Infection, Genetics and Evolution xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Reproduction in *Leishmania*: A focus on genetic exchange

V. Rougeron [a,*], T. De Meeûs [b], A.-L. Bañuls [a]

[a] MIVEGEC (Laboratoire Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle), UMR CNRS 5290-IRD 224-Université de Montpellier, Montpellier, France
[b] Institut de Recherche pour le Développement (IRD), UMR 177 INTERTRYP IRD-CIRAD, TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form 17 October 2016
Accepted 18 October 2016
Available online xxxx

Keywords:
Leishmania
Genetic exchange
Reproductive strategies

ABSTRACT

One key process of the life cycle of pathogens is their mode of reproduction. Indeed, this fundamental biological process conditions the multiplication and the transmission of genes and thus the propagation of diseases in the environment. Reproductive strategies of protozoan parasites have been a subject of debate for many years, principally due to the difficulty in making direct observations of sexual reproduction (i.e. genetic recombination). Traditionally, these parasites were considered as characterized by a preeminent clonal structure. Nevertheless, with the development of elaborate culture experiments, population genetics and evolutionary and population genomics, several studies suggested that most of these pathogens were also characterized by constitutive genetic recombination events. In this opinion, we focused on *Leishmania* parasites, pathogens responsible of leishmaniases, a major public health issue. We first discuss the evolutionary advantages of a mixed mating reproductive strategy, then we review the evidence of genetic exchange, and finally we detail available tools to detect naturally occurring genetic recombination in *Leishmania* parasites and more generally in protozoan parasites.

1. Introduction

Reproductive mode is fundamental in biological entities. It conditions the multiplication and the transmission of genes and thus the propagation and adaptation of individuals in the environment. The diversity of reproductive strategies in living organisms, including pathogens, is extremely wide. Some, like the emblematic Bdelloid rotifers are strictly clonal (Welch and Meselson, 1998), others, such as soil nematodes, Monogonont rotifers, *Cladocerans* sp. (Custacea), Aphid insects, Tardigrads, Mesostigmata or Prostigmata acarians, several plants, and fungi, display various ways to alternate sexual and clonal propagation (de Meeûs et al., 2007), while mammals or birds can only reproduce sexually, at the exception of armadillos ("The Tree of Life — Guillaume Lecointre, Hervé Le Guyader | Harvard University Press,", page 403, 2006). Parasites also display various strategies. *Trypanosoma brucei gambiense* type 1 is considered to be strictly clonal (Koffi et al., 2009; Weir et al., 2016). Other parasites are also able to reproduce sexually at some stages of their life cycle and asexually at other times in their life cycle. These include, *Plasmodium falciparum*, the malignant agent of malaria; *Toxoplasma gondii*, responsible of toxoplasmosis; *Schistosoma mansoni*, a trematode flatworm (Platyhelminthes), the agent of schistosomiasis (Ajzenberg et al., 2004; Criscione and Blouin, 2006; Paul and Day, 1998). Finally, some are even purely sexual like most cestodes (Platyhelminthes), Diplozoon flatworms (Monogenea), nematodes (roundworms) agent of filariases (Guinea worm, *Loa loa*, *Onchocerca volvulus*) and most tick species (Acaria). Understanding the reproductive strategies of pathogens is epidemiologically important to obtain clues on transmission patterns, evolution of virulence, drug resistance, and pathogenesis, and it is thus biomedically relevant.

Reproductive strategies of protozoan parasites have been a subject of debate for many years, principally due to the difficulty in making direct observations of sexual reproduction (i.e. genetic recombination). Traditionally protozoan parasites were believed to display a preeminent clonal structure (Weedall and Hall, 2015). Nevertheless, it is now known that meiotic sex was present in eukaryotes' common ancestor (Bernstein and Bernstein, 2010), meaning that meiotic sex was, and probably still is, a constitutive eukaryotic feature. With the development of elaborate culture experiments, population genetics and evolutionary and population genomics, several studies showed that most of the pathogens studied present sexual recombination events (Akopyants et al., 2009; Ehrenkaufer et al., 2013; Gibson et al., 2008; Malik et al., 2008; Peacock et al., 2014; Ramesh et al., 2005; Rogers et al., 2014; Weir et al., 2016, non-exhaustive list). Nevertheless, the frequency of sex still largely needs to be assessed.

