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Parasexuality and mosaic aneuploidy in *Leishmania*: alternative genetics

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Abstract

In most biological models, to reproduce as identical or similar organisms, mitosis is based on the extreme accuracy of the mechanisms involved in the even transmission of the genetic material in the two daughter cells. Character recombination and genotype diversification are insured 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 a 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 being essentially 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*, or 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 most algae. Polyploidy is different, as 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 many species analysed 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 approximately 4% of stillbirths and 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 a few 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 last 20 years, the parasite being considered 'mainly diploid' but with some authors suspecting aneuploidy [13]. The identification of two homologues 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, a trisomy was then demonstrated for certain chromosomes [15, 16]. Recently, Fluorescence *In Situ* Hybridization (FISH) analysis (Box 1), supported by High Throughput Sequencing data, demonstrated that all studied *in vitro* grown *Leishmania* strains and species exhibit a 'mosaic aneuploidy' [17-19]. Thus, within the cell population of a *Leishmania* strain, each of the ten studied chromosomes, in every cell, may be present in two or more ploidy states, that is, monosomic, disomic, or trisomic, yielding highly variable chromosomal contents among cells. Mosaic aneuploidy appears as a constitutive feature of this parasite [20]. The resulting genetic diversity is high: considering only seven of the chromosomes for which the ploidy was determined by FISH [17], and if the somy of a chromosome is independent of the somy of the other ones, the number of calculated different genotypes is >2000 (3^7); the frequency of the most prevalent genotype was therefore estimated at 10% and the rarest one would only be present in 1 out of 10^{11} cells [18]. When the whole set of chromosomes is considered, one may expect an even larger genotypic diversification. The variability of genetic contents among cells in the same population is one definition of mosaicism. Global approaches allow observing the whole genome, but they are less discriminant 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 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 of the chromosomes analysed 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 a number of observed kinetochores 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 predominant [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 high 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 (see below), we have proposed that mosaic aneuploidy primarily results from asymmetric chromosomal allotments during mitosis (Box 1) [17]. Using FISH analysis, and as the nuclear envelope persists during mitosis, the homologue copy number was determined for three different chromosomes both in interphasic and in dividing cells. A remarkably high proportion of asymmetric

chromosomal allotments was found in mitotic cells for these three chromosomes (Figure 1) [17]. In these 'unequal' divisions, the total number of homologues in both dividing nuclei was always an odd number, that is, '1+2' or '2+3' (see Figure in Box 1). Typically, aneuploidy arises as a result of errors in chromosome partitioning, *i.e.* segregation, during mitosis [30, 31]; yet, segregation defects are responsible for asymmetric figures with an even number of chromosome homologues among both dividing nuclei, as elegantly shown, for example, in *Trypanosoma brucei* after the knock-down of the protein SMC3 [32]. Therefore, the most parsimonious hypothesis for mosaic aneuploidy in *Leishmania* is a defect in the regulation of chromosomal replication [17]. In addition, the proportions of the different somies observed in interphasic and in mitotic cells suggest that under-replication and over-replication events frequently occur (Figure 1A-B). These data allow proposing a model in which the persistence of mosaic aneuploidy is assured by stable rates of asymmetric chromosomal allotments, yielding chromosome gains and losses at every mitotic generation [17].

Homozygosity at the cell level and intrastrain heterogeneity.

Two major and at first glance paradoxical genetic consequences can be drawn from this constitutive defect in the regulation of chromosomal replication in *Leishmania*: these are the gradual disappearance of heterozygous cells from the population and yet the persistence of genotypic diversity within the strain population [17, 18] (Figure 2). Indeed, chromosome loss in a disomic heterozygous cell leads to monosomy. The descending progeny, whatever the somy, and even in the case of an eventual chromosome copy gain, will remain homozygous. Yet, it should be stressed that in the absence of selection pressure, the different alleles are not eliminated and persist in the strain, which thus preserves all of its adaptive potential (Figure 2). In *Leishmania*, mosaic aneuploidy, in association with automixis, leads to highly original adaptive potentialities. (i) On the one hand, deleterious mutations occurring on monosomic chromosomes will be rapidly eliminated; in yeast, deleterious mutations are logically more efficiently eradicated from haploid than from diploid cell populations [33, 34]. On the other hand, beneficial mutations will be retained and, due to the natural fluctuation of the somy, may be amplified within the population through the passage from disomy to trisomy [18]. (ii) Mutations which are neutral in certain conditions may turn beneficial in another environment. This, together with the presence in the cell population of homozygotes with different somies, provides a strong adaptive potential to the population [18]. (iii) Finally, the persistence of an intra-strain genetic heterogeneity raises the hypothesis of a complementation between cells

which might be beneficial for the whole population. On the whole, one can imagine that the proportions of mono- di- or trisomic cells might vary according to the environment. Thus, the presence of aneuploidy and mosaicism “combines the features and advantages of both ploidy systems at the population level” [18].

Genetic exchange between aneuploid cells: a challenge?

