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Giardia Sex? Yes, but how and how much?

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Although *Giardia* is of practical importance as a pathogen and has theoretical importance in evolutionary biology, it is not known whether it ever reproduces sexually. Several recent papers have shed light on this problem, without completely solving it. One paper shows that nuclei in the encysted organism can temporarily fuse and exchange genes; this may explain the genetic similarity of the two nuclei in a cell. Another paper demonstrates that *Giardia* does undergo sexual reproduction with outcrossing. However, we still do not know whether this involves a meiotic or a parasexual cycle, when it occurs, or how common it is.

Giardia is an important pathogen with interesting genetics

Members of the genus *Giardia* are unicellular flagellated protists and intestinal parasites in humans and other mammals, birds and amphibians. The human pathogens *Giardia duodenalis* and *G. enterica* are major water-borne agents of diarrhea and serious health concerns in the developing world, especially for children. *G. duodenalis* is one of six species of *Giardia* defined by morphology, but genotype data divide the human pathogens into two genotypic assemblages, A and B, which a recent review [1] suggests should be called *G. duodenalis* and *G. enterica,* respectively. Hereafter, *Giardia* will refer to *G. duodenalis,* unless otherwise specified. The genus *Giardia* belongs to a group of protists known as Diplomonads because of their two nuclei and eight flagellae. The diplomonads were formerly placed at the base of the eukaryotic tree of life, but this is not consistently supported by recent phylogenies-based robust analytical methods, nor by the large numbers of genes [2–5]. The life cycle has two stages (Figure 1) [6]. The flagellated trophozoites inhabit the intestine of the host, attaching to the intestinal lining by means of the ventral disk. The trophozoites reproduce by binary fission, giving rise to more trophozoites, or form cysts that are excreted into the environment; if these are consumed by a host animal, they excyst in the intestine to liberate two trophozoites.

Giardia is of interest because of its medical importance, but also because of some remarkable features of its biology and genetics [7]. The *Giardia* genome is distributed over the haploid set of 5 chromosomes [8,9]. Trophozoites have two diploid nuclei [6,10,11] but aneuploidy has been detected [12], presumably resulting from mitotic nondisjunction. The small size of *Giardia* cells, nuclei, and chromosomes makes cytogenetic and cytological studies difficult, and some features of their reproduction have yet to be resolved. Even the process of cytokinesis in trophozoites remains controversial, with different investigators reporting different patterns of cell division [11,13–18].

Until recently, it has been unclear what role, if any, sexual reproduction plays in the life of *Giardia*. For the purpose of this review, 'sexual reproduction' and 'sex' are broadly defined as any process in which chromosomes from two cells, or two nuclei in the same cell, are combined in the same nucleus and undergo recombination to produce new genotypes, i.e. new combinations of alleles. The consequences of sex differ, depending on whether the recombining chromosomes come from the same cell or clone (selfing) or from different cells (outcrossing). In eukaryotes, two modes of sex are known, meiotic sex and parasexual cycles (Box 1). Here, I consider two recent papers and place them in the context of the field: one presents genetic evidence that *Giardia* undergoes some form of sex involving

