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Response to Rougeron *et al.*: *Leishmania* population genetics: clonality, selfing and aneuploidy

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The article by Rougeron *et al.* [1] makes relevant remarks about the population genetics and taxonomy of *Leishmania*. The authors in particular propose that linkage disequilibrium (LD) (nonrandom association of genotypes at different loci) could be explained by micro-Wahlund effects (separation by space and/or time), that recombination in *Leishmania* could be frequent, possibly in most lineages, and that aneuploidy could be transitory, with no implications for population genetic tests. We disagree with several of these statements.

Selfing and clonality

Rougeron *et al.* [1] consider that selfing (self-fertilization of an organism) and 'true' clonality (mitotic propagation) should be distinguished. This is a matter of definition. We have proposed that selfing is a particular case of our predominant clonal evolution (PCE) model (restrained recombination on an evolutionary scale) [2,3]. Indeed, most scientists working on pathogens consider selfing as a particular case of clonality [2]. Moreover, for applied research (e.g., strain typing or mapping of genes of interest), the distinction is not relevant, since the main fact, both in 'strict' clonality and in selfing, is that recombination is inhibited. Therefore, multilocus genotypes (MLGs) are transmitted as a whole and are stable in space and time.

Linkage disequilibrium, sampling strategies, and correlation between different markers

The authors [1] wrongly state that we consider clonality and LD as synonymous in the PCE model. Statistically significant LD is only one of the criteria proposed to define PCE [2]. Other criteria are the existence of stable, ubiquitous, overrepresented MLGs (which could be absent with respect to markers of high mutation rate), the presence of near-clades (phylogenetic subdivisions that are somewhat obscured by occasional genetic recombination), and Russian doll patterns (a miniature picture of the whole species within each near-clade, in which PCE operates too) [2,3]. The authors [1] state that the Wahlund effect bias (genetic isolation by space and/or time instead of by intrinsic biological properties of the organism under survey) could account for LD. This possible bias has been extensively discussed [4]. To discard it, in the framework of the PCE approach, we have recommended broad sampling across the whole ecogeographical range of the species, complemented by retrospective studies. If the same global pattern of widespread MLGs, near-clades, and Russian doll patterns is recorded in many different places and times, this cannnot be explained by a Wahlund effect and strongly suggests that PCE is verified. The authors cite several cases supporting this pattern [1]. They call the near-clades 'ecologically divergent entities' or 'different genetic taxa', vague terms based on imprecise working hypotheses, which is not the case for the near-clade concept. When Rougeron *et al.* note that 'some MLGs seem to coexist in the same region for very long periods of time' [1], this finely fits the PCE model. Such a persistent population structure, recorded in *Leishmania* [2,3], does not support the hypothesis that LD is caused by a Wahlund effect in a recombining species. If this were the case, overpresented MLGs and near-clades would tend to be geographically localized and/or limited in time. Lastly, it is wrong to state that multilocus enzyme electrophoresis (MLEE) and DNA markers are not linked. In *Leishmania donovani* [5], the MLEE strains MON 37 are not randomly distributed in the microsatellite tree. All but one are in the bottom cluster of the tree. The limited discrepancy between the two markers is easily explained by MLEE homoplasy, although it may also indicate occasional recombination, which is compatible with the PCE model [2,3]. Most times, MLEE and DNA markers exhibit a fair association: all *Leishmania infantum* strains MON 1 correspond to a unique microsatellite cluster, which comprises several genotypes ([6] for example). This is not a discrepancy; it shows only that microsatellites have a stronger resolution power than MLEE. We have cited many examples of agreement among different genetic markers in *Leishmania* [3]. Finally, a lack of correlation between population structure and drug resistance, although consistent with occasional recombination, is easily explained by independent emergences of resistance, a frequent pattern for a strongly selected phenotype (see [7] for an extreme case). The PCE model does not predict that all phenotypic characters should be strictly linked to population structure.

Aneuploidy and heterozygote deficit

Heterozygote deficit is a typical indication of selfing in diploid organisms. However, several causes are possible, which are not mutually exclusive [2]. Mitotic gene conversion is ruled out by Rougeron *et al.* [1], not by other authors [8]. *Leishmania* is thought to exhibit widespread aneuploidy,

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which should purge heterozygosity at each haploid cycle [9] and renders tests based on the hypothesis of diploidy invalid [2,3]. The view that microsatellite data do not fit the hypothesis of aneuploidy [1] is questionable [10]. Rougeron *et al.* [1] propose that aneuploidy could be transitory, which is not supported by a genomic analysis that deals with natural isolates and not experimental populations [11]. Even if it were true, heterozygosity purging at each haploid cycle [8] should remain.

Frequent recombination?

It is questionable to state that in some lineages (and possibly most lineages) of *Leishmania* sexual recombination is frequent [1], because the evidence against it is strong [2,3]. The studies cited to support sexual recombination [1], rather, deal with heterozygote deficit, which is considered as evidence for selfing. Selfing leads to lack of recombination and LD, not to sexual recombination [2].

The efforts by Rougeron *et al.* [1] to explore more finely the role played by selfing in *Leishmania* evolution are valuable. However, as recalled many times [2–4], selfing does not challenge the PCE model, since this model considers it as a particular case of clonality. Moreover, methodological difficulties (in particular the strong evidence for aneuploidy in *Leishmania*) make it tentative to evidence selfing. We consider that the PCE model by far fits the best *Leishmania* population genetic data, which do not show any evidence of frequent sexual recombination [1]. The development of whole-genome sequencing will certainly help in clarifying parasite evolutionary patterns, as it has done in several major bacterial species [2].

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Response to Tibayrenc *et al.*: can recombination in *Leishmania* parasites be so rare?

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The letter by Tibayrenc and Ayala [1] disagrees with several statements on the population genetics of *Leishmania* parasites that we recently published [2]. They consider that these parasites display a preponderant clonal evolution (PCE) model, suggesting no evidence of frequent sexual recombination, which is thus supposed to represent the best model fitting *Leishmania* population genetics data.

Confusing selfing and clonality

The first argument appearing in Tibayrenc and Ayala's letter is that 'most scientists working on pathogens consider selfing as a particular case of clonality'. Tibayrenc and Ayala self-cite their own paper [3] where, if we retrieve self-citations and some other papers where authors never

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