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Parasexuality and mosaic aneuploidy in *Leishmania*: alternative genetics

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Reproduction as identical or similar organisms in most biological systems depends on the extreme accuracy of the mitotic (and meiotic) mechanisms involved in the transmission of the genetic material to the two daughter cells. Character recombination and genotype diversification are ensured by the alternation between haploidy and diploidy, which corresponds to the most predominant model in sexually reproducing organisms. In *Leishmania*, the unique association of high levels of automixis and of constitutive 'mosaic aneuploidy' unexpectedly does not lead to loss of heterozygosity but constitutes an alternative for genotype recombination, hence a source of adaptability.

Aneuploidy and mosaicism in *Leishmania*

Throughout evolution, diploidy is strongly associated with the presence of a sexual cycle, the function of sex essentially being to shuffle genes. Genetic exchange typically takes place through the fusion of haploid cells, termed gametes. In most animals, including humans, as well as in the brown alga, *Fucus*, and even some protists (e.g., ciliates), these organisms develop in a diploid phase and only the gametes are haploid. By contrast, some organisms have an essentially haploid cycle, with a very short diploid phase; among them are protists, including *Plasmodium*, some molds and yeasts such as *Schizosaccharomyces pombe*, and multicellular organisms, for instance, the green algae *Ulothrix* or *Spirogyra*. Finally, the life cycle of some organisms is balanced between haploidy and diploidy; this is the case for mushrooms, the yeast *Saccharomyces cerevisiae*, as well as for most algae. Polyploidy is different because it corresponds to an exact multiple (>2) of the haploid chromosome number, and occurs widely in plants.

Aneuploidy (see Glossary) is generally considered to be deleterious and to have adverse effects on fitness. Indeed, whole-chromosome aneuploidy leads to severe developmental abnormalities or death in the many species analyzed to date [1,2]. Thus, in humans, the most common abnormalities are trisomy 21 and sex chromosome trisomies, including triple X syndrome, Klinefelter syndrome, and XYY syndrome, which are found in only 0.3% of live newborns but in approximately 4% of stillbirths and in 35% of all conceptions [3]. Moreover, aneuploidy is ubiquitous in cancer and has been linked to tumorigenesis [4,5]. However, this paradigm should be revisited because the situation appears to be slightly different in some plants [6], fungi [7], and in the protozoan parasite *Leishmania*. *Leishmania* are flagellated parasites that belong to the family of *Trypanosomatidae*, Order of *Kinetoplastea*, and are transmitted to mammals by the bite of an infected insect vector, a Phlebotomine sand fly. *Leishmania* spp. cause leishmaniases in 98 countries on four continents and are responsible for a broad spectrum of diseases ranging from skin lesions to potentially fatal organ damage [8,9]. The biology of *Kinetoplastea* is characterized by markedly original features, in particular in both the structure and the expression mechanisms of the genome; for example, they possess a unique form of gene arrays as large, directional single-stranded clusters [10], have a near absence of promoters for RNA polymerase II-directed transcription and hence of gene regulation at the post-transcriptional level [11], have no introns, and extensively edit mitochondrial mRNAs by insertion and deletion of uridines [12].

The question of ploidy in *Leishmania* has been controversial for the past 20 years, the parasite being considered 'mainly diploid' but with some authors suspecting aneuploidy [13]. The identification of two homologs of chromosome 2 differing in size by pulsed-field gel electrophoresis (PFGE), as well as of heterozygous phenotypes observed in isoenzyme and microsatellite typing (reviewed in [13]), at first sight supported the diploidy hypothesis [14]. However, trisomy was then demonstrated for particular chromosomes [15,16]. Recently, fluorescence *in situ* hybridization (FISH) analysis (Box 1), supported by high-throughput sequencing data, demonstrated that all studied *Leishmania* strains and species grown *in vitro* exhibit 'mosaic aneuploidy' [17–19]. Thus, within the cell population of a *Leishmania* strain, each of the 10 studied chromosomes, in every cell, may be present in two or more ploidy states – that is, monosomic, disomic, or trisomic – yielding highly

