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Mosaic aneuploidy in *Leishmania*: the perspective of whole genome sequencing

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In their recent opinion paper [1], Sterkers *et al.* review findings on intra-strain mosaic aneuploidy in *Leishmania* and present a challenging model predicting the consequences of this phenomenon for the genetic diversity observed within a given strain: this model leads to a loss of heterozygosity in clonal division, and to a gain of heterozygosity in automixy. They review results largely based on an elegant fluorescence *in situ* hybridization (FISH) that measured, at individual cell level, the somy of individual chromosomes within five laboratory strains from five different species propagated *in vitro* as promastigotes. While their results demonstrate the power of FISH, the method is time-consuming and only ten chromosomes (out of 34–36, depending on the species) are currently monitored at a time. Whole genome sequencing (WGS) is an alternative approach to assess ploidy, by measuring the relative depth of mapped reads [2]. While single-cell genomics approaches are becoming practicable [3], current sequencing approaches analyze a population of cells present in a given sample, thereby providing a cumulative view of the somy of each chromosome, which is averaged across the (potentially heterogeneous) parasite population included in the sample. As Sterkers *et al.* rightly point out, population averaging is an important drawback of WGS in the presence of intra-strain mosaic aneuploidy, but we nonetheless feel that the statement that global approaches like WGS are 'less discriminating than individual cell analysis' [1] should be interpreted with care, and that the two methods should be considered as complementary.

We think WGS offers a number of advantages, which can be used to complement FISH. First, in a single assay, WGS provides a snapshot of all the chromosomes, unlike FISH, which can only provide a view of chromosomes targeted by probes. Second, by measuring read-depth along the whole chromosome, WGS provides unbiased results not hindered by local copy number variations, frequently observed in *Leishmania* [2] and recently shown to occur widely in the genome [4]. This bias may likely affect regions targeted by FISH probes, which is why in *Leishmania* it might be recommended to use several FISH probes per chromosome. Third, although somy results from WGS are cumulative, they allow for the identification of chromosomes showing the largest deviations from disomy. For instance, a somy value of three either implies that a majority of cells are likely to be trisomic for that chromosome or that there are two sub-populations with equal number of cells showing disomic and tetrasomic chromosomes, resulting in a similar total chromosomal gene dosage in the parasite population. This total dosage is particularly relevant to detect adaptive changes occurring at the cell population level, such as during drug resistance induction experiments [5]. Fourth, in the context of studies on individual gene dosage, WGS allows for the correct identification of mechanisms beyond aneuploidy that may also contribute to adaptive changes such as circular episomes, amplifications within tandem gene arrays, or large linear amplicons [2,5]. Fifth, WGS allows for cheap and convenient high-throughput exploration of inter-strain diversity in natural populations. A report on 17 clinical isolates of *L. donovani* showed a cumulative somy level of two in nine chromosomes, a level of four in chromosome 31, and a variable (fluctuating between two and four) somy level in the 26 remaining chromosomes across the different lines [2]. Overall, each isolate showed a different aneuploidy pattern. A similar observation has also been made in *L. infantum* [6]. Finally, sequence data simultaneously reveal aneuploidy, genotype, and allele frequencies of the same population of cells. These allele frequencies can provide valuable information in interpreting read-depth estimates of somy when there is mosaicism in chromosome ploidy. We believe these types of data will be instrumental in fully understanding the population genetics of *Leishmania*, and to test ideas such as the parasexual model proposed by Sterkers *et al.* Indeed, WGS could allow direct testing of how changes in aneuploidy correlate with changes in heterozygosity.

FISH is currently the only method that would allow further dissection of these results at the single-cell level, but an emerging technology that might combine the ability of FISH to interrogate intra-strain mosaicism in somy with the advantages of a sequencing approach is single-cell genome sequencing (SCGS), where DNA is extracted from isolated, individual cells before amplification and sequencing. This approach has been successfully applied to bacterial cells [7], a number of microbial eukaryotes [8], including *Plasmodium* [9], and to human cells, specifically to detect aneuploidy and structural variation [10]. While great care is needed in both generating SCGS libraries and in the interpretation of results from these libraries, in light of a number of biases that can be introduced during the required DNA amplification step [3,10], developments in

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both data analysis and molecular biology methods [11] are making these approaches increasingly routine.

We agree that aneuploidy constitutes a large source of adaptability, both through gene dosage effects [12] and through the shaping of genetic heterogeneity present within a single species [1]. However, in order to fully understand ploidy, WGS and single-cell approaches like FISH or SCGS should currently be applied together as complementary methods to answer major biological questions.

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