PEARLS

Does the common sexually transmitted parasite *Trichomonas vaginalis* have sex?

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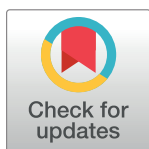
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Introduction

The parasite *Trichomonas vaginalis* is a haploid, flagellated, eukaryotic microbe that adheres to the human urogenital tract and causes the most common sexually transmitted parasitic infection, trichomoniasis ("trich"), with around 250 million new cases reported annually worldwide and about 7 million cases in the United States [1]. Previously considered a female "nuisance disease," *T. vaginalis* has now been associated with adverse pregnancy outcomes such as preterm delivery, low birth weight, increased risk of HIV infection, and cervical and prostate cancers [2]. In contrast to other parasitic protists that can encyst (e.g., *Giardia*), *T. vaginalis* appears to have only one morphological form, a sexually transmitted, mitotically dividing trophozoite. Besides its important role as the causative agent of trich, the parasite is of interest due to its unusually large genome size (around 160 Mb), of which 65% is made up of families of transposable elements (TEs), members of which are highly similar to each other [3]. The nature and size of the *T. vaginalis* genome raises questions about its evolution and, in particular, how this supposedly asexually reproducing organism survives the deleterious effects of so many active TE families. Indeed, several lines of evidence described below suggest that *T. vaginalis* may engage in genetic exchange or has done so in its recent evolutionary past. Elucidating a possible sexual cycle in *T. vaginalis* is crucial not only for learning about its biology and parasitism (e.g., virulence and spread of drug resistance), but also to provide generalizable models for the evolution of sex in other parasites.

What is sex and why is it important for parasitism?

There has been a debate about sexual cycles in eukaryotic microbes for many years. Sexual reproduction is a process by which specialized reproductive cells fuse, contributing genetic information to produce unique progeny. It is considered a major source of genetic diversity in a population and thus advantageous because it accelerates adaptation to fluctuating environments or purges deleterious mutations. There is a cost to sex, however, such as the disruption of well-adapted combinations of alleles. In fact, asexual reproduction is predicted to be advantageous as a short-term evolutionary strategy under many conditions. In an asexual (clonal) population, all members of the population carry the same genetic information, and only mutations, horizontal gene transfer, or genome rearrangements contribute to genetic variation. Only a few fungi are thought to be truly asexual. Cryptic sex, on the other hand, including parasexual or unisexual reproduction, is a common reproductive strategy in fungi (e.g., the *Candida* species complex) and some parasitic protists [4]. In a parasexual cycle, two cells and their nuclei fuse followed by chromosome loss, resulting in cells that can vary in their final ploidy [5]. Alternatively, unisexual reproduction introduces more limited genetic diversity

through mother–daughter cell–cell fusion or "endoreplication" and has been found in parasites such as *Giardia intestinalis* and *Leishmania* [6].

The success of parasites and the epidemiology of the diseases they cause is directly associated with their capacity to produce genetically variable infections. Sexually reproducing parasites create genetic variation through recombination (by merging genetic material from different parasites), thereby creating new combinations of genes that the parasite can use to overcome the host immune system or to develop drug resistance [7]. Thus, identifying the mode of reproduction of a parasite is important to determine how it will spread and how to treat it.

Population genetic evidence for sex in *T. vaginalis*

The genetic diversity of a parasite population and linkage disequilibrium (LD), the nonrandom segregation of alleles at different loci in a population, represent powerful metrics by which to evaluate evidence for sexual reproduction of an organism. Population genetics theory predicts that clonally reproducing organisms show low genetic diversity and high LD [8], whereas sexually reproducing organisms show high genetic diversity, population structuring, and independent segregation of alleles (linkage equilibrium) as a result of recombination. Several studies using a variety of genetic markers, including multi-locus strain typing (MLST) [9], microsatellites [10, 11], TE insertion polymorphisms [12], and single nucleotide polymorphisms (SNPs) [13, 14] have been used on global sets of *T. vaginalis* isolates to determine whether the parasite follows population genetic trends consistent with asexual reproduction. These studies have revealed high genetic diversity of *T. vaginalis* parasites and the presence of two global parasite subpopulations (Fig 1). Moreover, LD has been identified both within genes [10] and across the *T. vaginalis* genome (LD decay within 5 kb [13]), a pattern representative of frequently recombining organisms consistent with sexual reproduction. The rate of decay in LD is also a good indicator of recombination rates in the population. Our studies demonstrated faster LD decay and thus a higher recombination rate in one subpopulation over the other [10, 13]. In addition, isolates harboring alleles from both subpopulations have been identified (Fig 1), which may represent recombinant parasites between the two subpopulations and suggest *T. vaginalis* admixture (interbreeding between two isolated populations within a species). Thus, *T. vaginalis* population genetics strongly supports the ability of the parasite to undergo some form of genetic exchange or suggests the parasite could do so at some stage during its evolutionary past.