In this opinion paper, we will focus on the protozoan parasites of the *Leishmania* genus (Kinetoplastida: Trypanosomatidae), which are responsible for human leishmaniases. These diseases are neglected despite the serious public health problems they represent worldwide ("WHO Report on Global Surveillance of Epidemic-prone Infectious Diseases - Leishmaniasis," n.d., 2015). We first discuss the evolutionary advantages of a mixed mating reproductive strategy. Then, we review

* Corresponding author at: MIVEGEC (Laboratoire Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle), UMR CNRS 5290-IRD 224-Université de Montpellier, 34295 Montpellier, France.
E-mail address: rougeron.virginie@gmail.com (V. Rougeron).

http://dx.doi.org/10.1016/j.meegid.2016.10.013
1567-1348/

the state of knowledge on the reproduction model of *Leishmania* with a specific focus on the evidence of genetic exchange. Finally, we detail current available tools to detect genetic recombination in *Leishmania* parasites, and more generally in protozoan parasites, that will allow further understanding of the evolutionary mechanisms of these complex lifecycle parasites.

In this paper, we will use the term "sexual" as meiotic sex and "clonal" as absence of meiotic sex. As expounded elsewhere (Rougeron et al., 2015), we consider that any kind of system involving meiosis is sexual, hence selfing, sib-mating or very small effective population sizes, which have similar consequences together (inbreeding), are not equivalent to clonal reproduction (see also Ramírez and Llewellyn, 2014). Recently, Tibayrenc and Ayala proposed the PCE model (Preponderant Clonal Evolution) to characterize reproduction In some microorganisms, which reproduce mainly clonally but able to use other mode of reproduction such as meiotic sex. Readers may consult Tibayrenc and Ayala's papers and references therein (Tibayrenc et al., 2015; Tibayrenc and Ayala, 2013).

2. A mixed mating model of reproduction: what evolutionary advantages?

The most important life history trait of many microorganisms, particularly parasites with complex life cycles, is the ability within the same genus or even species to use different reproductive strategies depending on the life cycle or on the different environmental pressures to which pathogens are confronted. Data from the literature showed that *Leishmania* parasites, characterized by a digenetic lifecycle, have also evolved towards a complex mixed mating reproduction model. Specifically, based on the population genetic studies we performed on three different *Leishmania* species (Rougeron et al., 2011a, 2011b, 2009) and following results obtained in several other studies by colleagues (such as Gouzelou et al., 2012; Kuhls et al., 2013; Mahnaz et al., 2011; Rogers et al., 2014), it seems that *Leishmania* species alternate between modes of reproduction, in which clonality would occur in both vertebrate and invertebrate hosts and genetic recombination only within the insect vector (as formally demonstrated for other Kinetoplastida, such as *Trypanosoma brucei* sl) (Gibson et al., 2008; Peacock et al., 2014). It was also shown that sexual recombination often occurs between genetically related individuals (endogamy). Furthermore, on the basis of empirical data obtained in different *Leishmania* species, we suggested that these pathogens present different proportions of reproductive modes in relation to their diversity in terms of hosts with a continuum of mixed-mating reproductive strategies, from preponderant clonality with low level of endogamy and allogamy (corresponding to PCE model) to a model with high level of endogamy and clonality and quite frequent allogamic events (Rougeron et al., 2015, 2010).

To be maintained in natural populations, these multiple, complex and adaptive mechanisms of reproduction (clonality, endogamy, allogamy) certainly provide evolutionary advantages for short-term and long-term *Leishmania* parasite survival. *Leishmania* species need to adapt to extremely heterogeneous environments (such as different mammal hosts and/or vector species according to country or even environment). Parasites characterized by a mixed mating reproduction model probably benefit from the advantages inherent to the different types of reproduction. Indeed, being clonal is an advantage in a stable environment, where the parasite with the right genes combination is not confronted to any novel pressure and will thus be disseminated quickly. On the other side, even if sex through genetic recombination is cost effective, its benefits are undeniable for purging the genome of deleterious mutations (i.e. avoid the Muller's ratchet) and also repairing appropriately broken fitter combinations or creating new advantageous combinations. For *Leishmania* parasites, it has already been shown that sexual model such as allogamy contributes to the fitness of the parasite and results in the production of a wide library of genotypes enabling *Leishmania* to adapt to extremely variable environmental conditions (Lythgoe, 2000; Victoir and Dujardin, 2002). Other studies showed that inbreeding, as can be produced by sib-mating (endogamy), is in some cases an advantageous strategy (Lynch, 1991). Indeed, inbreeding may favor the cooperation between parents and offspring and between offspring since cooperation can result in enhanced fitness when cooperating entities are genetically related. Systematic sib-mating also occurs in *P. falciparum* and seems to enhance parasite success in the mosquito vector (Morlais et al., 2015).

