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Novel insights into genome plasticity in Eukaryotes: mosaic aneuploidy in *Leishmania*

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Summary

Leishmania are unicellular eukaryotes that have many markedly original molecular features compared to other uni- or multicellular eukaryotes like yeasts or mammals. Genome plasticity in this parasite has been the subject of many publications, and has been associated with drug resistance or adaptability. Aneuploidy has been suspected by several authors and it is now confirmed using state-of-the-art technologies such as high throughput DNA sequencing. The analysis of genome contents at the single cell level using Fluorescence In Situ Hybridization (FISH) has brought a new light on the genome organization: within a cell population, every chromosome, in every cell, may be present in at least two ploidy states (being either monosomic, disomic or trisomic), and the chromosomal content varies greatly from cell to cell, thus generating a constitutive intra-strain genomic heterogeneity, here termed 'mosaic aneuploidy'. Mosaic aneuploidy deeply affects the genetics of these organisms, leading for example to an extreme degree of intra-strain genomic diversity, as well as to a clearance of heterozygous cells in the population without however affecting genetic heterogeneity. Second, mosaic aneuploidy might be considered as a powerful strategy evolved by the parasite for adapting to modifications of environment conditions as well as for the emergence of drug resistance. On the whole, mosaic aneuploidy may be considered as a novel mechanism for generating phenotypic diversity driven by genomic plasticity.

Introduction

Leishmaniasis is one of the world's most neglected diseases, causing a broad spectrum of diseases (ranging from asymptomatic to lethal) in 98 countries on four continents. An estimated 350 million people are considered at risk of contracting leishmaniasis, with approximately 59 000 deaths due to visceral leishmaniasis per year. Mortality and morbidity from leishmaniasis worldwide show a worrying increasing trend; and there is a lack of potent, cost-effective and safe therapy (den Boer *et al.*, 2011, Croft *et al.*, 2011).

Leishmaniasis are due to protozoa of the genus *Leishmania*, digenetic parasites that are transmitted to mammals by the bite of an infected insect vector, a Phlebotomine sand fly. During its lifecycle, the parasite alternates between (i) motile promastigotes that live extracellularly in the digestive tract of the vector and develop into non-

dividing infective ('metacyclic') forms in the mouthparts of the sand fly and (ii) non-motile obligate intracellular amastigotes that live within macrophages after they were delivered to the mammalian host by the vector. Due to the mode of transmission (blood meal), the parasite needs to adapt rapidly to drastically different environments.

The haploid genomes of these parasites are organized in 36 chromosomes for the Old World species (*L. donovani*, *L. infantum*, *L. major*, *L. tropica*, *L. aethiopica*) (Wincker *et al.*, 1996) and 34 and 35 for the New World *L. mexicana* and *L. braziliensis*, respectively (Britto *et al.*, 1998). A striking feature of the *Leishmania* genome is the high degree of synteny among different species, including species that have been separated for some 20-100 millions years such as *L. tarentolae* (Raymond *et al.*, 2012) and extending to

other trypanosomatids such as *Trypanosoma brucei* (El-Sayed *et al.*, 2005). The comparison of the genomes of the different *Leishmania* species revealed a remarkably low number of species-specific genes (Raymond *et al.*, 2012, Rogers *et al.*, 2011). But also, a number of genes were found multicopy specifically in any given genome (Raymond *et al.*, 2012, Rogers *et al.*, 2011).

The question of the ploidy of *Leishmania* has been debated over the last 20 years, oscillating between diploidy and aneuploidy. The size monomorphism of homologous chromosomes observed within the same strain, contrasting with the multiple size forms of these homologues observed within a restricted population, was suggestive of either haploidy or diploidy with automixis (Blaineau *et al.*, 1992, Bastien *et al.*, 1992). The first clue suggesting diploidy was a disomic pattern of chromosome 2 with two homologues differing in size as seen by pulsed field electrophoresis (PFGE), a spontaneous amplification of a chromosomal region, the mini-exon gene array on chromosome 2, being responsible for this pattern (Iovannisci *et al.*, 1989). However, a trisomy has been demonstrated for certain chromosomes (Sunkin *et al.*, 2000, Ravel *et al.*, 1998, Scholler *et al.*, 1986). In certain cases, attempts to generate double-knockout null mutants have revealed the existence a third copy of the targeted chromosome. This was usually interpreted by the authors as deriving from an initial disomy and the subsequent selection of a supernumerary chromosome maintaining a copy of the targeted gene, which was then qualified essential (Martinez-Calvillo *et al.*, 2005, Cruz *et al.*, 1993, Dubessay *et al.*, 2002). Later, Marc Ouellette's group reported that a single round of allelic inactivation targeting *LinJ32_V3.2190*, a gene coding for an unclassified ABC protein, lead to a null mutant, demonstrating the monosomy of chromosome 32 in their *L. infantum* strain (Leprohon *et al.*, 2009). Even more recently,

whole genome analysis by high throughput sequencing of species and strains of *Leishmania* revealed a large extent of chromosome and gene copy number variation, thereby showing a large, although unspecified, degree of aneuploidy (Rogers *et al.*, 2011). This milestone paper, describing numerous gene amplifications, some of which are species-specific, actually came as corroborating previous findings which clearly demonstrated aneuploidy in *Leishmania* strains and explained both how it occurs and how ploidy can fluctuate over mitosis (Sterkers *et al.*, 2011). In parallel, data from Downing *et al.* based on 17 strains of *L. donovani* have demonstrated the instability of ploidy among strains (Downing *et al.*, 2011). Further, the chromosomal contents varying from cell to cell lead to the conclusion that *Leishmania* strains display a mosaic structure (Sterkers *et al.*, 2011). The term used for describing this highly peculiar genetic feature is 'mosaic aneuploidy' (Iourov *et al.*, 2008).