The presence of genetic exchange in *Leishmania* has for years been the subject of a controversy, which was fed by data from population genetic studies (reviewed in [25]) and by the report of genetic hybrids between divergent *Leishmania* species [35-37]. It was recently given experimental support by the recovery of hybrid parasites from sand flies co-infected with two recombinant strains of *Leishmania major* [23, 24]. However, the precise mechanisms of genetic exchange remain ill-known; although a classical sexuality with meiosis, implying the presence of gametes, was recently proposed [23, 24], a parasexual process was also envisaged by the same authors and remains a hypothesis consistent with their results. The genes associated with prometaphase I of the first meiotic division in higher eukaryotes can be found in the *Leishmania* [24] as well as *T. brucei* genomes [38]. The corresponding encoded proteins should classically be implicated in intra-chromosomal recombination events, although this function is not yet supported by experimental evidence. However, the presence of these genes does not automatically imply a typical meiotic process, that is, with the sequence of equational then reductional divisions. Indeed, all the identified genes are involved in the prometaphase I of meiosis, that is the equational division. Therefore, their presence cannot be considered as evidence for the presence of a reductional division step which would lead to gametes. This has been shown in *C. albicans* [39, 40] and the same question addressed in *Giardia*, another protozoan [41]. In *T. brucei*, which is considered as diploid for the megabase chromosomes, cells with an important reduction of their DNA contents have been observed in the salivary glands of the vector. In addition, these cells can fuse, which supports the presence of gametes in this trypanosomatid [42]. In contrast, the 'mosaic aneuploid' organization of the *Leishmania* genome does not really support the formation of gametes [18, 43]: the question here being how to conciliate such a high proportion of unequal mitotic divisions and the process of gametogenesis, which necessitates the precise reduction of ploidy to a haploid state. Moreover, the old observation of cell fusions of promastigotes by video microscopy, reminiscent of cell conjugation in yeast, supports the occurrence of a parasexual process [44] (see <http://www.parasitologie.univ->

montpl.fr/english_vers/en_doc_scienc.htm). In the model we propose (Figure 3), the fusion of 'parental' aneuploid cells, followed by karyogamy, would then be followed by a reductional mitotic division that would lead to two aneuploid daughter cells with different genomic/genetic contents, a process we would term 'chromosome shuffling'. Importantly, here, just as in parasexual processes, intrachromosomal recombination events – gene shuffling – may still occur during the cell fusion process, and not necessarily during a meiotic process, which would explain the presence of 'meiotic' genes in these organisms. Alternatives to meiosis have also been described in other organisms [27]. And an original non-meiotic evolution has been described recently in the bdelloid rotifer *Adineta vaga*, where oocytes are formed through mitotic division, without either chromosome pairing or chromosome number reduction [45]. Overall, particularly considering aneuploidy, parasexuality appears as a much more suitable and more probable reproduction mode for *Leishmania* than a classical sexual cycle involving the formation of gametes. It is noteworthy that a similar hypothesis has been strongly advocated in *Giardia* [41].

In *Leishmania*, automixis can paradoxically generate heterozygous cells.

A major hindrance imposed by the life cycle of the parasite to genetic exchange is the co-infection of the insect vector by two distinct parasite 'strains'. Because the vertebrate host appears to be predominantly infected by a single 'strain' and coinfection by two 'strains' is rare [46], this would imply that the vector successively feeds on two hosts infected with different 'strains', an infrequent event in general, and even more unlikely in areas of low endemicity. Therefore, automixis appears here as the most frequent genetic exchange event [13, 25, 26]. It should be stressed that in organisms that alternate between a diploid and a haploid stage, such as mammals, automixis inevitably leads to homozygosity over a few generations. Strikingly, in the context of the mosaic aneuploidy present in *Leishmania*, including the intra-strain genetic heterogeneity seen above, the consequences of automixis are just the opposite (Figure 4). In this context, automixis allows genetic shuffling between genetically heterogeneous cells, thus generating heterozygotes from a population made of homozygous cells [17, 18].

Concluding remarks

If mosaic aneuploidy likely constitutes an important asset for evolution, its consequences upon the biology of the parasite remain unknown. In particular, how and why *Leishmania* can tolerate aneuploidy remains elusive. One of the main consequences of aneuploidy is the

modification of the gene dosage. It is probable that the weak regulation of replication [47] and the predominantly post-transcriptional and translational regulation of gene expression [11] are factors that enable *Leishmania* to tolerate this generally deleterious process. Yet, it is difficult to figure out how cells which are monosomic for certain chromosomes can benefit from this haplo-insufficiency. It is striking to note that chromosome 2, which bears the mini-exon gene array, is predominantly monosomic in many strains. The transcriptional level of this sequence which plays a crucial role in mRNA processing might influence the global transcription level of the cell. More generally, a fundamental and yet unanswered question is whether the combination of somies may give the parasite a selective advantage. This problem is particularly difficult to solve because it would be necessary to establish the somy of every chromosome in every cell, which, today, is technically out of reach. Much more work remains to be done in that matter, not least the degree of extent of this phenomenon in natural populations of the parasite (Box 2).

