9. Stuphorn, V., Taylor, T.L., and Schall, J.D. (2000). Performance monitoring by the supplementary eye field. Nature *408*, 857–860.
10. Thaler, D., Chen, Y., Nixon, P.D., Stern, C., and Passingham, R.E. (1995). The functions of the medial premotor cortex (sma) I. Simple learned movements. Exp. Brain Res. *102*, 445–460.
11. Forstmann, B., Brass, M., Koch, I., and von Cramon, D.Y. (2005). Internally generated and directly cued task sets: an investigation with fMRI. Neuropsychologia, in press.
12. Kennerley, S.W., Sakai, K., and Rushworth, M.F. (2004). Organization of action sequences and the role of the pre-SMA. J. Neurophysiol. *91*, 978–993.
13. Brass, M., and von Cramon, D.Y. (2002). The role of the frontal cortex in task preparation. Cereb. Cortex *12*, 908–914.
14. Shima, K., Mushiake, H., Saito, N., and Tanji, J. (1996). Role for cells in the presupplementary motor area in updating motor plans. Proc. Natl. Acad. Sci. USA *93*, 8694–8698.
15. Rushworth, M.F.S., Walton, M.E., Kennerley, S.W., and Bannerman, D.M. (2004). Action sets and decisions in the medial frontal cortex. Trends Cogn. Sci. *8*, 410–417.
16. Deiber, M.-P., Honda, M., Ibanez, V., Sadato, N., and Hallett, M. (1999). Mesial motor areas in self-initiated versus externally triggered movements with fMRI: effect of movement type and rate. J. Neurophysiol. *81*, 3065–3077.
17. Walton, M.E., Devlin, J.T., and Rushworth, M.F. (2004). Interactions between decision making and performance monitoring within prefrontal cortex. Nat. Neurosci. *7*, 1259–1265.
18. Fujii, N., Mushiake, H., and Tanji, J. (2002). Distribution of eye- and arm-movement-related neuronal activity in the SEF and in the SMA and Pre-SMA of monkeys. J. Neurophysiol. *87*, 2158–2166.
19. Johansen-Berg, H., Behrens, T.E., Robson, M.D., Drobnjak, I., Rushworth, M.F., Brady, J.M., Smith, S.M., Higham, D.J., and Matthews, P.M. (2004). Changes in connectivity profiles define functionally distinct regions in human medial frontal cortex. Proc. Natl. Acad. Sci. USA *101*, 13335–13340.
20. Grosbras, M.H., Lobel, E., Van de Moortele, P.F., LeBihan, D., and Berthoz, A. (1999). An anatomical landmark for the supplementary eye fields in human revealed with functional magnetic resonance imaging. Cereb. Cortex *9*, 705–711.

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Sex: Is *Giardia* Doing It in the Dark?

The protist *Giardia* has long been considered strictly asexual. Now genes specific for meiotic recombination have been found in the *Giardia* genome, but their consequences for genetics, epidemiology and evolution remain unknown.

C. William Birky Jr.

Sexual reproduction is so nearly ubiquitous among vertebrates and plants that we tend to forget that many eukaryotes appear to have dispensed with meiotic sex altogether [1]. These range from parthenogenetic lizards to a wide array of eukaryotic protists, many of which are important parasites like *Giardia*. It is important to know if these organisms are truly asexual, because the answer will help determine when and how meiotic sex first evolved.

Early phylogenetic trees of eukaryotes based on sequences of the 18S ribosomal RNA gene suggested that the earliest branches are those leading to *Giardia* and some other species, such as *Euglena* and its relatives, that have been presumed to be asexual (Figure 1). If this is true, meiotic sex may have arisen after these organisms separated from the rest of the eukaryotes, and *Giardia* and its relatives may hold clues to the evolutionary origin of sex. However, recent studies using large numbers of protein sequences, or unique events such as gene fusions, to root the tree have tended to place the root on the branch connecting the opisthokonts and amoebozoa to the rest of the eukaryotes [2]. This tree would imply that meiotic sex arose in a common ancestor of all eukaryotes.