In this review, we will discuss how mosaic aneuploidy in *Leishmania* may occur and how it may have major genetic and biological consequences for the parasite.

From aneuploidy to mosaic aneuploidy

Using high throughput sequencing, Rogers *et al.* have generated a reference genome for *L. mexicana* and refined the reference genomes for *L. major*, *L. infantum* and *L. braziliensis*. These authors demonstrated that there is little variation in ortholog gene contents across *Leishmania* species; they identified a remarkably low number of species-specific genes (2, 14, 19, and 67, in *L. mexicana*, *L. major*, *L. infantum* and *L. braziliensis* respectively), and showed that large-scale genetic heterogeneity can result from gene amplification and chromosome number variation. From these data, the authors concluded that increased gene copy numbers due to chromosome amplification may contribute to alterations in gene expression in response to environmental conditions within the host, providing a genetic basis for disease tropism (Rogers *et al.*, 2011). This suggests that aneuploidy is

unstable within a strain in that sense that it may fluctuate in response to modifications of environmental conditions. In addition, whole genome sequencing of 17 strains of *L. donovani* clearly revealed extensive variation in chromosome copy number among strains of the same species, demonstrating the variability of ploidy (Downing *et al.*, 2011): thus, phylogenetically close strains (BPK173/Ocl3 and BPK275/Ocl18) exhibit a different somy for 8 chromosomes (*i.e.* chromosome 2, 8, 11, 12, 14, 20 and 33). Such a variability implies that aneuploidy is unstable in time. This instability of ploidy may explain the discrepancies observed in the ploidy of *L. major* Friedlin strain between Rogers *et al.* and Sterkers *et al.* (Rogers *et al.*, 2011, Sterkers *et al.*, 2011). Indeed, the first described a strain that is essentially diploid (with the noticeable exception of chromosome 31, that is tetrasomic), while the second (which examined the ploidy of seven chromosomes) found three chromosomes predominantly trisomic, three chromosomes predominantly disomic and one predominantly monosomic (Figure 1). This may be explained by the fact that the Friedlin strain (the *Leishmania* genome sequencing reference strain) is extensively cultivated in conditions that may vary widely among laboratories. Noticeable modifications of ploidy may occur during *in vitro* cultivation, as shown in cloning experiments yielding clones that possess a ploidy pattern different from that of the parental line (Sterkers *et al.*, 2011) and Figure 1F).

If aneuploidy is then unstable, a second question arises: what are the mechanisms that may explain this instability? Global genome analyses of unicellular organisms, including PFGE and genome sequencing, are based on the analysis of millions of cells taken together that are *a priori* considered identical or invariant (so-called "strains" or "stocks" or "isolates"). Intrinsically, they do not give much clues about single cell genomic organization; except for the cumulative ploidy inferred from high throughput sequencing for every

heterologue, which, when not a whole number, is suggestive of a ploidy heterogeneity in the population. Conversely, methods based on the analysis of single cells, like cytogenetics and Fluorescence In Situ Hybridization (FISH), bring another type of information. Indeed, using FISH, Sterkers and co-workers analyzed seven chromosomes in several *L. major* strains, and showed that these were present in at least two ploidy states, being either monosomic, disomic or trisomic depending on the cell and on the chromosome. For example, a single cell may be trisomic for chromosome 1, disomic for chromosome 2, disomic for chromosome 5 etc., while the neighboring cell may be disomic for chromosome 1, monosomic for chromosome 2, trisomic for chromosome 5 etc. (Figure 1 A-C). This amazing feature offers multiple possibilities of combinations that have not yet been explored. This was also confirmed for other *Leishmania* species (Bourgeois *et al.* in preparation). At this point, one can say that *Leishmania* strains are not only aneuploid but, since the chromosomal contents varies among cells, that they display a mosaic structure, hence the term 'mosaic aneuploidy' used for his phenomenon (Sterkers *et al.*, 2011).

Occurrence and continuous generation of mosaic aneuploidy in *Leishmania*

The data obtained by FISH have given a dynamic view of aneuploidy that is much more consistent with the idea that it is involved in response to modifications of the environment or in the regulation of pathogenicity and virulence. More specifically, the study of mitotic/dividing cells by FISH allowed determining both how mosaic aneuploidy originates and how it is maintained. Indeed, chromosome homologs were seen in the nuclei of dividing cells as distributing according to a classical symmetric '2+2' scheme, but also to a less classical symmetric '3+3' as well as to a totally unconventional asymmetric '1+2' and '2+3' patterns. Within one strain, the proportions of the different

patterns of division vary depending on each chromosome; but a large proportion of dividing cells (Sterkers *et al.*, 2011) exhibited asymmetric distributions of chromosome homologs into the two daughter nuclei. Of note, in all observed asymmetric chromosome allotments, the total number of homologs of a given chromosome was always an odd number (e.g. '1+2' '2+3') (Sterkers *et al.*, 2011). Segregation defects would also yield asymmetric allotments but with even numbers of chromosome homologs in the daughter nuclei ('1+3' or '0+4'; for example, see (Bessat *et al.*, 2009). Our data strongly suggest that these asymmetric chromosome allotments (ACAs) must originate from a defect in the regulation of chromosomal replication, yielding either an over- or an under-replication of the chromosome. The consequence of these ACAs, whether one considers them as 'positive' or 'negative', would be the gain or the loss of a chromosome copy, respectively. Several authors already proposed that, in *Leishmania*, the occurrence of supernumerary chromosomes may be explained by limited duplications of chromosomal segments (Singh *et al.*, 2001, Dubessay *et al.*, 2001, Ubeda *et al.*, 2008, Leprohon *et al.*, 2009, Mukherjee *et al.*, 2011), that may also be linked to replication defects. Like most molecular processes in these markedly original parasites, the origins of replication as well as the modalities of the regulation of replication remain largely uncharacterized in *Leishmania*.