Overall, the data obtained using a divergent eukaryote as a model shed new light on the participation of aneuploidy in a novel parasexual reproduction mode. More generally, these data are consistent with an increasing set of data, which lead us to revisit the concept of aneuploidy, such as the implication of aneuploidy in the differentiation of hepatic or neuronal cells in metazoans [29, 48] or in species evolution in plants [6].

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References

- 1 Torres, E.M., *et al.* (2008) Aneuploidy: cells losing their balance. *Genetics* 179, 737-746
- 2 Tang, Y.C. and Amon, A. (2013) Gene copy-number alterations: a cost-benefit analysis. *Cell* 152, 394-405
- 3 Hassold, T. and Hunt, P. (2001) To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2, 280-291
- 4 Stankiewicz, P. and Lupski, J.R. (2010) Structural variation in the human genome and its role in disease. *Annu Rev Med* 61, 437-455
- 5 Lv, L., *et al.* (2012) Tetraploid cells from cytokinesis failure induce aneuploidy and spontaneous transformation of mouse ovarian surface epithelial cells. *Cell Cycle* 11, 2864-2875

- 6 Chester, M., *et al.* (2012) Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). *Proc Natl Acad Sci U S A* 109, 1176-1181
- 7 Selmecki, A., *et al.* (2010) Genomic plasticity of the human fungal pathogen *Candida albicans*. *Eukaryot Cell* 9, 991-1008
- 8 Bastien, P. (2011) Leishmaniasis control: what part for development and what part for research? *Clin Microbiol Infect* 17, 1449-1450
- 9 Banuls, A.L., *et al.* (2011) Clinical pleiomorphism in human leishmaniasis, with special mention of asymptomatic infection. *Clin Microbiol Infect* 17, 1451-1461
- 10 Ivens, A.C., *et al.* (2005) The genome of the kinetoplastid parasite, *Leishmania major*. *Science* 309, 436-442
- 11 Clayton, C. and Shapira, M. (2007) Post-transcriptional regulation of gene expression in trypanosomes and leishmaniasis. *Mol Biochem Parasitol* 156, 93-101
- 12 Goring, H.U. (2012) 'Gestalt,' composition and function of the *Trypanosoma brucei* editosome. *Annu Rev Microbiol* 66, 65-82
- 13 Bastien, P., *et al.* (1992) *Leishmania*: sex, lies and karyotype. *Parasitol Today* 8, 174-177
- 14 Iovannisci, D.M. and Beverley, S.M. (1989) Structural alterations of chromosome 2 in *Leishmania major* as evidence for diploidy, including spontaneous amplification of the mini-exon array. *Mol Biochem Parasitol* 34, 177-188
- 15 Ravel, C., *et al.* (1998) The complete chromosomal organization of the reference strain of the *Leishmania* Genome Project, *L. major* 'Friedlin'. *Parasitol Today* 14, 301-303
- 16 Sunkin, S.M., *et al.* (2000) The size difference between *Leishmania major* 'friedlin' chromosome one homologues is localized to sub-telomeric repeats at one chromosomal end. *Mol Biochem Parasitol* 109, 1-15
- 17 Sterkers, Y., *et al.* (2011) FISH analysis reveals aneuploidy and continual generation of chromosomal mosaicism in *Leishmania major*. *Cell Microbiol* 13, 274-283
- 18 Sterkers, Y., *et al.* (2012) Novel insights into genome plasticity in Eukaryotes: mosaic aneuploidy in *Leishmania*. *Mol Microbiol* 86, 15-23
- 19 Rogers, M.B., *et al.* (2011) Chromosome and gene copy number variation allow major structural change between species and strains of *Leishmania*. *Genome Res* 21, 2129-2142
- 20 Lachaud, L., *et al.* (2014) Constitutive mosaic aneuploidy is a unique genetic feature widespread in the *Leishmania* genus. *Microbes Infect* 16, 61-66
- 21 Downing, T., *et al.* (2011) Whole genome sequencing of multiple *Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance. *Genome Res* 21, 2143-2156
- 22 Urena, F. (1985) Ultrastructure of the mitotic nucleus in *Leishmania mexicana* sp. tridimensional reconstruction of the mitotic spindle and dense plaques. *Microsc Electron Biol Celular* 9, 51-65
- 23 Akopyants, N.S., *et al.* (2009) Demonstration of genetic exchange during cyclical development of *Leishmania* in the sand fly vector. *Science* 324, 265-268
- 24 Inbar, E., *et al.* (2013) The Mating Competence of Geographically Diverse *Leishmania major* Strains in Their Natural and Unnatural Sand Fly Vectors. *PLoS Genet* 9, e1003672
- 25 Rougeron, V., *et al.* (2010) "Everything you always wanted to know about sex (but were afraid to ask)" in *Leishmania* after two decades of laboratory and field analyses. *PLoS Pathog* 6, e1001004
- 26 Blaineau, C., *et al.* (1992) Multiple forms of chromosome I, II and V in a restricted population of *Leishmania infantum* contrasting with monomorphism in individual strains suggest haploidy or automixy. *Mol Biochem Parasitol* 50, 197-204

