

## Aneuploidies are an ancestral feature in Trypanosomatids and could be related to parasite adaptation

Samuel Alexandre Pimenta Carvalho, Laila Viana de Almeida, Anderson Coqueiro-dos-Santos, Rodrigo P. Baptista, Gabriela F. Rodrigues-Luiz, Mariana Santos Cardoso, Costa CHN, Cooper A. Grace, Daniel Jeffares, Richard McCulloch, Daniella C. Bartholomeu, João Luís Reis-Cunha

Aneuploidy, the presence of an aberrant number of chromosomes in a cell, usually results in severe abnormalities in multicellular eukaryotes as humans. However, some unicellular eukaryotes rely on aneuploidy as a mechanism to allow rapid adaptation to changing environments, having a positive fitness in stress conditions and promoting drug resistance. Aneuploidies have been largely described in protozoan parasites as *Leishmania* and *Trypanosoma cruzi*, where duplicated chromosomes vary in different hosts and can promote drug resistance. Interestingly, their closely related parasite *Trypanosoma brucei*, is mainly euploid. Hence, to evaluate if aneuploidies are an ancestral or recent feature in trypanosomatids we estimated the chromosome copy number variation in 13 Trypanosomatidae species, including *Angomonas*, *Crithidia*, *Leptomonas* and *T. vivax*, using whole genome sequencing and read depth coverage variations. Aside from the *T. brucei*, *T. evansi* and *T. vivax*, all the remaining species have evidence of aneuploidies, including *Paratrypanosoma confusum*, an early-branching trypanosomatid, indicating that it is an ancestral character in these parasites. The presence of aneuploidies could be detrimental in *T. brucei* clade, as their genome is packed in a lower number of larger chromosomes. Next, we evaluated if there were consistent chromosomal duplications in the evaluated species. *Leishmania's* chromosome 31 is constantly supernumerary, a fact reassured by our analysis of ~200 isolates from *L. donovani* and *L. infantum* populations in Africa, Asia and Brazil. This chromosome had an increased nucleotide diversity ( $\pi$ ), which is expected, as having extra copies per cell results in more sites to be randomly mutated. Similarly, redundant copies of genes could allow a rapid adaptation and diversification without loss of function. Regarding the other trypanosomatid species, the chromosomes that have most of its genes orthologous to *Leishmania* chromosome 31 were also consistently supernumerary, even in the euploid *T. brucei* clade where regions of this chromosome are observed in two chromosomes, 4 and 8. We evaluated the function of these shared duplicated genes and we found genes involved in housekeeping functions as osmoregulation and response to stress, diverse cytoskeleton mediated processes such as cell morphogenesis, flagellar motility and cell division, energy obtaining pathways, host immune system evasion, infectivity and intracellular trafficking. We are now evaluating species-specific genes that were inserted in these duplicated regions specifically in each protozoan, as those can be important to each parasite adaptation.