Trends in Parasitology

CellPress REVIEWS

Spotlight

A Way Straight-Forward for *Leishmania* Genetics

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Genetic exchange between *Leishmania* parasites was demonstrated in sandflies over 10 years ago. Louradour *et al*. have shown *in vitro* hybridization of two *Leishmania tropica* isolates, with the potential to remove a major roadblock to using forward genetics in *Leishmania*, understanding *Leishmania* reproductive biology, and analyzing gene flow in natural populations.

There has long been interest in the sex lives of trypanosomatid parasites. For many years, trypanosomatids were considered to reproduce mostly or entirely clonally, but a great deal of evidence has accumulated that many natural populations of *Leishmania*, trypanosomes and their relatives, exchange genes more or less regularly [1]. Direct evidence of genetic exchange in laboratory infections of flies with *Trypanosoma brucei* is now over 30 years old and has subsequently been shown in *Trypanosoma cruzi* infecting mammalian cells. Genetic exchange between *Leishmania* parasites in their sandfly vectors was first demonstrated over 10 years ago [2] but only a handful of papers describing genetic crosses have since been reported from just three research groups. Only around 35 laboratories in the world are keeping sandflies [3], so the availability of colonies of these notoriously fussy insects represents a major roadblock in the wider use of genetics to understand *Leishmania* biology. In a recent report, Louradour *et al.* [4] have shown that *Leishmania* cells can also form stable hybrids entirely *in vitro*. By removing the need to maintain sandfly colonies, this work promises to make *Leishmania* among the most tractable parasite species for forward genetics research.

Forward genetics attempts to identify the genetic basis for specific phenotypes ('gene mapping') that vary between parasite strains. While it is well known that reverse genetics tools have a growing scope and convenience – including in *Leishmania* – classical forward genetics is also undergoing a renaissance as the widespread availability of whole-genome sequence data greatly facilitates the identification of genetic variation affecting phenotypes of interest. One well known approach – the genome-wide association study (GWAS) – has become widely used in human genetics, but other approaches, for example, experiments combining selection after a cross with sequencing and 'bulk segregant analysis' are also extremely powerful. Perhaps the best known parasitological examples are crosses between *Plasmodium falciparum* strains, but genetic crosses have also been performed in *Toxoplasma*, *Cryptosporidium*, and a number of helminth parasites. However, we know of only a single study [5] that has mapped a trypanosome trait using a genetic cross.

While the first demonstration of *in vitro* hybridization promises an expanded toolset to study *Leishmania* biology [4], important practical limitations remain. First, the authors only investigated *in vitro* hybridization between two specific strains of *Leishmania tropica*, and these were chosen based on hybridizing with higher frequency than other combinations of strains or species in sandfly-infection experiments [4,6]. Whether other *Leishmania* strains will also hybridize *in vitro* remains unknown, and even in these carefully selected isolates, *in vitro* efficiencies are estimated to be reduced by a factor of ~10^2 to 10^3 compared with hybridization in sandflies [4]. Moreover, the current work shows the ability to form first generation (F1) hybrids. While some traits could be mapped by phenotyping these strains, crossing designs that investigate genetic variation between isolates require that these F1 hybrids are themselves able to hybridize – either with each other (for intercross designs) or with one of the parents (a backcross). Backcrosses have been achieved in *Leishmania* via sandflies [6], but it remains to be shown whether either of these approaches works *in vitro*. Lastly, as for 'in-fly' approaches, different selectable markers need to be present in the parental strains to efficiently identify hybrid offspring, so only genetically engineered parental strains can be used.

Regardless of the suitability of *in vitro* hybridization for genetic mapping in *Leishmania*, the demonstration by Louradour *et al.* [4] promises a more accessible experimental approach to understanding *Leishmania* reproductive biology. It is clear that *Leishmania* can reproduce clonally by mitotic cell division as well as through hybridization of genetic material of two parental cells in the promastigote stage, but the molecular mechanism of hybrid formation is not understood. Possible hybridization mechanisms debated so far include typical meiosis, a tetraploid meiotic cycle, and parasexual reproduction (Figure 1). Typical meiosis requires the generation and subsequent hybridization of gametes, which has recently been argued for based on indirect evidence [6]. However, while direct evidence of classical meiosis through the observation of haploid gametes is available for *T. brucei* [7], these cells have not been seen in *Leishmania*-infected sandflies. Indeed, while *Leishmania* chromosomes are predominantly diploid in most strains, the concept of 'haploid' gametes is complicated due to pervasive mosaic aneuploidy in *Leishmania* [8]. The two additional models of hybridization both assume cell fusion of two typically diploid cells resulting in a transient tetraploid state. The model involving a