- 27 Forche, A., *et al.* (2008) The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. *PLoS Biol* 6, e110
- 28 Bennett, R.J. and Johnson, A.D. (2003) Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. *Embo J* 22, 2505-2515
- 29 Duncan, A.W., *et al.* (2010) The ploidy conveyor of mature hepatocytes as a source of genetic variation. *Nature* 467, 707-710
- 30 Jones, K.T. and Lane, S.I. (2013) Molecular causes of aneuploidy in mammalian eggs. *Development* 140, 3719-3730
- 31 Holland, A.J. and Cleveland, D.W. (2012) Losing balance: the origin and impact of aneuploidy in cancer. *EMBO Rep* 13, 501-514
- 32 Bessat, M. and Ersfeld, K. (2009) Functional characterization of cohesin SMC3 and segregation of large and minichromosomes in *Trypanosoma brucei*. *Mol Microbiol.* 71, 1371-1385
- 33 Otto, S.P. and Gerstein, A.C. (2008) The evolution of haploidy and diploidy. *Curr Biol* 18, R1121-1124
- 34 Zeyl, C., *et al.* (2003) An evolutionary advantage of haploidy in large yeast populations. *Science* 299, 555-558
- 35 Ravel, C., *et al.* (2006) First report of genetic hybrids between two very divergent *Leishmania* species: *Leishmania infantum* and *Leishmania major*. *Int J Parasitol* 36, 1383-1388
- 36 El Baidouri, F., *et al.* (2013) Genetic structure and evolution of the *Leishmania* genus in Africa and Eurasia: what does MLSA tell us. *PLoS Negl Trop Dis* 7, e2255
- 37 Lukes, J., *et al.* (2007) Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. *Proc Natl Acad Sci U S A* 104, 9375-9380
- 38 Peacock, L., *et al.* (2011) Identification of the meiotic life cycle stage of *Trypanosoma brucei* in the tsetse fly. *Proc Natl Acad Sci U S A* 108, 3671-3676
- 39 Heitman, J. (2010) Evolution of eukaryotic microbial pathogens via covert sexual reproduction. *Cell Host Microbe* 8, 86-99
- 40 Hickman, M.A., *et al.* (2013) The 'obligate diploid' *Candida albicans* forms mating-competent haploids. *Nature* 494, 55-59
- 41 Birky, C.W., Jr. (2010) *Giardia* sex? Yes, but how and how much? *Trends Parasitol* 26, 70-74
- 42 Peacock, L., *et al.* (2014) Meiosis and haploid gametes in the pathogen *Trypanosoma brucei*. *Curr Biol* 24, 181-186
- 43 Mannaert, A., *et al.* (2012) Adaptive mechanisms in pathogens: universal aneuploidy in *Leishmania*. *Trends Parasitol* 28, 370-376
- 44 Lanotte, G. and Rioux, J.A. (1990) [Cell fusion in *Leishmania* (*Kinetoplastida*, *Trypanosomatidae*)]. *C R Acad Sci III* 310, 285-288
- 45 Flot, J.F., *et al.* (2013) Genomic evidence for ameiotic evolution in the bdelloid rotifer *Adineta vaga*. *Nature* 500, 453-457
- 46 Pratlong, F., *et al.* (1989) [Simultaneous presence in dogs of 2 zymodemes of the *Leishmania infantum* complex]. *Ann Parasitol Hum Comp* 64, 312-314
- 47 Clayton, C.E. (2002) Life without transcriptional control? From fly to man and back again. *EMBO J* 21, 1881-1888
- 48 McConnell, M.J., *et al.* (2013) Mosaic copy number variation in human neurons. *Science* 342, 632-637
- 49 Duncan, A.W. (2013) Aneuploidy, polyploidy and ploidy reversal in the liver. *Semin Cell Dev Biol* 24, 347-356

Glossary

Aneuploidy:

Corresponds to a chromosome number which is not an exact multiple of the haploid number. Aneuploidy is considered as "whole chromosomal" aneuploidy in this Opinion, as opposed to copy number changes affecting only parts of chromosomes, described as "partial", "segmental" or "structural" aneuploidy.

Asymmetrical division :

Unequal chromosome allotment during mitosis (see also symmetrical chromosomal allotment).

Automixis:

Several 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 in which the number of chromosomes is preserved: during meiosis, it corresponds to the second division that follows the reductional division (see also this term).

Fluorescence in situ hybridization (FISH):

A cytogenetic technique that is most often used to microscopically detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH allows examination of nuclear chromosomal contents cell by cell.

Gene dosage:

Corresponds to the total copy number of a gene in a genome.

Gene shuffling:

During meiosis, chromosome homologues 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 this term) and 'chromosome shuffling' (see text).

Genotype: the genetic constitution – the whole of the alleles in a genome – of a cell, an individual, or an organism. By extension, it is used as the allelic constitution of a defined number of genes.

Homozygote:

Refers to diploid cells in which identical alleles of a gene are present on both homologous chromosomes.

Heterozygote:

Refers to diploid cells in which the alleles of a gene present on both homologous chromosomes differ.

Karyogamy:

Fusion of two nuclei from different cells; typically occurs following the fusion of gametic cells.

