Episomal gene amplification and copy number variation in natural populations of *Leishmania donovani*

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Structural variation has gained attention as contributor to genomic diversity in addition to sequence variation. This is particularly prevalent in *Leishmania donovani* from the Indian subcontinent, where the large amount of copy number variation (CNV) contrasts with the high level of sequence conservation. One mechanism responsible for CNV is the formation of extra-chromosomal circular episomes. Such episomes were previously evidenced in experimental conditions, e.g. drug resistance induction, and found to be unstable in the absence of drug pressure. We present a bioinformatics method to predict the occurrence of episomes from whole-genome sequencing data. We applied our method on a population of 17 drug resistant and sensitive *L.donovani* clinical lines, detected two potential episomes and experimentally verified our approach. The first episome was already described (H-locus containing the ABC-thiol MRPA gene), the second one harbored 4 genes, among which a MAPK homologue. We expanded the analysis of these loci in >100 clinical lines and found that (i) both episomes were present in most lines and (ii) the copy number of the MAPK-locus was linked with treatment failure of visceral leishmaniasis in the corresponding patients. We also tested in vitro and in vivo episome stability.