Molecular genetic evidence for sex in *T. vaginalis*

What are the lines of molecular genetic evidence for a sexual cycle in *T. vaginalis*? Malik et al. [15] mined the genome sequence to identify a nearly full complement of meiosis genes (27 of 29) in the *T. vaginalis* genome, suggesting that the parasite may be equipped to perform meiotic recombination or a similar parasexual process by using its meiotic gene homologs. Moreover, eight genes specific for meiosis in model organisms and known to exist mostly in sexually reproducing species were also present in *T. vaginalis*. Potential morphological evidence for recombination has also been described, such as multinucleated cells in *T. vaginalis* and "budding" in other species of trichomonads (indicating polyploidy and the potential for recombination [16]). We evaluated expression of the eight meiosis-specific genes (Spo11, Hop1, Hop2, Mnd1, Dmc1, Mer3, Msh4, Msh5) in *T. vaginalis* using RNA-seq from the reference *T. vaginalis* strain G3 [13]. None of the eight genes contains stop codons or nonsense mutations, and six of them were transcribed at levels above the average expression of all genes in *T. vaginalis*, suggesting meiosis to be an active process (Fig 1). Although the presence and
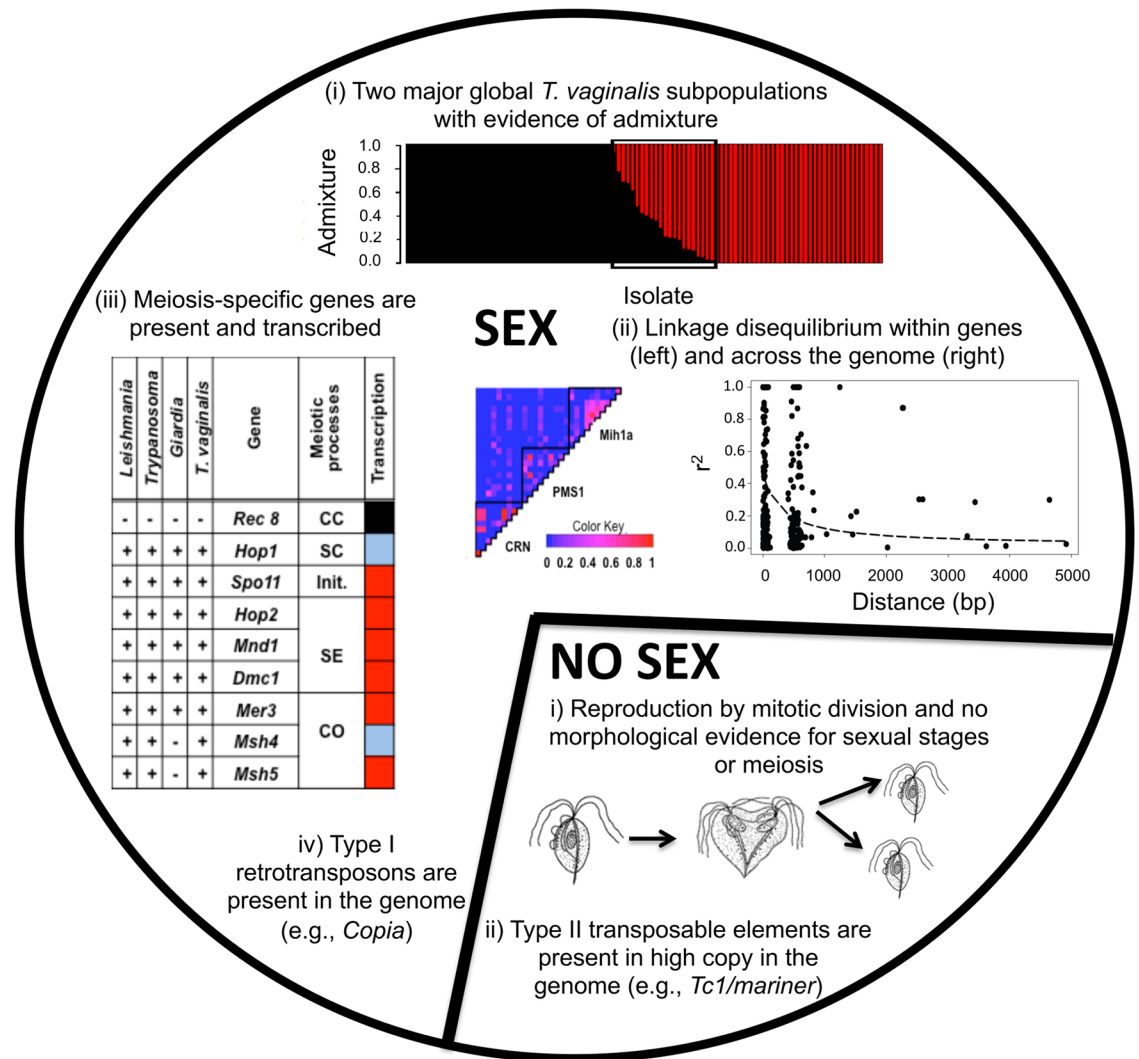
Fig 1. Summary of evidence suggesting sexual or asexual reproduction in *T. vaginalis*. The large slice (SEX) presents evidence for sexual reproduction: (i) Population admixture plot based on analysis of 3,923 SNP markers in 102 global *T. vaginalis* isolates (data from [13]). Each column represents a single *T. vaginalis* isolate and shows the proportion of its genotype as a part of each of two subpopulations (black and red). Isolates containing both black and red genotypes represent potential recombinants between the two subpopulations. (ii) LD decays fast within genes [10], as well as across the genome [13]. A heat map represents the degree of LD between 49 SNPs in three single-copy genes, with r^2 (the standardized measure of LD between pairs of SNPs) colored according to low LD (0; blue) and high LD (1; red); black lines indicate gene boundaries. The graph shows LD decay (r^2, y-axis) calculated over 5-kb intervals (distance, x-axis) in 2,837 SNPs from 872 *T. vaginalis* contigs. Each point represents the average LD between two SNPs that are 5 kb apart. (iii) Homologs of nine major meiosis-specific genes are present in *T. vaginalis* and other sexually reproducing protozoan parasites [15]. Transcription of eight of these genes (excluding Rec8 [black square]) is detectable at levels above (red squares) or below (blue squares) the average expression of all *T. vaginalis* genes (RNA next generation sequencing [RNA-seq] data from [13]). (iv) The presence of Type I retro TEs (e.g., Copia, a family of long terminal repeat elements that move by means of an RNA intermediate, common in animals, fungi, protista, and plants), suggests sexual recombination in *T. vaginalis*. The small slice (NO SEX) presents evidence for asexual reproduction: (i) No visible sexual stages have been identified for *T. vaginalis* under any conditions tested. (ii) A high abundance of extremely similar Type II TEs (e.g., Tc1/mariner, a family of transposons found throughout metazoa that use a cut-and-paste mechanism to transpose) implies their accumulation due to a lack of sexual reproduction. CC, cohesin complex; CO, crossover; Init., initiation of double-strand break; LD, linkage disequilibrium; SC, synaptonemal complex; SE, strand exchange; SNPs, single nucleotide polymorphisms.