Glossary

Aneuploidy: aneuploid cells or nuclei have different numbers of different chromosomes, usually as a result of nondisjunction. A well-known example is Down's syndrome in humans in which affected individuals have three copies of chromosome 21, but two copies of all other chromosomes.
Crossing-over: the type of recombination in which chromosomes exchange segments resulting in reciprocal reombinant genotypes. For example, in the heterozygote *Ab*/*aB* a crossover between the *a* and *b* genes would produce the recombinant chronosomes *AB* and *ab*.
Gene conversion: the type of recombination in which a gene in a heterozygote of genotype *Aa* is converted from one allele to the other, resulting in a homozygote *AA* or *aa*.
Horizontal gene transfer (also called 'lateral gene transfer'): rare transfers of DNA segments, which may include genes, between individuals belonging to different species and by mechanisms other than meiotic or parasexual cycles.
Mating types: many protists and fungi exist in two or more mating types, such that a cell can only mate with members of a different mating type. Unlike male and female sexes, different mating types are usually not distinguishable by morphology.
Meiotic recombination: recombination that takes place during meiosis.
Mitotic recombination: recombination that takes place in cells that are dividing only by mitosis. A mitotic crossover causes all loci distal to the crossover to become homozygous. Mitotic gene conversion makes individual genes or short segments of chromosomes homozygous.
Nondisjunction: the failure of homologous chromosomes to segregate properly during the first meiotic division, or the failure of sister chromatids to segregate properly during the second meiotic division or during mitosis. Nondisjunction produces aneuploid cells.
Ploidy: the ploidy of a cell is the number of copies of a complete genome in a cell or nucleus. Haploid means 1 copy, diploid means 2, tetraploid means 4.
Recombination: the production of new combinations of alleles, or genotypes, by independent assortment of different chromosomes, by crossing-over, or by gene conversion. Homologous chromosomes undergo crossing-over and gene conversion with high frequency during meiosis, but these forms of recombination can also occur with low frequency in any cell.

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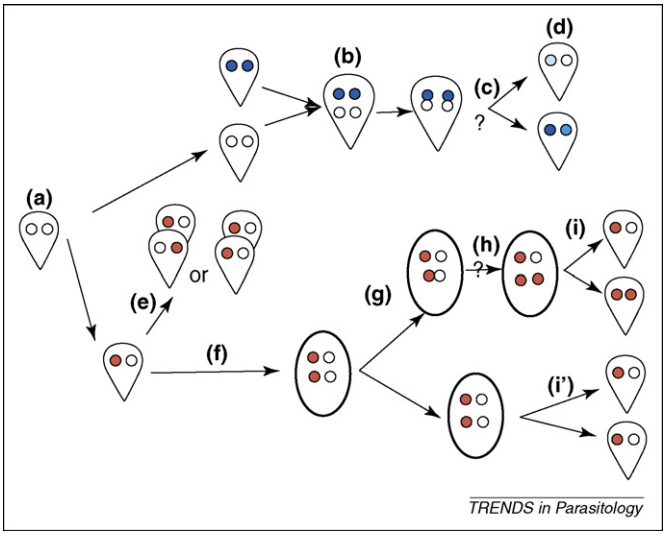

Figure 1. *Giardia* life cycle. **(a)**. The two nuclei of the trophozoite are each approximately diploid, with the 2C amount of DNA in G1 (before DNA replication) and 4C in G2 (after DNA replication), where C is the amount of DNA in one copy of the genome. **(b)**. During outcrossing, trophozoites must fuse and their nuclei must exchange chromosomes to allow recombination; in eukaryotes this usually involves nuclear fusion as shown here. **(c)**. The fused nuclei must divide by meiosis or mitosis to allow chromosome segregation. This is followed by cytokinesis to produce daughter cells **(d)** with recombinant genomes indicated by varying shades of blue. **(e)**. When a trophozoite divides, the four daughter nuclei are partitioned equationally so that each daughter cell receives on one division product of each nucleus as shown by the inheritance of plasmids (red). Reports differ as to whether the nuclei retain the orientation they had in the parent cell or are switched right to left; both cases are shown **(f)**. Trophozoites in G2 undergo encystation. Mature cysts have 4 nuclei, each with 4 copies of the genome or 4C amount of DNA. **(g)**. In some cysts, two nuclei fuse, exchange plasmid markers and probably chromosomes as well, and **(h)** separate by an unknown process. **(i)**. Upon excystation the trophozoite undergoes cytokinesis without DNA replication to produce two cells, each with two diploid nuclei [6].

outcrossing [19], whereas the other paper [20] provides physical evidence for the exchange of plasmids, and presumably genes, between two nuclei in the same cell.

Is *Giardia* having sex?