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Glossary

Aneuploidy: describes a chromosome number which is not an exact multiple of the haploid number. Aneuploidy is considered as 'whole-chromosome' aneuploidy in this Opinion, as opposed to copy-number changes affecting only parts of chromosomes, which are described as 'partial', 'segmental', or 'structural' aneuploidy.
Asymmetric division: unequal chromosome allotment to daughter cells during mitosis (see also symmetric chromosome allotment).
Automixis: reproductive, sexual, and/or asexual mechanisms in which offspring are derived from genetically related or identical cells; automixis is particularly adapted when individuals are geographically isolated (e.g., desert plants).
Equational division: nuclear division is which the number of chromosomes is preserved: during meiosis, it corresponds to the second division that follows the reductional division (see below).
Fluorescence in situ hybridization (FISH): a cytogenetic technique often used to detect and localize microscopically the presence or absence of specific DNA sequences on chromosomes. FISH allows examination of nuclear chromosomal contents cell by cell.
Gene dosage: the total copy number of a particular gene in the genome of a cell.
Gene shuffling: during meiosis, chromosome homologs recombine by crossing-over and exchange stretches of DNA, which in turn shuffles the genes among both chromosomes.
Genetic shuffling: generic term that includes 'gene shuffling' (see above) and 'chromosome shuffling' (see text).
Genotype: the genetic constitution – the ensemble of alleles in a genome – of a cell, an individual, or an organism. By extension, the term is used to describe the allelic constitution of a defined set of genes.
Homozygote: a diploid cell in which identical alleles of a gene are present on both homologous chromosomes.
Heterozygote: a diploid cell in which different alleles of a gene are present on the two homologous chromosomes.
Karyogamy: fusion of two nuclei from different cells; typically occurs following the fusion of gametic cells.
Loss of heterozygosity (LOH): allelic homozygosity, affecting small or large portions of a chromosome, that results from either partial deletion or whole-chromosome loss and subsequent duplication of the remaining homolog.
Mosaic aneuploidy: varying numbers of chromosome homologs among cells within the same population/strain.
Parasexuality: a non-conventional (non-sexual) mechanism for transferring genetic material and allowing gene recombination without meiosis or fertilization. Similar to a sexual cycle, parasexuality gives a species the opportunity to recombine its genome and produce new genotypes in its offspring.
Phenotype: observable expressed features, including morphology, development, biochemical, or physiological properties; includes the molecular phenotype as determined by molecular methods.
Ploidy conveyor: a dynamic model of chromosome copy-number variation which encompasses polyploidy, aneuploidy, and ploidy reversal (see below), as described by Duncan *et al.* in hepatocytes [29] and reviewed in [49]. This mechanism evolved in hepatic cells to promote genetic diversity and adaptation in response to liver injury.
Ploidy reversal: a term forged by Duncan *et al.* [29] to refer to the restoration of diploidy when polyploid hepatic cells produce diploid daughter cells through multipolar mitosis.
Polyploidy: an increase (>2) in the number of complete sets of chromosomes; autopolyploidy corresponds to the fusion of genomes within the same species; alloploidy, to the fusion of genomes of different species. In polyploid cells, gene dosage (see above) remains balanced.
Prometaphase I: in reference to the sequence of mitosis, prometaphase is the second stage, following prophase and preceding metaphase. Prometaphase I refers to the first meiotic division or meiosis I. Recombination between homologous chromosomes occurs at this stage.
Reductional division: corresponds to the first meiotic division, or meiosis I, in which the number of chromosomes is halved. Of note, during the whole of this process the chromosomes possess two sister chromatids; gametes, which are haploid and of which the chromosomes are formed by a single chromatid, emerge from the second, equational, meiotic division.
Recombination: gene shuffling between two chromosome homologs by crossing-over or by gene conversion; takes place during meiosis I.
Symmetric chromosome allotment: to reproduce as identical or similar organisms, which is a paradigm in biology, the genetic material is evenly distributed to the two sister nuclei during mitosis.

variable chromosomal contents among cells. Mosaic aneuploidy appears to be a constitutive feature of this parasite [20]. The resulting genetic diversity is high: considering only the seven chromosomes for which the ploidy was determined by FISH [17], and if the somy of one chromosome is independent of the somy of the others, the number of calculated different genotypes is >2000 (3^7); the frequency of the most prevalent genotype was therefore estimated at 10% whereas the rarest genotype would only be present in 1 of 10^{11} cells [18]. When the whole set of chromosomes is considered, one may expect even greater genotypic diversification. The variability of genetic contents among cells in the same population is one definition of mosaicism. Global approaches allow the whole genome to be observed, but they are less discriminating than individual cell analysis: they give a 'cumulative' view of ploidy and consequently miss the mosaic structure. Normalized or relative chromosomal read-depths obtained by whole-genome sequencing were found to be close to 2.0 for most chromosomes [19,21], but similarly, the cumulative ploidy inferred from FISH data was also close to 2.0 [18]. The whole of the presently available data indicate that all chromosomes are probably subject to some degree of mosaic aneuploidy. The percentage of strictly diploid cells is probably extremely low in the population: considering only seven out of the ten chromosomes analyzed by FISH [17], their frequency can be calculated in *Leishmania major* Friedlin as <1%. This calculation is based on the assumption that all chromosomes segregate independently. The association of several physical chromosomes during segregation remains an open question in *Leishmania*, in particular in the absence of characterization of centromeres and with the number of observed kinetochores being far below the number of chromosomes [22].

The question of ploidy in *Leishmania* has intimately and logically been linked to the question of genetic exchange, itself associated with major questions such as the transmission of virulence or drug-resistance features. With respect to genetic exchange, the generation of hybrid progeny in *Leishmania* has recently been experimentally demonstrated in the invertebrate host [23,24] but, in natural conditions, automixis appears to predominate [13,25,26]. Thus, after 20 years of debate, novel data allow rejuvenation of the hypotheses about this essential part of the life cycle.

Primary mechanisms for mosaic aneuploidy

Candida albicans is a model for the study of parasexual processes. In this organism the fusion of two parental diploid cells leads to a tetraploid stage, which is then followed by a progressive and random reduction of the chromosome number, leading to a large number of different genotypes [27,28] (reviewed in [7]). Therefore, although this was not demonstrated, the resulting strain also probably exhibits 'mosaic aneuploidy'. This progressive chromosome loss in *C. albicans* recalls, to a different extent, the extensive chromosomal variation observed in a recently formed natural allopolyploid plant species, *Tragopogon miscellus* (Asteraceae) [6] as well as the dynamic model of hepatocyte polyploidization, ploidy reversal, and aneuploidy, a phenomenon termed the 'ploidy conveyor' [29].

In *Leishmania*, although this does not rule out the cell fusion model (discussed below), we have proposed that mosaic aneuploidy primarily results from asymmetric chromosome allotments during mitosis (Box 1) [17]. Using FISH analysis, and because the nuclear envelope persists during mitosis, the homolog copy number was determined for three different chromosomes both in interphase and in dividing cells. A remarkably high proportion of asymmetric