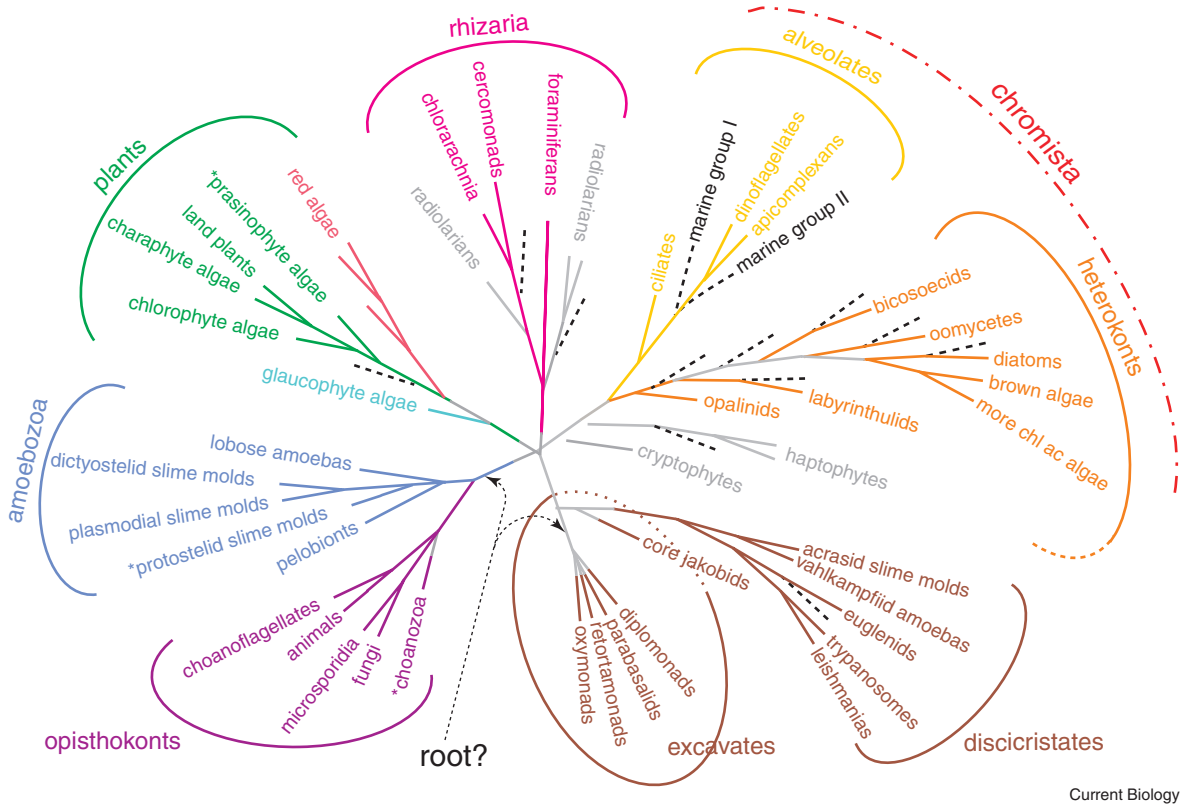
Another reason for wanting to know which organisms truly lack sex is that they can be used in comparative studies to identify the evolutionary advantages of sex, which hitherto have been limited mainly to animals, plants and viruses [3–6]. Finally, knowing whether or not parasites like *Giardia* are having sex will have implications for their epidemiology and treatment [7].

Our failure to observe meiosis or fertilization in an organism does not, however, mean that it never occurs. An apparently asexual organism might: be having sex so infrequently that it has not yet been observed; engage in furtive sex, under conditions in which we have not yet observed it; or have cryptic sex, readily observed but not easily recognized as sexual reproduction.

These problems are especially severe in protistan parasites such as *Giardia*, which grows and reproduces as tiny binucleated, flagellated trophozoites in the dark of animal intestines, and then forms a cyst with an opaque wall. Although all of the stages of the life cycle can be reproduced in the lab, their small size and requirement for anaerobic conditions have prevented direct observation of nuclear divisions.

When sexual reproduction cannot be observed directly, its effects can be detected using genetic markers. Unfortunately, most of these tests can show that sexual reproduction with outcrossing occurs, but cannot prove its absence. For example, observations of high levels of neutral sequence divergence between alleles — the Meselson effect — was used to demonstrate that bdelloid rotifers are anciently asexual [8–11]. Sequence heterozygosity is very low in *Giardia* [12], but the absence of the Meselson effect is not definitive evidence of sex, because allele sequences can be homogenized by mitotic recombination or by cycles of chromosome loss and duplication due to nondisjunction [13].

The advent of genomics suggested a new test for sex: screening complete genomes for



Current Biology

Figure 1. A phylogenetic tree of the eukaryotes.

Two possible positions of the root (common ancestor of all eukaryotes) are shown, but several others have not been excluded. (Tree reproduced with permission from [2], with some modifications by S. Baldauf.)

genes that function specifically in sexual reproduction. If most or all of these genes are absent, it is likely that the organism is truly asexual, but if they are present, the organism almost certainly has been engaging in meiosis recently. This approach was first used to show that the putatively asexual pathogenic yeast *Candida albicans* has a locus similar to the mating type locus of *Saccharomyces cerevisiae* [14]. But further studies did not distinguish whether mating is followed by a sexual or a parasexual cycle [15].