Why we should care

The origin and evolution of sexual and asexual reproduction in eukaryotes is a major conundrum in evolutionary biology. It is of more than academic interest to parasitologists because whether a parasite reproduces sexually (and, if so, when, how and how often) has a significant impact on the epidemiology and treatment of parasitic diseases. In an asexual parasite, only new mutations, gene or chromosome rearrangements, or exceptionally rare horizontal gene transfers can provide genotypes that confer resistance to antibiotics or host immune response. A sexual organism has all of these mechanisms for producing new genotypes, plus highly efficient recombination during meiosis and the opportunity to combine alleles from genetically different individuals during outcrossing. Thus, the adaptation of a parasite to host defenses or to a new host is greatly facilitated by sexual reproduction, especially with outcrossing. When tracking the course of an epidemic, it is important to know how much variation one would expect to see among the causal agents, and how they differ from other similar organisms.

Sex in Giardia is rare, furtive or cryptic

To date, no sexual reproduction has been directly observed in *Giardia*. No one has documented two trophozoites fusing, as would be required if there is outcrossing. Nuclear fusion has not been seen in trophozoites, as would be expected if they regularly underwent self-fertilization. One could imagine that sexual reproduction occurs in cysts, but cysts always seem to be formed by single trophozoites, indicating that, if there is sex in cysts, it must be selfing. However, the failure to detect these possible signs of sexual reproduction is not conclusive evidence for exclusively asexual reproduction, especially in protists. *Giardia* could be having sex that is difficult to detect because it is rare, furtive (done under conditions where we are unlikely to see it) or cryptic (it can be directly observed but is not recognizable). Rare sex can be detected only by examining very large numbers of individuals, but this is difficult when all observations must be done at high magnification. Furtive sex is especially likely in parasites that reproduce while hidden in the intestine and are covered with an opaque cyst wall when outside the cell wall. Cryptic sex must be considered a possibility in any protist for which there are no close sexual relatives that could give us a hint of what sexual reproduction would look like.

This is very different from some asexual animals and plants in which sex is not cryptic. In these organisms, we are reasonably certain that sex is not furtive because we know enough about the organisms in nature to be able to observe them in all of the situations in which they live. Moreover, if we examine a sufficiently large number of individuals without finding males or hermaphrodites with sperm, we can conclude with a reasonable certainty that the frequency of sexual individuals is too low to allow for effective sex with outcrossing. But, in *Giardia,* our failure to observe sexual reproduction is only weak evidence that reproduction is exclusively asexual, and so we must rely on indirect evidence.

Indirect tests for sexual reproduction in Giardia

In the absence of direct observations, a variety of kinds of indirect evidence have been used to detect sexual reproduction. Historically, this began with the application of

Box 1. Contrasting sex with meiosis and parasexual cycles

Traditionally, *sexual reproduction* refers to meiosis in a diploid cell producing haploid cells (gametes), which subsequently fuse to recreate a diploid cell. Most sexual eukaryotes have life cycles that include varying numbers of mitotic divisions of the haploid cells, diploid cells, or both.

Another form of sexual reproduction, called the parasexual cycle, was discovered in fungi. This cycle begins with the fusion of two cells or hyphae to produce a heterokaryon with nuclei of two different genotypes, e.g. *Ab* and *aB* where the *a* and *b* loci are on different chromosomes. These nuclei can fuse to produce a diploid heterozygous nucleus. Mitotic recombination between genes on the same chromosome occurs in the diploid nucleus. Also, the diploid nucleus is unstable and undergoes haploidization, randomly losing one or another chromosome by nondisjunction. Thus, a diploid nucleus with chromosome pairs *Ab* and *aB* will produce haploid nuclei with the parental genotypes *Ab* and *aB* as well as recombinant genotypes *AB* and *ab* in approximately equal numbers. This is the equivalent of independent assortment of unlinked genes in meiosis, and it is very difficult to distinguish the outcome of a parasexual cross from that of a meiotic sexual cross.