In Figure 2, we propose a model where a defect in the regulation of DNA replication may explain a partial or total duplication of a chromosome. Double firing of several replication origins along the chromosome would explain complete chromosome duplication; obviously, this would be followed with an asymmetric segregation.

A model for the kinetics of mosaic aneuploidy

The kinetics of the occurrence of mosaic aneuploidy depends directly on the

frequency of ACAs during mitosis. Considering that the rates of 'positive' and 'negative' ACAs are stable, a simple mathematical formula (see Figure 3) explains how a steady state of mosaic aneuploidy (*i.e.* a stable proportion of mono-, di- and trisomic cells within the population) may be attained. This equilibrium will be reached more or less rapidly depending on the value of the positive and negative ACA rates. For chr. 5 of *L. major* Friedlin strain, considering the two predominant ploidy states, the equilibrium between disomic and trisomic cells in the population is set roughly at 30%/70% and, due to the high ACA rates, can be reached in less than 10 generations (Figure 3). This simply explains how mosaic aneuploidy can be rapidly observed in cell clones derived from a *Leishmania* strain in the absence of drug pressure and selective advantage (Sterkers *et al.*, 2011). It should be stressed that a steady state of mosaic aneuploidy will be reached whatever the values of the ACA rates are. Therefore, the exceptional fact is not so the mosaicism observed in *Leishmania* than its scarcity in the living world. Indeed, what we can expect from these data is that mosaic aneuploidy might be more frequent than rare in the living world (Iourov *et al.*, 2008), the most notable feature there being rather the extreme accuracy of the processes involved in the transmission of the genetic material. The apparent scarcity of this phenomenon might thus be due to the fact that aneuploidy is deleterious and that mechanisms aiming at eliminating aneuploid cells are highly prevalent and/or efficient in higher eukaryotes.

Heritability of aneuploidy

It came as a surprise that the mosaic aneuploidy observed in strains was essentially conserved in derived clones and even subclones (Sterkers *et al.*, 2011). Strikingly indeed, the ploidy pattern in the parental strain and its clones was found either almost identical or substantially different. This raises the question of how a strain-specific pattern of ploidy can be

transmitted through mitotic generations. Since the majority of sibling clones and subclones exhibited a ploidy pattern similar to that of the parental strain, the most prominent feature is an apparent stability of mosaic aneuploidy across mitotic generations. Nevertheless, a low proportion of clones (2/6) exhibited a different ploidy pattern (Figure 1D). This suggests a fluctuating process rather than the presence of a genetic determinism for the transmission of mosaic aneuploidy. Taken together, both the stability and the fluctuation observed among sibling clones favor the hypothesis that an epigenetic mechanism is involved in the transmission of the ploidy pattern. Similarly to the transcription start and stop sites of the polycistronic transcription units in Trypanosomatids (reviewed in (Kramer, 2012)), we assume that autoreplicating sequences (ARS) might be marked epigenetically by histone variants/posttranslationally modified histones. Therefore, one could consider that there is a dialectic equilibrium between reproductive invariance and fluctuation in this model.

An extreme genotypic diversity

Mosaic aneuploidy has two essential genetic consequences: (i) an extreme genotypic/karyotypic diversity and (ii) a conserved intra-strain genetic heterogeneity in spite of the fact that the population almost exclusively comprises homozygous cells.

With respect to genotypic diversity, taking into account the proportions of mono-, di- and trisomic cells for the seven chromosomes studied in the *L. major* Friedlin strain (Sterkers *et al.*, 2011), we calculated that this would yield more than two thousand of different possible genotypes (see Figure 4 and legend for the calculation). The statistically most frequent genotype, *i.e.* trisomy of chromosome 1, 5 and 17, monosomy of chromosome 2 and disomy of chromosome 12, 27 and 36, would be present in 10% of the cells. The rarest genotypes could even be absent from

a population of 10^{11} cells (Figure 4). Of course, considering the whole of the 36 chromosomes constituting the genome, the number of possible genotype would be even much higher and the most frequent genotype would then present in less than 1% of cells.

Strain genetic heterogeneity versus cellular homozygosity

The second major genetic consequence of mosaic aneuploidy is the loss of heterozygosity. Indeed, in the absence of selective pressure, the fluctuations of ploidy ineluctably lead to a reduction in the number of heterozygous cells. At every cell division, a given chromosome homolog stands a chance of being lost or perpetuated. The lineage of a cell that passed through a monosomic stage is necessarily homozygous for the alleles borne by the corresponding chromosome, even if the ploidy of the descendant cells subsequently increases. The tendency toward homozygosity depends on the positive and negative ACA rates, and occurs more or less rapidly depending on their values. With ACA rates at 10%, heterozygous cells would have almost disappeared from the population in less than 100 generations (Figure 5). However, it must be kept in mind that (in the absence of selective advantage), the whole of the alleles, hence the genetic heterogeneity of the strain, can be maintained and may still constitute a reservoir of phenotypic diversity.

As said above, global approaches such as PFGE, strain DNA analysis (*e.g.* RFLP) or high throughput sequencing are not relevant here, as they cannot tell if a genetically heterogeneous cell population is made of heterozygous cells or of homozygous cells comprising different alleles and ploidy contents. Only techniques analyzing single cells, such as cytogenetics, FISH, or single cell PCR, allow this distinction.

