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Highlight

The *Leishmania* chromosome lottery☆

Many items come in pairs: socks, mittens, chopsticks, earrings, and in the case of the majority of eukaryotic organisms, chromosomes. This diploid state is considered the common standard of the eurkaryotic karyotype, although many plants and fungi may switch between a diploid state and a haploid state depending on the stage in their life cycle. Polyploidy, which involves duplication of the whole set of chromosomes is relatively well tolerated across a number of species and is believed to be an important driving force of evolution and diversification in many organisms [2]. However, alterations in chromosome number whereby chromosome pairs behave differently from their comrades usually have detrimental consequences on organism development in many eukaryotes [3]. Such gains or losses in chromosomes is termed aneuploidy and is the leading cause of miscarriage in humans [4]. Playing with chromosome numbers results in changes to gene dosage leading to stoichiometric imbalances in essential gene products and reduced cellular fitness [5]. Surely such chromosomal imbalances cannot be the norm for any organism? In this issue of Microbes and Infection, Sterkers and colleagues show that aneuploidy is a widespread feature of the eukaryotic *Leishmania* genus [1] and suggest that far from spelling developmental disaster, chaotically organised chromosomes may offer certain advantages to this unconventional micro-organism.

Leishmania is a genus of single-celled parasites transmitted to human hosts via sand flies that cause the disease leishmaniasis, which shows a broad clinical spectrum ranging from particularly unpleasant, disfiguring cutaneous lesions to potentially fatal visceral leishmaniasis [6]. Around 20 species of *Leishmania* are thought to cause the disease in humans. Cutaneous *Leishmania* is caused by species such as the Old World *Leishmania major* and the New World *Leishmania mexicana* whereas the more severe visceral leishmaniasis is caused by species of the *Leishmania donovani* complex. Despite an estimated divergence of 20–100 million years, gene content amongst species is extremely well conserved, with Old and New World species possessing similar numbers of chromosomes (36 and 35 or 34 respectively), and genome sequencing revealing very few species specific genes [7]. Instead, diversity within the realm of these parasites comes from gene amplification and deletion events giving rise to homologous chromosomes of varying sizes [8]. It was long suspected that further alteration to gene dosage in *Leishmania* was also achieved by natural aneuploidy. The debate over these parasites employment of what should be considered a developmentally insane strategy has largely been put to rest by microscopy experiments in *L. major* [9], and by sequencing across a range of other species [7,10]. Analysis of chromosome copy number by sequencing read depth revealed large differences for eight strains and species of *Leishmania*, with trisomic read depth observed for up to nine chromosomes [7] (Note that earlier clues as to the presence of trisomy were previously provided by the frustrations of scientists trying to create double-knockout mutants for the presumed disomic chromosomes).

However, the story of *Leishmania*'s dealings with aneuploidy does not end here. Sequencing analysis further showed the peculiar presence of chromosomes with 'intermediate' read depth that were neither disomic nor trisomic. The problem with this type of analysis however is that it gives information on a population of cells pooled together into one big pot that may be (wrongfully) considered as genetically identical. Sterkers and colleagues used DNA FISH to examine single cell genomic organisation [9]. Of seven analysed chromosomes of *L. major* derived clones, none were exclusively disomic; they were all monosomic, disomic, or trisomic. In other words, aneuploidy in the population of cells was mosaic. Sterkers and colleagues now demonstrate this 'mosaic aneuploidy' using single cell resolution techniques for six different chromosomes in three Old World species (*Leishmania infantum*, *L. donovani*, and *Leishmania tropica*) and one New World species (*Leishmania amazonensis*) [1]. All chromosomes examined were monosomic, disomic, or trisomic, and less frequently tetrasomic in proportions that varied depending on the chromosome and species examined.

To abate the outcry of anyone who has ever worked with HeLa, or indeed with any extensively cultivated cell line, who would rightly plead that genetic divergence in cell culture is unremarkable, Downing and colleagues measured genetic diversity in *Leishmania* derived from clinical samples [10]. They found extensive aneuploidy in the genomes of 17 phylogenetically close strains of *L. donovani*. Each isolate had a unique karyotype. Vive la (*Leishmania*) revolution.

☆ Article highlight of "Constitutive mosaic aneuploidy is a unique genetic feature widespread in the *Leishmania* genus." by Lachaud, L. et al. [1].