Parasex is probably common in fungi, and could occur in many apparently asexual protists.

classical population-genetic theory, which showed that genotype frequencies in randomly mating sexual organisms will be in Hardy–Weinberg equilibrium at a single locus, so long as allele frequencies are constant. Moreover, genotype frequencies at two or more loci will be in linkage equilibrium if they recombine. Tests for equilibrium applied to isozyme data from *Giardia* showed that these organisms are either asexual or strongly inbreeding [19]. In either case, the genotypes of the progeny of a single cell are identical to each other and to the mother cell, barring mutation and mitotic recombination; hence, asexual reproduction and sexual reproduction with strong inbreeding are both referred to as 'clonal.' However, tests for deviations from equilibrium frequencies are not very sensitive to violations of the assumptions, and can be positively misleading if there is selection, drift, and interactions between different genes. Moreover, electrophoretic variants represent a rather 'blunt instrument' for studying genetic variation. Since those studies were carried out, DNA sequencing has become routine, along with methods for using the more informative sequence data to detect outcrossing and recombination.

At the molecular level, a related approach is to look for the Meselson effect, i.e. unexpectedly high synonymous sequence differences between alleles of a protein-coding gene. In outcrossing sexual organisms, the two alleles of a gene in a diploid individual are drawn approximately randomly from the population at large; in an asexual organism, the two alleles in any individual are effectively contained together and inherited together in the parent of that individual and so on back to the first asexual ancestor of the species. Consequently, the two alleles in an asexual lineage should accumulate different mutations. Selection will either eliminate or fix the mutations that affect the phenotype but neutral mutations are free to accumulate indefinitely. Thus far, the Meselson effect has been detected only in the bdelloid rotifers [20], where it is strong evidence for ancient asexuality. In other putatively asexual organisms, including *Giardia* [21–23], the sequence difference between alleles in an individual or a nucleus is similar to that seen in sexual organisms, a few percent or less. Unfortunately, this is not strong evidence for sex, despite assertions to the contrary. Two mechanisms could reduce or eliminate heterozygosity periodically during asexual reproduction of the trophozoites [24,25]. One is mitotic recombination, including both crossing-over and gene conversion. The other is occasional ploidy cycles, in which one of the two chromosomes is lost by nondisjunction, and subsequently the other chromosome is duplicated by a second nondisjunction event, making the nucleus homozygous.

The presence of two diploid nuclei in *Giardia* lends an additional dimension to the problem. If *Giardia* is truly asexual, one would expect that the two nuclei would acquire and accumulate mutations independently. As a result, their genes would gradually accumulate different synonymous mutations and diverge in sequence, even if the two homologous chromosomes in each nucleus are kept similar by mitotic recombination or ploidy cycles. But the total heterozygosity in *Giardia*, including both differences between alleles within a nucleus and differences between nuclei, is less than 0.01%, which means that the two nuclei are kept similar by sex reproduction or some other mechanism. One possibility is that, when cells divide, the nuclei are partitioned reductionally, with one daughter cell receiving the two products of mitosis in one of the two nuclei in the parent cell, and the other daughter receiving the two products of meiosis from the other parental nucleus. Thus, the two nuclei in each daughter will be genetically identical. To distinguish between these two alternatives, *Giardia* were transfected with multi-copy plasmids that could be detected by fluorescence *in situ* hybridization [11,14,17]. After transfection, the plasmid was found in only one nucleus in each cell, and this pattern was maintained even after many generations of division. This shows that nuclei are partitioned equationally, such that each daughter cell receives one copy of each parental nucleus. Reductional partitioning would produce cells with label in both nuclei. It would also produce daughter cells with label in neither nucleus, but these would have been selected against by drug pressure and, in any event, cannot be distinguished from cells that failed to be stained.

Another indirect test for meiotic sex makes use of the completely sequenced *Giadia duodenalis* genome. Ramesh *et al.* [26] screened the genome for homologs of 17 genes implicated in meiosis in other eukaryotes; knockout experiments showed that seven of these genes are specific for meiosis in yeast. Of these seven genes, homologs of 55 were found in *Giardia* and showed clear signs of being functional, leading to the conclusion that *Giardia* undergoes meiotic sex. There are several problems with this conclusion [25]. One is that the selection of meiosis genes did not focus on those whose protein products determine the difference between the segregation of homologous chromosomes that characterizes the first meiotic division and the segregation of sister chromatids, which occurs in mitosis and the second meiotic division. Another major problem is that the specificity of these genes for meiosis was determined in organisms that are very distantly related and biologically very different from *Giardia*. In fact, many (if not all) of the genes have potential or known functions in other processes. For example, a number of pathogenic eukaryotic protists, including *Candida albicans* and *Trypanosoma cruzi*, retain 'meiosis-specific' genes [26], but have a parasexual cycle rather than meiotic sex (see Box 1 for a comparison of meiotic sex and parasex). Recently, one such gene (*SPO11*) was shown to be required for recombination during the parasexual cycle in *Candida* [27]. This is not surprising, given the general observation that evolution is constantly re-using old genes for new purposes.