Loss of heterozygosity (LOH):

Homozygosity of alleles affecting more or less large portions of a chromosome, owing to either partial deletion or whole chromosome loss and subsequent duplication of the remaining homologue.

Mosaic aneuploidy:

varying numbers of chromosome homologues among cells within the same population/strain.

Parasexuality:

Non-conventional (non-sexual) mechanism for transferring genetic material and allowing gene recombination without meiosis and fecundation. 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 molecular phenotype as determined by PCR or any molecular method.

Ploidy conveyor:

Dynamic model of chromosome copy number variation, which englobe polyploidy, aneuploidy and ploidy reversal (see also this term), described by Duncan et al. in hepatocytes [29] and reviewed in [49]. This mechanism was evolved by hepatic cells to promote genetic diversity and adaptation in response to liver injury.

Ploidy reversal :

Term forged by Duncan et al. [29], which refers to the restoration of diploidy, when polyploid hepatic cells produce diploid daughter cells through a 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; allopolyploidy, to the fusion of genomes of different species. In polyploid cells, gene dosage (see this term) 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; and gametes, which are haploid and of which chromosomes are formed by a single chromatid, emerge from the second, equational, meiotic division.

Recombination:

Gene shuffling between two chromosome homologues by crossing-over or by gene conversion; occurs during meiosis I

Symmetrical chromosome allotments

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.

Box 1. Fluorescence in situ hybridization and the ploidy of *Leishmania*.

Fluorescence in situ hybridization (FISH) is one cytogenetic technique able to provide data about the chromosomal contents of individual cells, hence to give a direct view of the cell ploidy/somy. FISH allowed not only the direct observation of aneuploid *Leishmania* cells, but more importantly, that of the mosaic structure of the strain population. The principle of the technique is simple, although it is often awkward to perform: it comprises four steps (Fig. A). The most critical point are the DNA probes which have to be sensitive, yet specific for a given chromosome, the parasite preparation and mastering the in situ hybridization. FISH must include 'Z-stack image acquisitions', that is pictures taken in a number of different planes (Fig. B) to allow a 3D-representation of the nucleus contents, here chromosome homologues (Fig. C; see also Movies in [17]). One of the greatest challenges in this application, apart from a low fluorescence background, is that FISH must label >99% of the nuclei, otherwise the chromosome counting would be worthless. Another limitation is the arduousness of the chromosome counting, in 200-400 cells per chromosome and strain.

In addition to observing interphasic cells, FISH allowed analysing dividing cells. This gave an insight into the mechanisms underlying mosaic aneuploidy (Fig. D, and [17]).

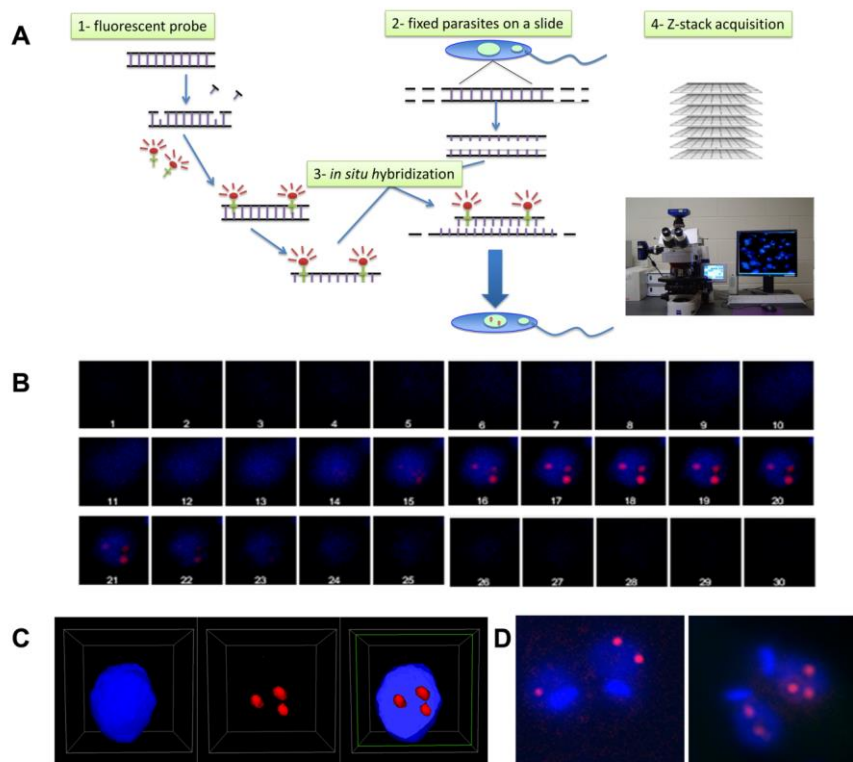


Figure I. Fluorescence in situ hybridization

(A) The four steps of FISH. (B) Gallery of Z-stack acquisitions. (C) Three-dimensional reconstruction of a trisomic nucleus. Three chromosome 5 homologues (red) are specifically labelled, the nucleus is labelled by DAPI (in blue). (D) Asymmetrical chromosome allotment during mitosis, with an odd total number of chromosomes in the daughter cells, either '1+2' (left) or '2+3' (right).