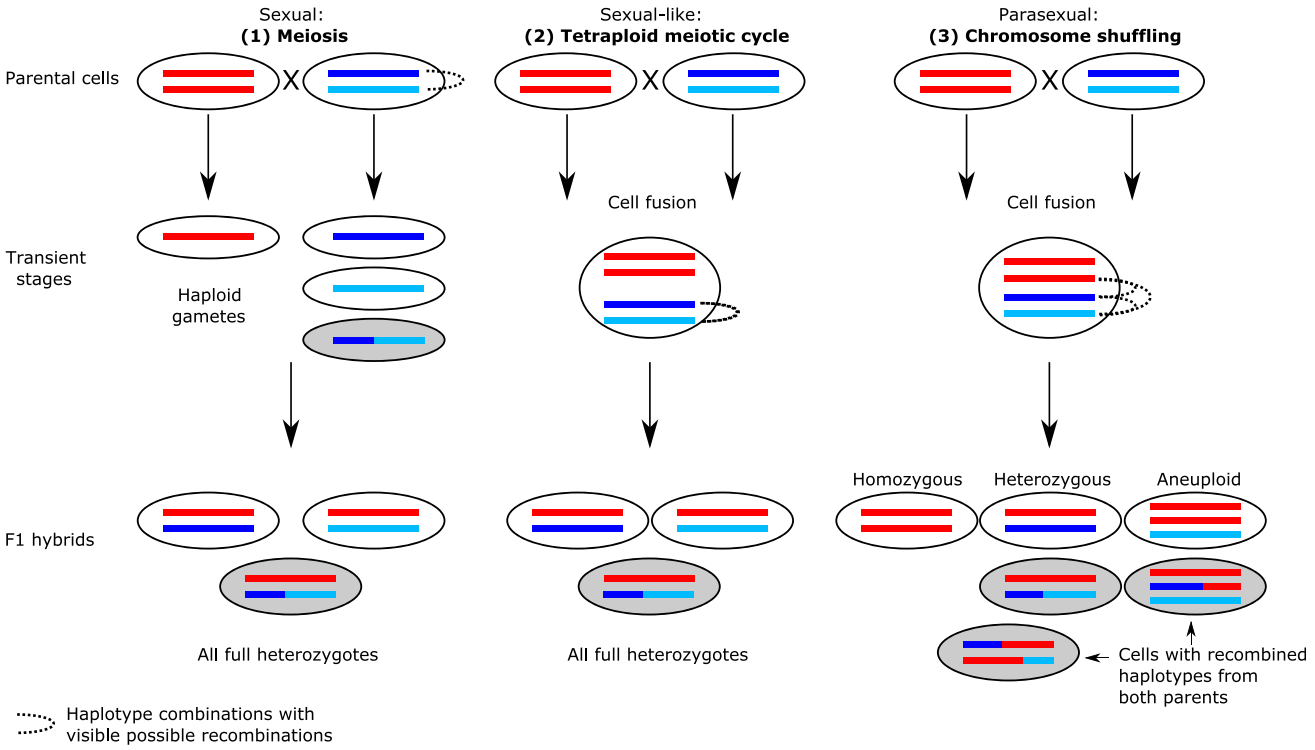

Figure 1. Genomic Patterns of Segregation Expected from Different Models of Hybridization. We show three possible hybridization scenarios between diploid parental cells: (1) sexual reproduction including classical meiosis, (2) fusion of parental cells initiating a tetraploid meiotic cycle [6], and (3) fusion of parental cells followed by karyogamy, chromosome shuffling, and subsequent loss of chromosomes or through mitotic division of shuffled chromosomes [8]. Combinations resulting in visible recombination events are indicated by dotted lines and shaded cells; only exemplary cases out of very many possible outcomes are shown here. Models (1) and (2) both imply the presence of full heterozygotes throughout the chromosome in diploid F1 hybrids but may be distinguished if transient stages can be detected. Only in model (3) recombinant haplotypes between both parental lines could be present in F1 hybrids. Aneuploidy can be generated and maintained mitotically in *Leishmania* [8] and could complicate the illustrated scenarios, as could mitotic recombination.

tetraploid cycle, as known for *Saccharomyces*, was argued for by Inbar *et al.* [6]. In the alternative parasexual model, cell fusion is followed by karyogamy and reshuffling of chromosome copies during subsequent mitotic division. This ultimately leads to 're-diploidization': either immediately in a reduction division as originally proposed [8] or through more gradual chromosome loss – a process well described in *Candida albicans*. Both *in vitro* and in flies, dominantly diploid F1 *Leishmania* hybrids generally have equal contributions from both parents [4,6], arguing for the options involving well regulated, meiosis-like processes. However, hybrid cells generated under both experimental conditions may be diploid, triploid, or even tetraploid [2,4,9].

The availability of an *in vitro* system will make further work to resolve the details of genetic exchange in *Leishmania* much more convenient and may make some novel experiments tractable. For example, if gametes can be cultured *in vitro* to a high enough density, single-cell sequencing might identify those key cells by resolving the ploidy and gene expression patterns of cells involved in genetic exchange. The observed low frequency of hybridization so far does mean that this will probably require either increasing the frequency of hybridization or some way to select for cells of interest. Additionally, an efficient *in vitro* system could even allow testing of enough strains to permit genetic screens. Those might ultimately identify the genetic basis for hybridization compatibility and efficiency in *Leishmania*. It would be of particular interest to investigate whether differences in the frequency of recombination observed between different populations in nature (e.g., [10]) are due to intrinsic differences in the proclivity of the local *Leishmania* to hybridize, or to other factors such as differences in vector species. Understanding the causes of regional differences in observed hybridization frequencies might allow us to better predict geographic regions of future gene flow, and so prevent the spread of drug resistance or enhanced virulence.

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