Giardia nuclei fuse and exchange genes

Although the two nuclei in trophozoites have not been observed to fuse and exchange genes, Poxleitner *et al.* [28] recently showed that this does happen in cysts. Trophozoites in which one nucleus contained a multicopy plasmid were induced to undergo encystation and stained for the plasmid at intervals during the encystations and excystation process. Many cysts had plasmid in two of the four nuclei, but, in others, the plasmid was detected in three nuclei. These arose when one pair of non-sister nuclei

fused and shared plasmids, then divided again. The fusion of two nuclei was verified by electron microscopy. The authors [28] called the process of nuclear fusion and division 'diplomixis' and referred to it as a parasexual process. This is premature; they did not show that the nuclear fusion and separation is part of a canonical parasexual cycle involving mitotic recombination and haploidization. Importantly, no outcrossing was involved.

Poxleitner *et al*. hypothesized that the 'meiosis-specific' genes detected in *Giardia* by Ramesh *et al*. [26] might promote mitotic recombination between chromosomes in the fused nuclei. To test whether, and when, these genes are expressed, five putative meiosis genes fused to the GFP reporter were transfected into trophozoites. Three of these genes were expressed only in cysts (in all four nuclei), whereas two were expressed in trophozoites as well as cysts. The authors suggested that mitotic recombination in the fused nuclei could explain why the copies of each gene in the two nuclei remain similar to each other in spite of incurring different mutations. The division by which the fusion nucleus gives rise to two nuclei is probably a mitosis, because the fusion nucleus is an autopolyploid, with four sets of chromosomes from the same species. Consequently, if the divsion was a meiosis, the chromosomes would often segregate three or four to one pole instead of two to each pole.

Giardia has sex with outcrossing

As noted above, traditional population genetic analyses of isozyme data found no convincing evidence for sex with outcrossing in *Giardia*. However, two more powerful tests were recently used by Cooper *et al*. [29] to obtain convincing evidence for outcrossing and recombination in *Giardia duodenalis* (see also the commentary on this paper by Logsdon [30]). In order to maximize their chances of detecting sex with outcrossing, the authors chose isolates from the same putative species, genotype A2 (see Box 2). Furthermore, they used five isolates from four households in a location where *Giardia* infections are endemic, resulting in a high frequency of individuals being infected with two or more genotypes. They also sequenced the same region from isolate JH from the same putative species. From each isolate, they sequenced segments of chromosomes 3, 4, and 5, totaling more than 135 kbp, and containing 14 different protein-coding genes. The corresponding regions of isolate WB, which belongs to genotype A1 and has been completely sequenced, served as outgroups for rooting the trees.

Two methods were used to test for sex and recombination. The first method tests whether different genes have phylogenetic trees that are topologically different. It is assumed that, if two or more genes are perfectly linked, a phylogenetic tree showing the relationship of different isolates of one gene (or any segment of the genome) will be the same as the tree for all other genes or segments. To look for recombination between genes on different chromosomes, Cooper *et al*. [29] made trees of the complete sequences of each of the three genome segments. Bootstrapping was used to assess statistical significance of the topologies of the trees; all nodes had at least 78% bootstrap support. Conflicting phylogenetic signals, i.e. topological differences, were found for the sequenced regions on different chromosomes. For example, in the tree for chromosome 3, isolate 55 was part of a clade that included isolates 303, 305 and 246, whereas in the tree for the chromosome 4, it formed a clade with isolate 335. The conflicting phylogenies demonstrate recombination of genes on different chromosomes, which is almost certainly due to independent assortment of the chromosomes.