Biological significance of mosaicism: Mosaic aneuploidy as an adaptative means to changing environments

Like other authors (Singh *et al.*, 2001, Dubessay *et al.*, 2001, Ubeda *et al.*, 2008, Leprohon *et al.*, 2009, Mukherjee *et al.*, 2011, Rogers *et al.*, 2011), we consider that genotypic variation/ chromosomal dosage plasticity is a potential source of phenotypic diversity. Hence, the parasite might use this mechanism to adapt to the different environments encountered during its life cycle.

With this unique system of genomic plasticity, *Leishmania* appears to cumulate the advantages of both haploidy and diploidy. In haploids, (non-silent) mutations may immediately have phenotypic consequences. Although this can be lethal for an individual cell, this adaptability may confer an advantage to the population: during rapid environmental changes, such populations may more rapidly select the appropriate surviving mutants. It has actually been shown that

In total, more than aneuploidy *per se*, it is the mosaic structure of *Leishmania* strains that appears so exceptional and confers to the parasite a large intra-strain genotypic diversity.

Future studies should investigate how this genome instability is involved in the adaptation of the parasite to the different conditions it has to struggle with and should aim at dissecting the mechanisms and regulation of chromosomal replication in these organisms.

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References

- Bastien, P., Blaineau, C. and Pages, M. (1992). Leishmania: sex, lies and karyotype. *Parasitol Today* **8**, 174-177.
- 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.
- Blaineau, C., Bastien, P. and Pages, M. (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.
- Britto, C., Ravel, C., Bastien, P., Blaineau, C., Pages, M., Dedet, J.P. and Wincker, P. (1998). Conserved linkage groups associated with large-scale chromosomal rearrangements between Old World and New World *Leishmania* genomes. *Gene* **222**, 107-117.
- Croft, S.L. and Olliaro, P. (2011). Leishmaniasis chemotherapy--challenges and opportunities. *Clin Microbiol Infect* **17**, 1478-1483.
- Cruz, A.K., Titus, R. and Beverley, S.M. (1993). Plasticity in chromosome number and testing of essential genes in *Leishmania* by targeting. *Proc Natl Acad Sci U S A* **90**, 1599-1603.

deleterious mutations are more efficiently eliminated, and beneficial mutations more easily spread, in haploid populations than in diploids (Zeyl, 2004). On the other hand, diploids adapt less rapidly than haploids, since mutations can remain masked by the heterozygous state. But (a) diploids may take advantage of heterozygosity and (b) they benefit from a more stable phenotype, in particular with respect to harmful mutations. Trisomic cells are even more stable in that sense: the probability that the same mutation occurs on three homologues is very low. In a *Leishmania* strain, the proportions of coexisting mono-, di- and trisomic cells might vary according to the environment, combining the advantages of all ploidy systems at the population level. Similarly, in the diploid *Candida albicans*, the reversible loss or gain of one homolog of a specific chromosome was found to be a prevalent means of adaptation (Rustchenko, 2007).

den Boer, M., Argaw, D., Jannin, J. and Alvar, J. (2011). Leishmaniasis impact and treatment access. *Clin Microbiol Infect* **17**, 1471-1477.

Downing, T., Imamura, H., Decuypere, S., Clark, T.G., Coombs, G.H., Cotton, J.A., *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.

Dubessay, P., Ravel, C., Bastien, P., Crobu, L., Dedet, J.P., Pages, M. and Blaineau, C. (2002). The switch region on *Leishmania major* chromosome 1 is not required for mitotic stability or gene expression, but appears to be essential. *Nucleic Acids Res* **30**, 3692-3697.

Dubessay, P., Ravel, C., Bastien, P., Lignon, M.F., Ullman, B., Pages, M. and Blaineau, C. (2001). Effect of large targeted deletions on the mitotic stability of an extra chromosome mediating drug resistance in *Leishmania*. *Nucleic Acids Res* **29**, 3231-3240.

El-Sayed, N.M., Myler, P.J., Blandin, G., Berriman, M., Crabtree, J., Aggarwal, G., *et al.* (2005). Comparative genomics of trypanosomatid parasitic protozoa. *Science* **309**, 404-409.

Iourov, I.Y., Vorsanova, S.G. and Yurov, Y.B. (2008). Chromosomal mosaicism goes global. *Mol Cytogenet* **1**, 26.

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.

Kramer, S. (2012). Developmental regulation of gene expression in the absence of transcriptional control: the case of kinetoplastids. *Mol Biochem Parasitol* **181**, 61-72.

Leprohon, P., Legare, D., Raymond, F., Madore, E., Hardiman, G., Corbeil, J. and Ouellette, M. (2009). Gene expression modulation is associated with gene amplification, supernumerary chromosomes and chromosome loss in antimony-resistant *Leishmania infantum*. *Nucleic Acids Res* **37**, 1387-1399.

Martinez-Calvillo, S., Stuart, K. and Myler, P.J. (2005). Ploidy changes associated with disruption of two adjacent genes on *Leishmania major* chromosome 1. *Int J Parasitol* **35**, 419-429.

Mukherjee, A., Langston, L.D. and Ouellette, M. (2011). Intrachromosomal tandem duplication and repeat expansion during attempts to inactivate the subtelomeric essential gene GSH1 in *Leishmania*. *Nucleic Acids Res* **39**, 7499-7511.

Ravel, C., Dubessay, P., Bastien, P., Blackwell, J. and Ivens, A. (1998). The Complete Chromosomal Organization of the Reference Strain of the *Leishmania* Genome Project, *L. major* 'Friedlin'. *Parasitol Today* **14**, 301-303.

