Title
Response to Rougeron et al.: Leishmania population genetics: Clonality, selfing and 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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wrote that selfing was a particular case of clonality (e.g., [4]) or even clearly made the distinction between clonality and selfing [5], we are left with only few remaining articles (most of which refer to Tibayrenc and Ayala's papers) asserting that selfing and clonality are the same thing. Then, Tibayrenc and Ayala's PCE model proposes that the evolutionary consequences of strict clonality and strong selfing are similar in that they lead to linkage disequilibrium (LD) and propagation of unchanged multilocus repeated genotypes (MLGs) [3]. In other words, the authors assimilate individual inbreeding as evidence of clonality [3]. The only common feature between clonality and selfing is that strongly inbred populations (i.e., small populations of extremely small sizes) will produce the MLGs that clonality classically generates [6]. It should be noted that genetic drift in small populations will also lead finally to LD as well as repeated MLGs over time, even in sexually reproducing populations that display no selfing (T. de Meeûs, personal communication). Thus, considering LD and repeated MLGs as key clues for defining clonality or selfing is not always appropriate. Moreover, it is relevant to distinguish between selfing and clonality because population genetic studies will use different models that will lead to different inferences. This is obvious in diploids, where clonality generates heterozygote excesses [7], while selfing leads to homozygosity excesses [8]. From an evolutionary point of view, selfing and clonality are different. Selfers undertake meiosis at each generation, which leads to higher mutation rates compared with mitotic propagation [9]. Thus, recurrent genome-wide reshuffling will recombine those new and more numerous mutations in selfers, while, for clonal propagators, the only way to restore damaged (mutated) important sites will be through localized mitotic recombination, such as gene conversion.

LD and inappropriate sampling strategies

Tibayrenc and Ayala state again that one of the criteria to be taken into account for the PCE model is LD. To avoid that Wahlund effect in LD, the authors recommend performing broad sampling across the whole ecogeographical range of species [1]. Computer simulations showed that LD does not provide reliable measurements to make the distinction between clonal and sexual reproductions [10]. Moreover, in our review, we specifically underlined that spatiotemporal effects, combined with inbreeding and/or clonality, produce unpredictable effects on LDs. By considering a broad range of sampling, how would the PCE model proposed by Tibayrenc and Ayala give any valuable information on the reproductive strategies used by *Leishmania* parasites based on this genetic parameter? We do not understand how the near clade and Russian doll criteria proposed would unravel such combined spatiotemporal Wahlund effects with selfing and/or clonal propagation and small effective local population sizes. In any case, such criteria would need to be validated by analytical modeling or simulations.

Aneuploidy and population genetics in Leishmania

Tibayrenc and Ayala explain that, even if *Leishmania* are aneuploid organisms, heterozygosity purging at each haploid stage would remain and, thus, the recurrent heterozygote deficits that we (and several others) obtained in population genetic studies are irrelevant. Nevertheless, they fail to explain why more than two alleles were never reported in any population genetics survey (and there are many), as expected in cases of hereditary aneuploidy, because aneuploidy is known to involve numerous cases of tri-, tetra-, and pentazomic chromosomes [11]. This is why we suggest that these aneuploidy states are more likely transient. Moreover, the life-stage specificity of aneuploidy deduced by Inbar *et al.* from their experimental studies strongly supports this assumption [12].

Recombination in Leishmania *parasites*

Given the above arguments, those illustrated in our review, and in the absence of any theoretical or experimentally controlled validation of Tibayrenc and Ayala's PCE, near clade, and Russian dolls criteria [1], we are left with the reasonable conclusion that most *Leishmania* populations recurrently experience sexual recombination, mainly endogamic and less frequently allogamic. These assessments are in agreement with the results obtained in most empirical population genetics studies with accurate sampling strategies, as well as experimental studies.

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