Independently, Teodorovic *et al*. [23] also detected recombination between three genes on different chromosomes using the same method, but a different set of isolates. Surprisingly, they also found evidence for exchange of genes between strains of groups A and B, which are believed to be different species [1]. This could be due to horizontal transfer rather than meiotic sex or parasex. Alternatively, it could mean that these groups are not distinct biological species, or are recently diverged species that can hybridize occasionally without completely losing their distinctiveness, as is notoriously the case with some plant species. It is also possible that the recombination occurred long ago, before groups A and B became completely reproductively isolated.

Cooper *et al*. [29] also tested for recombination between genes or segments of genes on the same chromosome using genomic analyses. A visual examination of the distribution of polymorphic sites showed a number of likely recombination breakpoints, which were verified by amplifying and sequencing across the breakpoints. In addition, Sawyer's runs test was used to detect long regions in which two sequences are more similar to each other than expected by chance. One or more such regions were found on each chromosome. Bootstrapped phylogenetic trees were constructed for each of these regions and confirmed that they showed conflicting phylogenies.

These papers clearly demonstrate that *Giardia* is having sex with outcrossing. Although this is a very important result, the data do not differentiate between meiotic sex and parasex. Even more importantly, they do not tell us

Box 2. Defining species before testing for recombination

Sexual reproduction is the exchange of genes among members of a species; the exchange of genes between different species is called horizontal transmission. In eukaryotes, these are very different processes with different mechanisms. In particular, horizontal transfer generally involves one or a few genes at a time. Unfortunately, it is common to assign individuals to species without indicating how one defines species. This causes confusion in the literature, makes it difficult to know how one should identify new isolates, and can result in comparing incomparable phenomena. For useful systematic work, it is necessary to use an appropriate species concept (a model of what species are) and criterion (an operational procedure for assigning individuals into species) [31]. For the purpose of testing for recombination, it would be most appropriate to use the general lineage species concept that defines species as independently evolving populations and lineages [32]. Thus, each evolutionary species is an independent arena for mutation, random drift, natural selection, and (in sexual organisms) recombination. Evolutionary species can be defined and detected in both asexual and sexual species, so populations of organisms can be divided into evolutionary species in advance of testing them for recombination. Unfortunately, the suggestion that *Giardia* can be divided into 11 different species (reviewed in Ref. [1]) is made without clear reference to any of the various explicit species concepts or criteria used in systematic biology.

how frequent the sexual reproduction is. Presumably the same mechanism could also result in selfing, which cannot be detected using these methods.

Conclusions

The recent paper by Poxleitner *et al*. [28] clearly demonstrates genetic exchange between two nuclei in the cysts of *Giardia*, whereas Cooper *et al*. [29] provides convincing evidence that *Giardia* does have sex with outcrossing. These papers confirm the occurrence of two different phenomena that can explain the remarkable sequence similarity of the two nuclei within a cell. But the mechanism of sexual reproduction, whether it is meiosis and fertilization or a parasexual cycle, remains cryptic. Neither do we know how frequent sex is, or how often it is selfing as opposed to outcrossing. Until these parameters are known, it is difficult to say how significant sexual reproduction might be for the evolution, pathogenicity, and epidemiology of this remarkable organism.