Raymond, F., Boisvert, S., Roy, G., Ritt, J.F., Legare, D., Isnard, A., *et al.* (2012). Genome sequencing of the lizard parasite *Leishmania tarentolae* reveals loss of genes associated to the intracellular stage of human pathogenic species. *Nucleic Acids Res* **40**, 1131-1147.

Rogers, M.B., Hilley, J.D., Dickens, N.J., Wilkes, J., Bates, P.A., Depledge, D.P., *et al.* (2011) Chromosome and gene copy number variation allow major structural change between species and strains of *Leishmania*. In *Genome Res*.

Rustchenko, E. (2007). Chromosome instability in *Candida albicans*. *FEMS Yeast Res* **7**, 2-11.

Scholler, J.K., Reed, S.G. and Stuart, K. (1986). Molecular karyotype of species and subspecies of *Leishmania*. *Mol Biochem Parasitol* **20**, 279-293.

Singh, A.K., Papadopoulou, B. and Ouellette, M. (2001). Gene amplification in amphotericin B-resistant *Leishmania tarentolae*. *Exp Parasitol* **99**, 141-147.

Sterkers, Y., Lachaud, L., Crobu, L., Bastien, P. and Pagès, M. (2011). FISH analysis reveals aneuploidy and continual generation of chromosomal mosaicism in *Leishmania major*. *Cell Microbiol* **13**, 274-283.

Sunkin, S.M., Kiser, P., Myler, P.J. and Stuart, K. (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.

Ubeda, J.M., Legare, D., Raymond, F., Ouameur, A.A., Boisvert, S., Rigault, P., *et al.* (2008). Modulation of gene expression in drug resistant *Leishmania* is associated with gene amplification, gene deletion and chromosome aneuploidy. *Genome Biol* **9**, R115.

Wincker, P., Ravel, C., Blaineau, C., Pages, M., Jauffret, Y., Dedet, J. and Bastien, P. (1996). The *Leishmania* genome comprises 36 chromosomes conserved across widely divergent human pathogenic species. *Nucleic Acids Res* **24**, 1688-1694.

Zeyl, C. (2004). Experimental studies on ploidy evolution in yeast. *FEMS Microbiol Lett* **233**, 187-192.

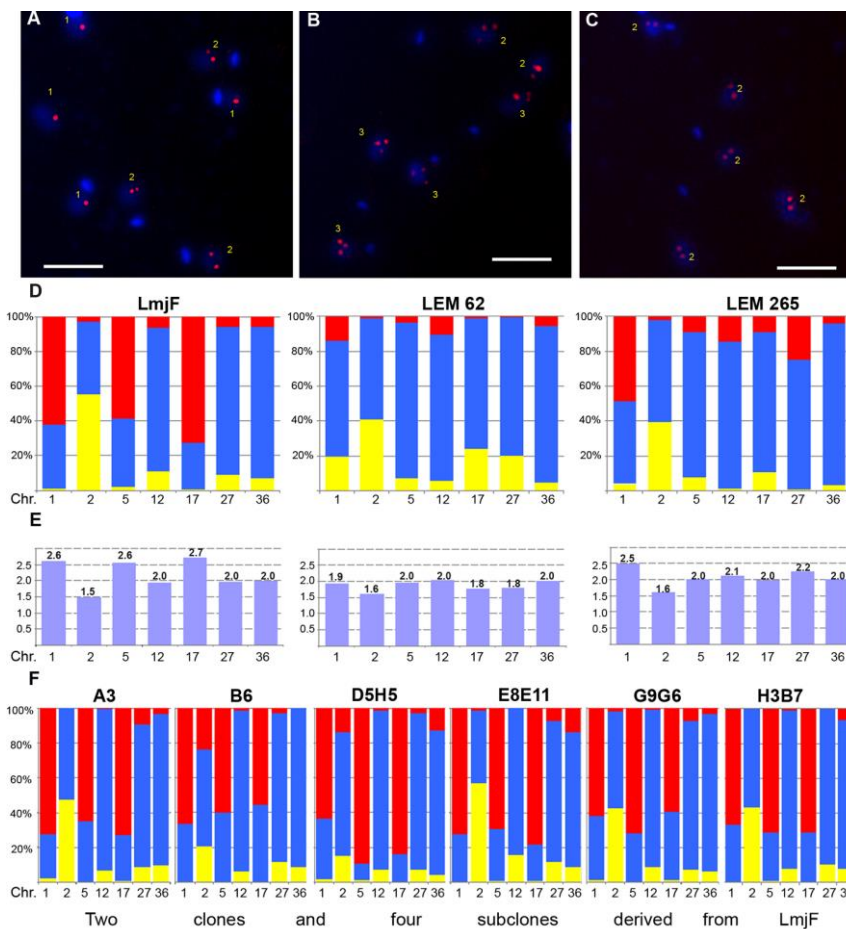


Figure 1: Mosaic aneuploidy in *Leishmania*.