However, many questions remain about this mixed-mating, especially concerning the ploidy variations of these organisms (Downing et al., 2011; Sterkers et al., 2014, 2011). Several studies based on FISH (Fluorescent In Situ Hybridization) and next-generation sequencing data showed a constitutive aneuploidy in *Leishmania*, with chromosomes found in single, two, three, or even in 4 or 5 copies (Downing et al., 2011; Romano et al., 2014; Sterkers et al., 2011). These patterns are globally in apparent contradiction with all the microsatellite data published in the literature. Theoretically, the low heterozygosity detected in *Leishmania* population could be due to single copy aneuploidies but cannot be reconciled with triploidy or higher levels of ploidy. Indeed, to our knowledge, individuals displaying three, four, or five alleles were never reported in population genetic studies (i.e. Ferreira et al., 2012; Kuhls et al., 2013; Mahnaz et al., 2011; Rougeron et al., 2011a, 2011b, 2009; Segatto et al., 2012). This means that such profiles may be very rare at best, or even absent. One explanation, that will need to be explored in the future, could be a transient state of aneuploidy in *Leishmania*, as already proposed for other organisms such as yeasts (Berman, 2016). This would alter DNA quantities but not the intra-individual polymorphism. This hypothesis is in agreement with the ability of *Leishmania* parasites to undergo gene amplification at a genome-wide scale to offset the absence of regulated transcriptional control in these parasites and probably to adapt to a changing environment (Downing et al., 2011; Ubeda et al., 2014). Finally, another explanation to consider lays on technical bias of PCR resulting in the amplification of only one or two alleles (Lachaud et al., 2014).

3. Evidences of genetic recombination in *Leishmania* parasites

Currently, the existence of genetic exchange is not questioned anymore for *Leishmania* even in the PCE model. Indeed, it is supported by at least four lines of evidence. The first line of evidence lays on the direct observation of nuclear fusion in *Leishmania* parasites, with a video depicting spontaneous fusion between two promastigotes (corresponding to the vector stage) of L. *tropica* and a quantitative demonstration through microspectrophotometry of nuclear fusion in the intracellular amastigote form within the mammalian host (Lanotte and Rioux, 1990; Kreutzer et al., 1994). These studies strongly support the existence of sex in *Leishmania* parasites, even if the location of sexual events is still not fully determined. The second line of evidence is based on the demonstration of hybrid isolates between and within *Leishmania* species. The existence of interspecific hybrids among *Leishmania* parasites has been known for decades (Bañuls et al., 1999, 1997; Belli et al., 1994; Cortes et al., 2012; Dujardin et al., 1995; Hide et al., 2007; Kelly et al., 1991; Ravel et al., 2006; Rogers et al., 2014) and has been recently confirmed experimentally in sand flies by the production of hybrids from laboratory crosses of *Leishmania major*, *Leishmania infantum* and *Leishmania donovani* (Akopyants et al., 2009; Sadlova et al., 2011; Calvo-Álvarez et al., 2014). The third line of evidence of genetic recombination is the identification in the genome of *Leishmania* parasites of some conserved meiotic orthologous genes (Ramesh et al., 2005). Even if the meiotic genes do not obligatory suggest the existence of sexual recombination or its frequency in natural populations, they should be considered as clues. Finally, the fourth line of evidence is based on the high homozygosity observed in population genetic studies of different *Leishmania* species (Adaui et al., 2011; Al-Jawabreh et al., 2008; Ferreira et al., 2012; Gelanew et al., 2010; Kuhls et al., 2008; Rougeron et al., 2011a, 2011b, 2009; Schwenkenbecher et al., 2006; Segatto et

Please cite this article as: Rougeron, V., et al., Reproduction in *Leishmania*: A focus on genetic exchange, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.10.013