Box 2. Outstanding questions : where next to go ?

To establish the extent of mosaic aneuploidy (i) in amastigotes, and (ii) in *Leishmania* populations in *natura*.

To establish the evolution in time of mosaic aneuploidy, preferably *in vivo*.

To evaluate the different existing combinations of aneuploidy of different chromosomes in a cell population, using multicolour FISH.

To determine the incidence of the parasite differentiation on the pattern of ploidy/somy of chromosomes.

To decipher the mechanisms of activation and maturation of replication origins in *Leishmania*.

To measure the transcription and the translation levels in single cells, and to correlate these with gene dosage.

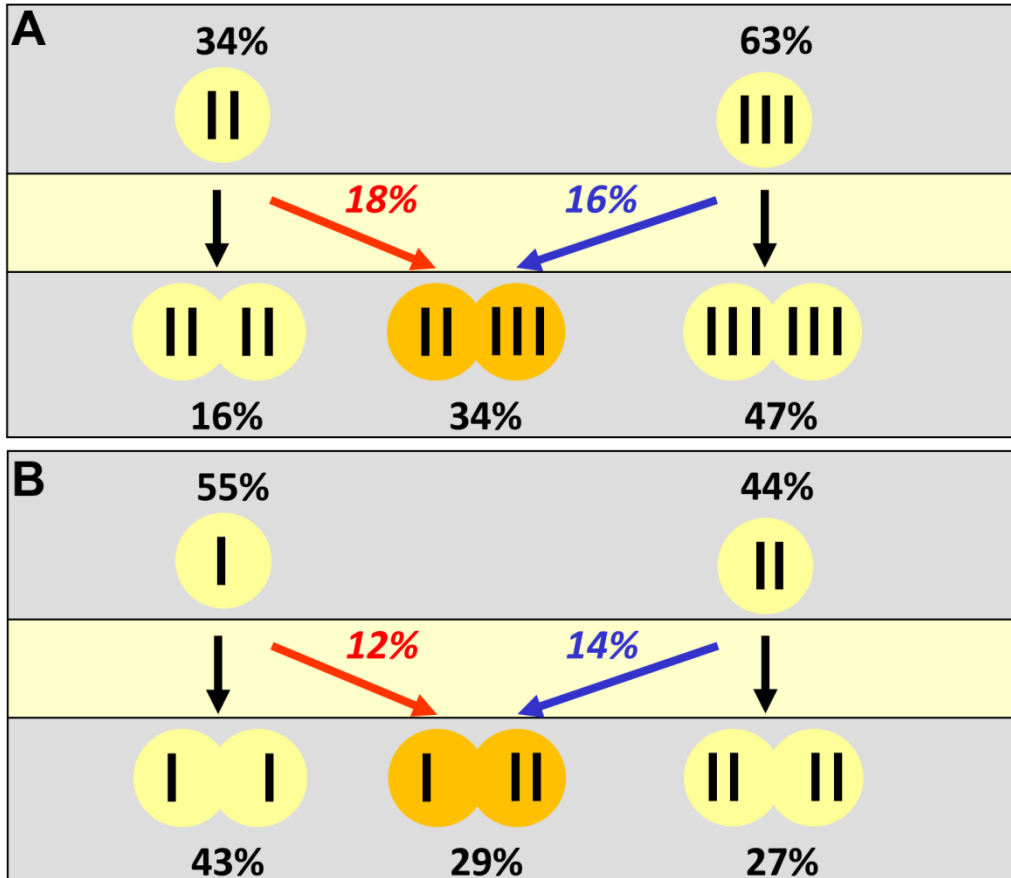


Figure 1. Mechanistic origin of mosaic aneuploidy.

(A) During mitosis, a high rate of asymmetric ‘2+3’ divisions generates mosaic aneuploidy of chromosome 5 [17]. In the *Leishmania major* ‘Friedlin’ strain, counting chromosome 5 homologues in interphasic cells using FISH revealed a high proportion of trisomic cells (63%) and only 34% of disomic cells (top line) [17]. When examining the dividing cells, the proportions of ‘regular’ symmetrical chromosome allotments were much lower than expected (bottom line): these constituted only 16% of the total of divisions for classical disomic ‘2+2’ patterns and 47% for trisomic ‘3+3’ patterns; conversely, the proportions of asymmetric figures of division (‘2+3’)(orange) were remarkably high: 34%. Therefore, about half (18%/34%) of the disomic cells and a quarter (16%/63%) of the trisomic cells underwent asymmetric chromosome allotment events.

(B) A high rate of asymmetric ‘1+2’ divisions generates mosaic aneuploidy of chromosome 2. In the same *Leishmania* strain, chromosome 2 was found predominantly (55%) monosomic [17]; low proportions of symmetrical chromosome allotments were found (43% for the ‘1+1’ division pattern and 27% for the ‘2+2’ division pattern), again accompanied with high proportions of asymmetric figures of division (‘1+2’) in 29% of the cases.

