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Publication Date

2018-11-01

DOI

10.1016/j.actatropica.2018.07.015

Peer reviewed



A misleading description of the predominant clonal evolution model in *Trypanosoma cruzi*



We read with great interest the fine review by Zingales (2018) about *Trypanosoma cruzi* genetic diversity. It presents many relevant updated facts about the evolution of the agent of Chagas disease and its epidemiological consequences. Unfortunately, this article conveys in a very misleading way the predominant clonal evolution (PCE) model proposed by us for *T. cruzi* (Tibayrenc et al., 1986; Tibayrenc and Ayala, 2015) as well as for many other pathogens (Tibayrenc and Ayala, 2012, 2017). As several other authors, Zingales (2018) does not challenge this model as it is, that is to say: posited by its authors, but rather, on the basis of her erroneous understanding of it. This is all the more unexpected, since this model is quite correctly presented in Zingales et al. (2012).

It is totally untrue that we considered *T. cruzi* as an “organism that replicates by binary fission”. Certainly, it does, but certainly too, not only. As recalled many times, the PCE model focuses, not on the precise mating process of the organism considered, but rather, on the population genetic consequences of it. The PCE model only states that genetic recombination in the organism under survey is not prevalent enough to break the main pattern of clonal population structure. This population structure manifests itself by: (i) the widespread propagation of multilocus clonal genotypes; (ii) a statistically significant linkage disequilibrium (nonrandom association of genotypes occurring at different loci); and (iii) the presence of discrete genetic subdivisions or “near-clades” that are stable in space and time. In this model, lack or scarcity of recombination can be caused, not only by mitotic propagation (= binary fission), but also by selfing, strong homogamy and several kinds of parthenogenesis known from unisexual vertebrates (Avisé, 2008).

It is incoherent to state that “the current evidence challenges the traditional paradigm of the prevalent clonal evolution model in *T. cruzi*” (that is to say if the PCE model is correctly understood: scarcity of recombination), and later, to recall the existence of six genetic lineages or discrete typing units. It has been suggested that the discreteness of *T. cruzi* DTUs/near-clades was “self-evident” (Ramírez and Llewellyn, 2015). However, we did not have this feeling when we established it after long studies (Brisse et al., 2000). If *T. cruzi* DTUs (= near-clades) are “discrete”, it does imply that recombination is strictly limited among them, and therefore, that the species *T. cruzi* exhibits a PCE pattern at the level of its major sub-lineages.

It is not correct to state that *T. cruzi* is “unaffected” by rare events of genetic exchanges. We have repeatedly insisted on the fact that: (i) the PCE model does not state that recombination is absent, but only that it is not frequent enough to break up the prevalent clonal population structure; (ii) occasional bouts of recombination and hybridization could have strong consequences on *T. cruzi* genetic and genomic diversity at evolutionary scales.

We have insisted on the fact that imprecise and subjective terms such as “quite frequent” recombination are poorly informative and could be misleading. Pathogen evolutionary studies should not be based on vague assertions, but rather on appropriate statistical tests supported

by significant *p* levels of signification and phylogenetic analyses adapted to the specific cases under study. The PCE model aims at replacing this imprecise terminology by a clear-cut criterion, the “clonality threshold”. When the PCE pattern is reinforced more and more by adding more relevant data (sample size, number of loci, markers with a higher resolution), it means that genetic recombination is efficiently countered by predominant clonality and separate genetic lines (near-clades) will irreversibly diverge from one another. This is what has been repeatedly observed in *T. cruzi*: adding more genetic markers and more strains has constantly reinforced the robustness of the six DTUs/near-clades.

Lastly, although this point is not considered by Zingales (2018), an important component of the PCE model is the “Russian doll” hypothesis (Tibayrenc and Ayala, 2013), which states that within the DTUs/near-clades, the population structure is similar to that of the whole species, with widespread multilocus clonal genotypes, statistically significant linkage disequilibrium, and lesser near-clades. We have cited several examples in Tibayrenc and Ayala (2015, 2017) showing Russian doll structures within the *T. cruzi* DTU/near-clade “TCI”. Contrary to what has been claimed (Ramírez and Llewellyn, 2015), the tests used to support the Russian doll model are not “highly sensitive to anything but completely random associations between loci at a population level”, not more than the tests used at the level of the whole species, since they are the same. As an example, in Messenger et al. (2015), although this is not emphasized by the authors, the patterns observed in sylvatic Bolivian TCI isolates tick all the right Russian doll boxes: (i) repeated multilocus genotypes that would be quite improbable in case of random mating; as a matter of fact, in the case of random recombination, the probability of a given multilocus genotype is the product of the probabilities of the unilocus genotypes it is composed of. With a very simple model with two loci A and B, each having two genotypes A1 and A2, B1 and B2, equally frequent (0.5), the probabilities of the bilocus genotypes A1/B1, A1/B2, A2/B1 and A2/B2 is $0.5 \times 0.5 = 0.25$ each. The probability to have twice the genotype A1/B1 is $0.25 \times 0.25 = 0.0625$. The probability to have it three times is 0.015625. When more loci are considered, the probability to observe repeated genotypes rapidly becomes close to zero (Tibayrenc et al., 1990); (ii) strong linkage disequilibrium in all subpopulations; and (iii) “deep internal nuclear branching patterns in both lowland groups”, in other words: reliable genetic subdivisions traducing a clear phylogenetic signal = lesser near-clades. Messenger et al. (2015) do not propose specific hypotheses to explain these patterns. A Wahlund effect (physical separation by space and/or time) could contribute to generate these PCE manifestations; however, such a Wahlund effect cannot account for the observed patterns as a whole.

For the reasons stated above, we do claim that, in the present state of art, the PCE model is by no means “challenged” for the agent of Chagas disease. However, intra-near-clade/DTU population genetics in *T. cruzi* calls for additional studies. Although present data support the

Russian doll hypothesis in *T. cruzi* for all cases conveniently explored, data on *T. cruzi* intra-near-clade genomic diversity still are scarce by comparison with *Leishmania* (Imamura et al., 2016) and bacteria (Alnajar and Gupta, 2017; Dyson et al., 2017). Future genomic studies on *T. cruzi* intra-near-clade population genetics should take as models such approaches based on fine typing using thousands of SNP markers. They should explore the diversity of the six main *T. cruzi* near-clades (= DTUs) on their whole ecogeographical range. After discarding the biases due to the Wahlund effect, the right boxes to tick for supporting the PCE model are: (i) statistically significant linkage disequilibrium; (ii) widespread multilocus genotypes which probability do not fit panmictic expectations; (iii) genetic subdivisions that are stable in space and time (lesser near-clades)

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