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transcription of meiosis-specific genes suggests the parasite has a sexual cycle, other parasitic protists (e.g., *G. intestinalis*, *Trypanosoma cruzi*) also contain meiosis genes but undergo a cryptic sexual cycle [17] or unisexual reproduction [6]. Further studies in *T. vaginalis* will be needed to learn about the function of the meiosis-specific genes.

Presence of retrotransposons as evidence for sex in *T. vaginalis*

TEs are ubiquitous and present in many living organisms, and their type, diversity, and frequency of occurrence in a genome are some predictors of the type of reproduction. Asexual organisms frequently lack Class I TEs (also called retrotransposons, derived from RNA), while organisms that undergo a sexual cycle can contain Class II (DNA-derived) TEs as well as Class I TEs [18]. The *T. vaginalis* genome is unique among parasitic protists because it contains 30,000–40,000 Class I and II TEs in more than 50 different families, with TEs making up close to 40% of the genome. In addition, there are far fewer Type I than Type II TEs, and members of each family show very little genetic diversity [3]. Studies have shown that members of the Type II *Tc1/mariner* family are active [19], show insertion-site polymorphisms among different strains, and exhibit reduced expression of *T. vaginalis* genes in close proximity to a *mariner* insertion [12]. Thus, the deleterious effects of active and transposing TEs in the *T. vaginalis* haploid genome have the potential to be exceptionally high, especially if the parasite is asexual, and could potentially lead to its extinction. While *T. vaginalis* has most likely developed a mechanism to mitigate the deleterious effects of TEs, it seems most likely that stable TE copy numbers in *T. vaginalis* are maintained through the interplay of recombination, sexual reproduction, and natural selection, as has been hypothesized [20].

Sexual reproduction is highly common among eukaryotes, and many eukaryotic microbial pathogens have recently been found to have extant cryptic sexual cycles, enabling them to increase genetic diversity, purge deleterious mutations, and be successful in the face of host immunity or drug pressure. While we know that *T. vaginalis* is transmitted during sexual contact, we don't know whether the parasite itself has sex. Here we have summarized some of the recent advances in *T. vaginalis* biology that provide compelling evidence that the parasite has an active sexual cycle—possibly cryptic—or had sex recently in its evolutionary past.

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