by the clonal model is not what specifically happens in the Pyrénées Orientales focus, but rather: is *L. infantum* considered, as a whole, a normal sexual species (like man and fruitflies) or should it be better considered a predominantly clonal species? My answer still is: the latter.

### References

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