An unconventional regulation of chromosomal replication probably explains these unequal division patterns, leading to chromosome loss or gain at every mitotic generation: for example, the over-replication of chromosome 5 in about half the disomic cells and its under-replication in a quarter of the trisomic cells would lead to the 34% of asymmetric ‘2+3’ division patterns (A).

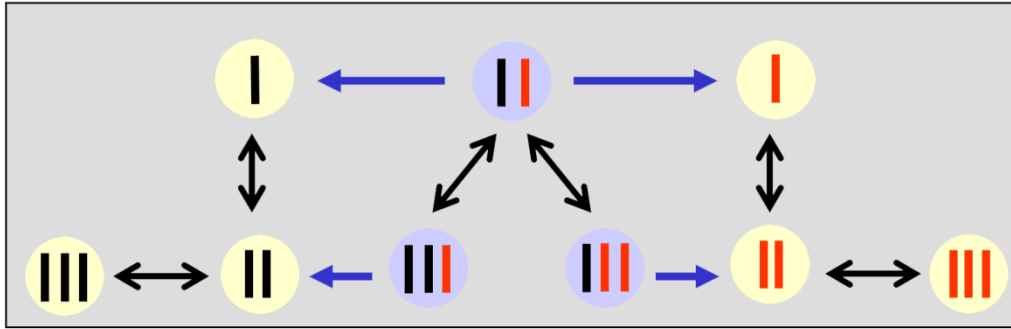


Figure 2. Mosaic aneuploidy leads to the loss of heterozygous cells, yet maintaining intra-strain genetic heterogeneity.

Different allelic homologues of the same chromosome are represented in either black or red. Monoallelic or homozygous cells are coloured yellow and heterozygous cells blue. Only asymmetric divisions are represented here. Single arrows indicate non-reversible karyotype evolutions during mitosis; double arrows reversible ones. In the course of successive mitoses, underreplication events lead to the loss of heterozygous cells in the population. Heterozygous disomic/trisomic cells will generate a proportion of homozygous monosomic/disomic cells. Subsequently, the descending lineage will be homozygous, whichever the evolution of ploidy thereafter, including regaining a chromosome copy. As this occurs in every generation, heterozygotes gradually disappear from the population. Yet, in the absence of selection, the intra-strain genetic heterogeneity, or mosaicism, of the strain is maintained; for example, in this figure, the two alleles present at the start in heterozygotes persist in the resulting subpopulations.