A, B and C: Diversity of ploidy patterns. The chromosomal contents vary from cell to cell, cells being either mono-, di- or trisomic for a given chromosome. The *Leishmania major* Friedlin (LmjF) strain chromosome 2 is predominantly monosomic (**A**), chromosome 1 is predominantly trisomic (**B**) and chromosome 27 predominantly disomic (**C**). Bar 5 μm . **D: Ploidy patterns of three *L. major* strains.** The proportions of monosomic (yellow), disomic (blue) and trisomic (red) cells for chromosome (chr.) 1, 2, 5, 12, 17, 27 and 36 are represented in *L. major* Friedlin and two other *L. major* strains LEM 62 (MHOM/YEM/76/LEM62) and LEM265 (MHOM/MA/81/LEM265). y-ordinates: ploidy proportions (0-100%); x-abscissas: the seven chromosomes analyzed. In *L. major* Friedlin, all chromosomes are present in at least two major ploidy stages among trisomy, disomy and monosomy. Chromosome 1, 5 and 17 are predominantly trisomic, chromosome 2 is predominantly monosomic and chromosome 12, 27 and 36 are predominantly disomic. The high percentages observed are statistically significant given the size of the population studied (200-500 cells). Mosaic aneuploidy is observed in the three strains but the ploidy pattern differs among strains; therefore, there is no species-specific pattern. **E: Cumulative ploidy inferred from FISH data.** Similarly to global approaches such as deep sequencing, a 'cumulative ploidy' may be deduced from the individual cell ploidy determined by FISH. y-ordinates: cumulative ploidy inferred from FISH (0-3); x-abscissas: the seven studied chromosomes. FISH gives a vision of the population at the single cell level whereas deep sequencing allows an overall view of the mean ploidy for each of the 36 chromosomes. However, the latter does not give much clue about the mosaic structure of the strain, particularly when the mean value is a whole number. Thus, the cumulative ploidy inferred from FISH for most chromosomes (Chr. 12, 27 and 36 in LmjF; Chr. 1, 5, 12, 17, 27, 36 in LEM62 and Chr. 5, 12, 17, 27 and 36 in LEM265) is close to 2.0 despite the fact that they are present in at least two ploidy stages. **F: Heritability of ploidy patterns through cell generations.** Proportions of monosomic (yellow), disomic (blue) and trisomic (red) cells for chromosome (chr.) 1, 2, 5, 12, 17, 27 and 36 are represented in six cell clones or subclones derived from the parental line LmjF. y-ordinate: ploidy proportions (0-100%); x-abscissas: the seven chromosomes studied in each of the clones/subclones. Mosaicism is not lost in the clones, and the pattern of ploidy is essentially similar between the parental strain and clones as well as among clones. Thus, all six clones derived from LmjF are predominantly trisomic for chromosome 1, the proportion ranging from 64 to 72% of cells, which is very close to the 63% observed for LmjF. Similarly, chr. 12, 27 and 36 are mainly disomic in LmjF and its clones. By contrast, the monosomy of chr. 2 (at 62% LmjF) is found between 43% and 57% in four clones, but at only 15% in clone D5H5 and 21% in B6.

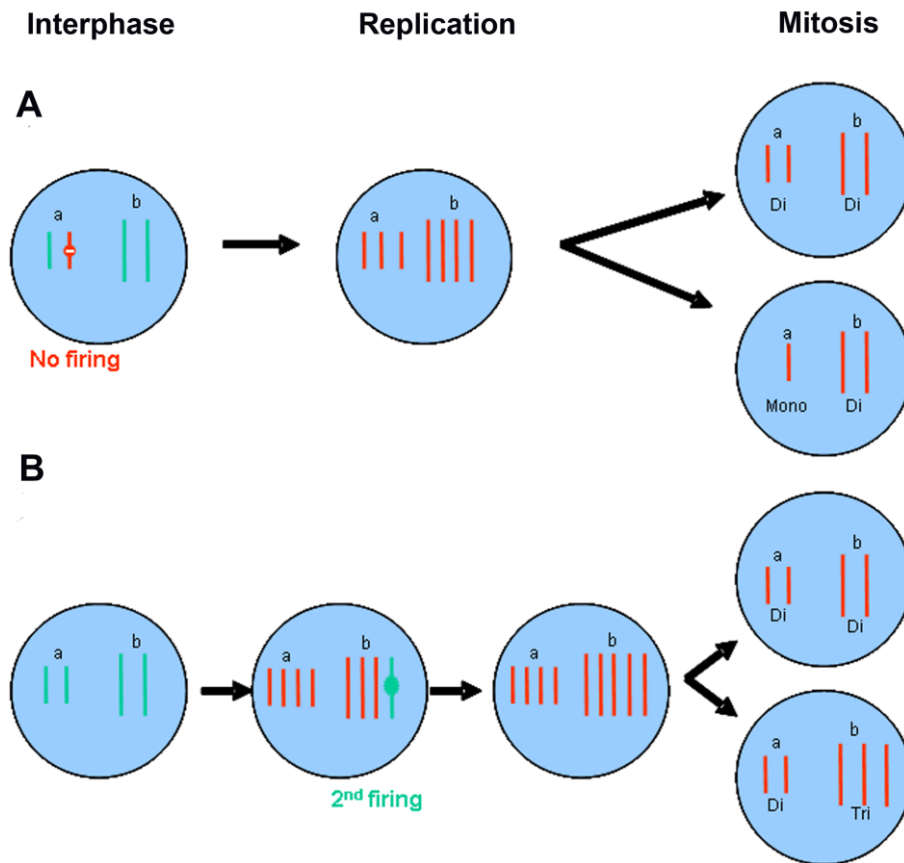


Figure 2: Occurrence of mosaic aneuploidy

Every chromosome is present in the *Leishmania* cell population at least two ploidy states (monosomic, disomic or trisomic). In the *L. major* Friedlin strain, for example, chromosome 2 is 60% monosomic and 40% disomic, chromosome 5 is 30% disomic and 70% trisomic and chromosome 27 is 80% disomic, 10% mono- and 10% trisomic (Sterkers *et al.*, 2011). These variable chromosomal contents from cell to cell establish a strongly mosaic structure of the population. In the presented model, two homologs of chromosome ‘a’ and ‘b’ are represented in interphase in a diploid hypothesis. **In A**, chromosome ‘b’ replicates properly and, during mitosis, segregates in a symmetric manner. Chromosome ‘b’ was disomic (Di) before cell division and remains disomic afterwards. Chromosome ‘a’ does not replicate properly due to the non-firing of a replication origin: following the S phase, there are only three copies of chromosome ‘a’; an asymmetric segregation occurs, leading to a cell disomic for chromosome ‘a’ and another one monosomic (Mono) for chromosome ‘a’. **In B**, chromosome ‘a’ replicates and segregates properly. For chromosome ‘b’, replication begins properly but restarts for one of the sister chromatid an over-replication occurs, so that a trisomic (Tri) cell is generated following asymmetrical segregation.