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So how does mosaic aneuploidy arise and why is it tolerated? Possible mechanisms include defective segregation of sister chromatids during cell division or chromosome replication error. DNA FISH experiments on mitotic cells support the latter possibility [9]. Gain or losses in chromosomes may be authorised since the *Leishmania* the haploid genome is small (approx. 32 Mb) and is distributed over a large number of chromosomes such that altering the copy number of one chromosome does not affect many genes.

Perhaps the most compelling question however is how might the parasite benefit from such unorthodox chromosome biology? In *Leishmania*, genes are organised in large polycistronic transcription units made of functionally unrelated genes containing no promoter for RNA polymerase II. This organisation precludes typical mechanisms of transcriptional regulation and instead the parasite relies largely on post-transcriptional control [11]. Increasing or decreasing gene copy number could be a way of regulating gene expression. Indeed, control of genomic output is predicted to be a robust feature of the parasite's biology since it has to cope with two very different host environments: the fly gut, and mammalian macrophages. These have very different pH, temperature, and nutrients and thus require substantial changes in gene expression during the parasite's life cycle. Furthermore, adaptivity to changing environments may be greatly aided by the peculiar feature of mosaic aneuploidy. In haploids, detrimental mutations may spell disaster for an individual cell, but this ensures that such unfit mutants can be quickly weeded out to the benefit of the population as a whole ensuring the rapid selection of appropriate surviving mutants. Hence, deleterious mutations are more effectively eliminated and beneficial mutations are more easily propagated in haploid rather than diploid populations.

Leishmaniasis represents a substantial global burden with around 350 million people at risk of contracting the disease and around 59,000 deaths due to visceral leishmaniasis each year [12]. There is currently no available vaccine. Mosaic aneuploidy has serious implications on strategies of disease prevention and control since any potential vaccine or drug target needs to be on a stable chromosome. Sterkers and colleagues calculated that in the *L. major* Friedlin strain there could be up to 2000 different genotypes, with the most frequent being present in 10% of the cells and the rarest being absent from a population of 10^{11} cells. This endless possibility of combinations paints a worrying picture for attempts to conquer this parasite. Perhaps mechanisms creating aneuploidy themselves could be a viable drug target. In this sense, the study of a little-known protozoan may be highly relevant to one of nature's darkest demons, cancer, since despite the seemingly detrimental effects of a fluctuating karyotype, aneuploidy is a hallmark of cancer and several lines of evidence suggest that aneuploidy has a casual role in tumourgenesis.

Model organisms taught us that two is company, but three's a crowd. Then along came the *Leishmania* to get the party started.

1. Biosketch – Yvon Sterkers

Dr. Yvon Sterkers completed a medical degree between 1989 and 1995 in the Faculty of Medicine Necker (Paris V) and became a resident (Interne des Hôpitaux de Paris) specialising in clinical hematology. In 2001, he joined the Scherf laboratory in the Pasteur Institute for a PhD entitled "*Plasmodium falciparum* parasitized red blood cells surface molecules and their role in malaria pathogenesis". He defended his thesis in 2005, and in 2006 he joined the department of Parasitology Mycology and Prof. Patrick Bastien in Montpellier, where he obtained a permanent position as a lecturer in 2010. Today he shares his time amongst (i) the molecular diagnosis of toxoplasmosis both in routine and in the molecular pole of the reference national center for toxoplasmosis (coordinated by Prof. P. Bastien; http://cnrtoxoplasmose.chu-reims.fr/); (ii) lectures for the medical school and for the master of science (http://www.masterbs.univ-montp2.fr/); (iii) basic science on core molecular and cellular processes of *Leishmania* in UMR MIVEGEC (CNRS5290-IRD224-UM1) together with Laurence Lachaud (MCU-PH) and Michel Pagès (CR CNRS).

2. Interview with Yvon Sterkers

1. What triggered your interest in mosaic aneuploidy in Leishmania sp?

We are interested in core cellular processes, and work on *Leishmania* sp., a protozoan parasite responsible for a large spectrum of diseases over four continents. We use molecular methods with a particular emphasis on single cell methods (Fluorescent *in situ* hybridization (FISH), Immunofluorescence assay). If core cellular processes are heavily studied in higher eukaryotes, they remain ill-known in *Leishmania*, considered as 'ancestral' or 'divergent'