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References

1 Monis, P.T. *et al.* (2008) Variation in *Giardia*: towards a taxonomic revision of the genus. *Trends Parasitol.* 25, 93–100
2 Burki, F. *et al.* (2008) Phylogenomics reveals a new 'megagroup' including most photosynthetic eukaryotes. *Biol. Lett.* 4, 366–369
3 Dacks, J.B. *et al.* (2008) Implications of the new eukaryotic systematics for parasitologists. *Parasitol. Int.* 57, 97–104
4 Hampl, V. *et al.* (2009) Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic "supergroups". *Proc. Natl. Acad. Sci. U. S. A.* 106, 3859–3864
5 Yoon, H.S. *et al.* (2008) Broadly sampled multigene trees of eukaryotes. *BMC Evol. Biol.* 8, 14–26
6 Bernander, R. *et al.* (2001) Genome ploidy in different stages of the *Giardia lamblia* life cycle. *Cell. Microbiol.* 3, 55–62
7 Ortega-Pierres, G. *et al.* (2009) New tools provide further insights into *Giardia* and *Cryptosporidium* biology. *Trends Parasitol.* 25, 410–416
8 Adam, R.D. (2001) Biology of *Giardia lamblia*. *Clin. Microbiol. Rev.* 14, 447–475
9 Morrison, H.G. *et al.* (2007) Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. *Science* 317, 1921–1926
10 Kabnick, K.S. and Peattie, D.A. (1990) *In situ* analyses reveal that the two nuclei of *Giardia lamblia* are equivalent. *J. Cell Sci.* 95, 353–360
11 Yu, L.Z. *et al.* (2002) The two nuclei of *Giardia* each have complete copies of the genome and are partitioned equationally at cytokinesis. *Eukaryot. Cell* 1, 191–199
12 Tumova, P. *et al.* (2007) Cytogenetic evidence for diversity of two nuclei within a single diplomonad cell of *Giardia*. *Chromosoma* 116, 65–78
13 Filice, F.P. (1952) Studies on the cytology and life history of a *Giardia* from the laboratory rat. *Univ. Calif. Publ. Zool.* 57, 53–146
14 Ghosh, S. *et al.* (2001) How Giardia swim and divide. *Infect. Immun.* 69, 7866–7872
15 Solari, A.J. *et al.* (2003) A unique mechanism of nuclear division in *Giardia lamblia* involves components of the ventral disk and the nuclear envelope. *Biocell* 27, 329–346
16 Benchimol, M. (2004) Mitosis in *Giardia lamblia*: multiple modes of cytokinesis. *Protist* 155, 33–44
17 Sagolla, M.S. *et al.* (2006) Three-dimensional analysis of mitosis and cytokinesis in the binucleate parasite *Giardia intestinalis*. *J. Cell Sci.* 119, 4889–4900
18 Tumova, P. *et al.* (2007) Cell division of *Giardia intestinalis*: assembly and disassembly of the adhesive disc, and the cytokinesis. *Cell Motil. Cytoskeleton* 64, 288–298
19 Tibayrenc, M. *et al.* (1991) Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. *Proc. Natl. Acad. Sci. U. S. A.* 88, 5129–5133
20 Mark Welch, D. and Meselson, M. (2000) Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science* 288, 1211–1215
21 Baruch, A.C. *et al.* (1996) The molecular epidemiology of *Giardia lamblia*: a sequence-based approach. *J. Infect. Dis.* 174, 233–236
22 Prabhu, A. *et al.* (2006) Characterisation of the subtelomeric regions of *Giardia lamblia* genome isolate WBC6. *Int. J. Parasitol.* 37, 503–513
23 Teodorovic, S. *et al.* (2007) Unusually low levels of genetic variation among *Giardia lamblia* isolates. *Eukaryot. Cell* 6, 1421–1430
24 Birky, C.W., Jr (1996) Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics* 144, 427–437
25 Birky, C.W., Jr (2005) Sex: is *Giardia* doing it in the dark? *Curr. Biol.* 15 (2), R56–R58
26 Ramesh, M.A. *et al.* (2005) A pylogenomic inventory of meiotic genes: evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. *Curr. Biol.* 15, 185–191
27 Forche, A. *et al.* (2008) The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. *PLoS Biol.* 6, 1084–1097
28 Poxleitner, M.K. *et al.* (2008) Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*. *Science* 319, 1530–1533
29 Cooper, M.A. *et al.* (2007) Population genetics provides evidence for recombination in *Giardia*. *Curr. Biol.* 17, 1984–1988
30 Logsdon, J.M., Jr (2007) Evolutionary genetics: sex happens in *Giardia*. *Curr. Biol.* 18, R66–R68
31 Coyne, J.A. and Orr, H.A. (2004) *Speciation*, Sinauer Associates, Inc.
32 de Queiroz, K. (2007) Species concepts and species delimitation. *Syst. Biol.* 56, 879–886