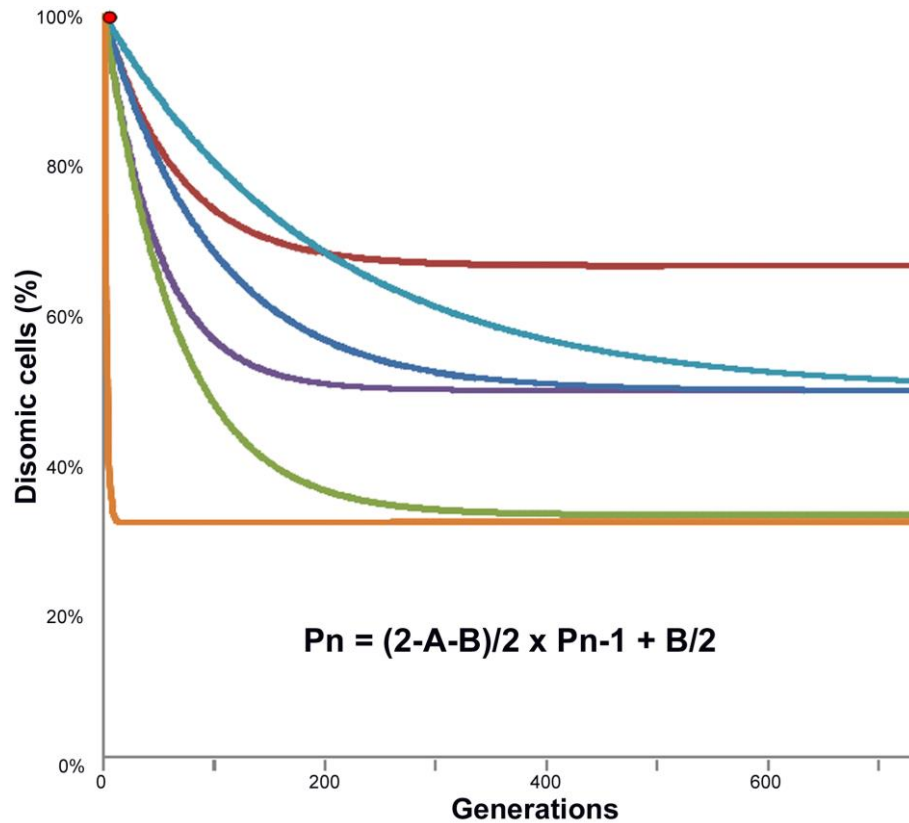


Figure 3: Kinetics of mosaic aneuploidy

We proposed a model for the kinetics and a mathematical formula for the occurrence and maintenance of mosaic aneuploidy in *Leishmania* that implies of 'positive' and 'negative' asymmetric chromosome allotments (ACAs) rates. In mitosis allotment of chromosomes in daughter nuclei are usually symmetrical. In *Leishmania*, FISH studies have demonstrated that it is not the case in large percentages of division, instead there are asymmetrical allotment of sister chromatids: 1+2, 2+3. With respect to the formula, P_n represents the percentage of disomic cells at generation n , A the rate of '2+3' ACAs issued from a disomic cell (i.e. positive ACA rate), B the rate of '2+3' ACAs issued from a trisomic cell (i.e. negative ACA rate).

Whatever ACA rates are, a steady state is reached. If positive and negative ACA rates are equal, the steady state is attained with 50% of disomic cells; if they are not equal the equilibrium is shifted upwards or downwards. With positive and negative ACA rates at 2% and 1%, respectively, the proportion of disomic cells is 33%; conversely, if positive and negative ACA rates are at 1% and 2%, respectively, the proportion of disomic cells is then 66%. Considering positive and negative ACA rates equals at 0.5, 1 or 2%, the equilibrium is still set at 50% of disomic cells, but it is reached all the more rapidly as the ACA rates are higher. In the example of chromosome 5 of *L. major* Friedlin (Sterkers *et al.*, 2011), the steady state should be reached in less than 10 generations.