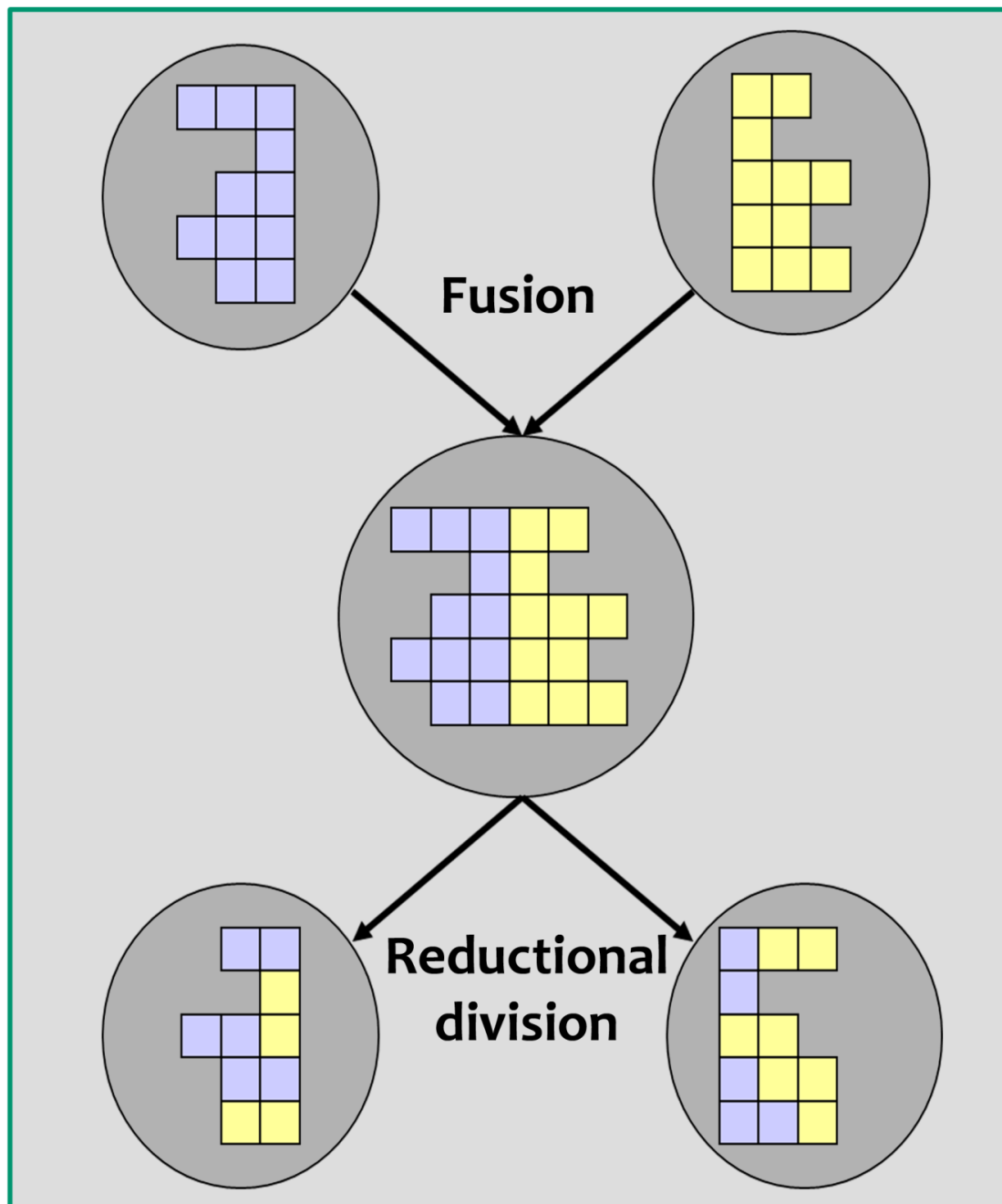


Figure 3. A parasexual model for *Leishmania*, involving 'chromosome shuffling'.

In this model, horizontal bars represent the genomic contents of two different cells (grey circles) as five heterologous chromosomes, which are homozygous at every locus, a consequence of mosaic aneuploidy [18]. These chromosomes, from top to bottom in the cell on the left, are trisomic, monosomic, disomic, trisomic, and disomic, and, in the cell on the right, disomic, monosomic, trisomic, disomic, and trisomic. The fusion of these two 'parental' aneuploid cells gives rise to an aneuploid cell with high DNA content, which will itself again divide. During the reductional mitotic division that follows, chromosome homologues are redistributed in daughter cells, resulting in 'chromosome shuffling'. Intra-chromosomal recombination, gene shuffling, may also occur at the cell fusion stage (not represented here).

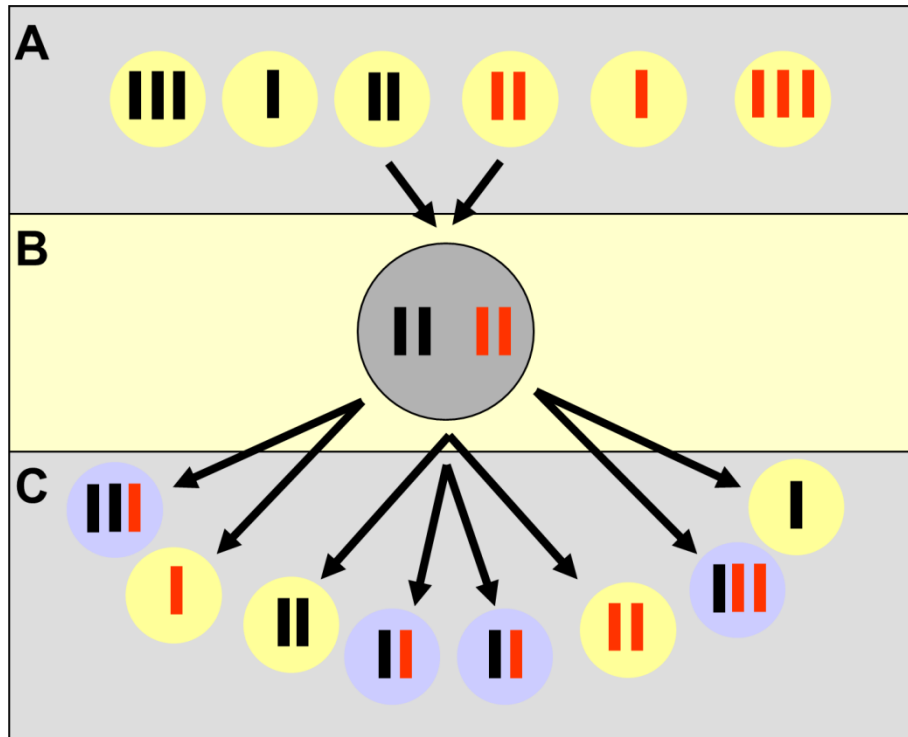


Figure 4. In *Leishmania*, automixis generate heterozygotes rather than homozygosity.

(A) *Leishmania* strains exhibit a peculiar genomic organization termed mosaic aneuploidy. Mosaic aneuploidy inevitably leads to a strain population constituted by homozygous cells (yellow) with different somies (among mono-, di- and trisomy), yet retaining genetic heterogeneity in the strain [18]. Different allelic homologues of the same chromosome are represented in either black or red (Figure 2). (B) The fusion of two homozygous disomic cells leads to a tetrasomic heterozygous stage. (C) Following a reductional division with random and asymmetric chromosome redistribution among the daughter cells, heterozygous cells (blue) may arise. Therefore, automixis allows generating heterozygotes from a genetically heterogeneous population of homozygous cells. In contrast, in diploid organisms, automixis leads to homozygosity over a few generations.