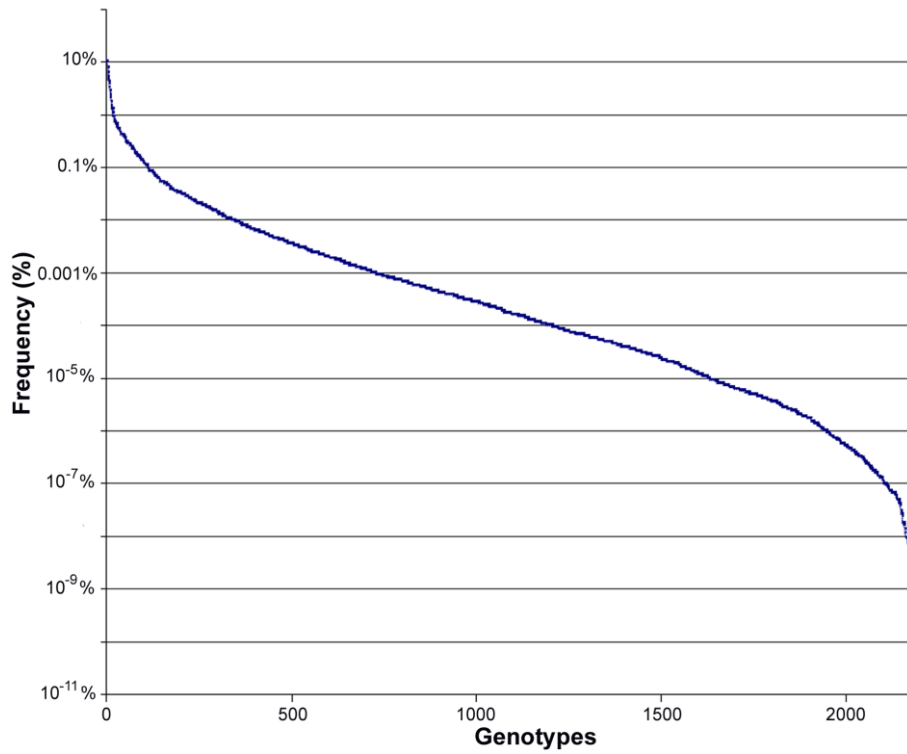


Figure 4: An extreme diversity of genotypes within the population

Considering the 7 chromosomes that have been studied by FISH, and all chromosomes being possibly present in three ploidy states (among mono-, di-, and trisomy) in any given cell, the number of genotypes expected in the population can be calculated at 3^7 , *i.e.* 2187.

In the figure, the different genotypes (x-*abscissa*) are plotted against the frequency of each genotype in the population (y-*ordinates*). The frequencies of the different genotypes have been ranked in decreasing order: the most predominant one is observed in 10% of the cells (left), whereas the frequency of the rarest one is 10^{-9} % (right), *i.e.* present in one cell out of 10^{11} .

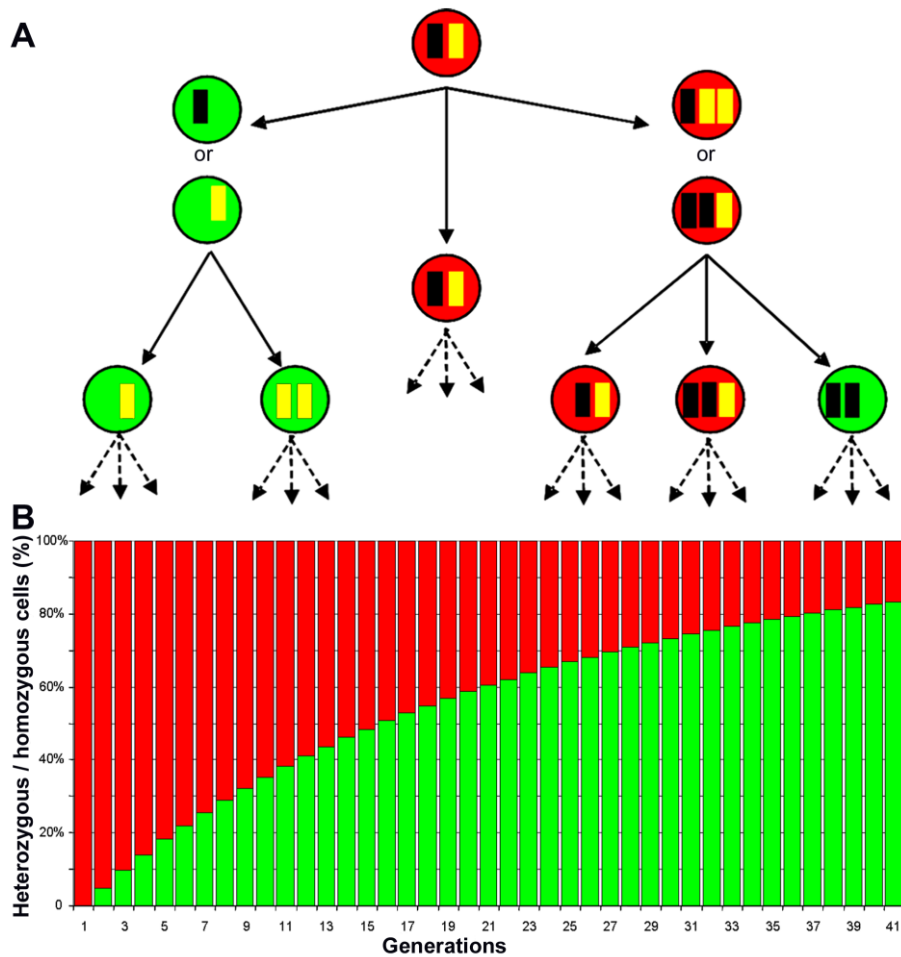


Figure 5: Loss of heterozygous cells in *Leishmania* strains

A: Schematic representation of the evolution of a heterozygous cell throughout mitotic divisions. According to the data of Sterkers *et al.* (2011), due to frequent asymmetric chromosomal allotments (ACAs) in daughter nuclei during mitosis, every chromosome stands a chance of being lost or gained at each division. One consequence of this is the loss of heterozygous cells, which depends upon the value of positive and negative ACA rates (see Figure 3). Thus, starting from a population of heterozygous cells (in red) for a given chromosome/allele (in black and yellow), the proportion of heterozygotes decreases and that of homozygotes (in green) increases generation after generation. However, the loss of heterozygous cells does not eliminate variant alleles from the population, as those will be maintained in the absence of selective pressure. Thus the strain will still appear as ‘heterozygote’ when global analysis methods are used. **B:** Evolution of the proportion (%) of heterozygous cells within a *Leishmania* cell population according to ‘positive’ and ‘negative’ ACA rates. Here is represented a population with both positive and negative ACA rates at 10 %. The frequency of heterozygous cells in the population after 40 divisions would then be 17 %.