A paper [16] in this issue of *Current Biology* extends this approach to the detection of meiotic recombination genes in *Giardia lamblia* (synonym *G. intestinalis*). Ramesh *et al.* [16] identified 17 genes encoding homologues of known components of the 'core meiotic recombination machinery'; of those, seven have been shown to be meiosis-specific by gene-knockout and other experiments in *S. cerevisiae*.

DNA sequences of these genes and protein sequences of their products were used in BLAST and keyword searches of the draft genome sequences from the *Giardia* genome project database [17]. To verify that the genes constructed from fragments in the sequence database are present as complete genes in *Giardia*, primers designed from the gene sequences were used to amplify, clone and sequence the genes from genomic DNA. Finally, Ramesh *et al.* [16] made phylogenetic trees with the genes found in *Giardia* and their putative homologues in other eukaryotes, showing that the *Giardia* genes really are orthologs of their counterparts in sexual organisms.

All but four of the 17 meiotic recombination genes were found in *Giardia*. As controls, Ramesh *et al.* [16] searched for the same genes in databases of animals, plants and fungi known to have meiotic sex; each of the four genes not found in *Giardia* is also missing from one or more sexual organisms. None of the genes contains a mutation that would interrupt its open reading frame. This shows that the genes are functional, or have been until recently, because such mutations would accumulate in nonfunctional genes. Ramesh *et al.* [16] have thus provided convincing evidence that *Giardia* contains functional homologues of genes that function specifically in meiotic recombination in sexual eukaryotes.

Does this mean that *Giardia* undergoes canonical meiosis? Probably, but not necessarily, because meiosis involves more than just pairing and recombination of homologous chromosomes. An elaborate machinery is required to ensure that homologous chromosomes separate and go to opposite poles while sister chromatids remain tightly bound in anaphase of the first meiotic division. This must be followed by a second

division in which sister chromatids separate.

The difference between the reductional first meiotic division and the equational second division (and the similarly equational mitotic divisions) depends on the protein products of a number of genes [18], none of which was investigated by Ramesh *et al*. [16]. Until at least a few of those genes are located, the possibility remains that the recombination genes are functional in an unusual meiosis, or perhaps a primitive precursor of meiosis. One can even imagine that the recombination between homologues is not followed by a reduction division at all.

Ramesh *et al*. [16] also found recombination genes in the genomes of several other organisms for which meiosis is unknown — the amoebozoan *Entamoeba*, the microsporidian *Encephalitozoa*, and the trypanosomatids *Leishmania major and L. donovoni* — or suspected from genetic grounds but not demonstrated cytologically — *Trypanosoma brucei* [19].

Even if a canonical meiosis is demonstrated in *Giardia*, we will still need to know how diploidy is restored. This could take place between nuclei from two different individuals (outcrossing) or from the same individual (selfing). The distinction is crucial, because outcrossing is far more effective in producing new recombinant genotypes and breaking down the linkage disequilibria that retard natural selection. Two subspecies of *Trypanosoma brucei* appear to differ in this respect [20].

Evidently, molecular methods can only take us so far. If genome searches show that an organism contains a set of genes sufficient to carry out the important stages of meiosis, we can use this result to locate the origin of meiosis on the eukaryotic tree. But before the organism can be used to study the evolutionary significance of sex, and before we can understand its population genetic structure and epidemiology, we need to know whether the organism is outcrossing. This will require a combination of genetic and cytogenetic studies, such as using transfected markers to track nuclei with fluorescent *in situ* hybridization, and to select recombinants. Population genetic analyses are required to determine the extent of outcrossing. Modern molecular genetic and optical methods should make all of this feasible.

References

1. Bell, G. (1982). The Masterpiece of Nature (London: Croom Helm).
2. Baldauf, S.L. (2003). The deep roots of eukaryotes. Science *300*, 1703–1706.
3. Chao, L. (1997). The advantage of sex in the RNA virus ϕ6. Genetics *147*, 953–959.
4. Cutter, A.D., and Payseur, B.A. (2003). Rates of deleterious mutation and the volution of sex in *Caenorhabditis.* J. Evol. Biol. *16*, 812–822.
5. Lively, C.M., and Jokela, J. (2002). Temporal and spatial distributions of parasites and sex in a freshwater snail. Evol. Ecol. Res. *4*, 219–226.
6. Mark Welch, D.B., and Meselson, M. (2001). Rates of nucleotide substitution in sexual and anciently asexual rotifers. Proc. Natl. Acad. Sci. USA *98*, 6720–6724.
7. Ayala, F.J. (1998). Is sex better? Parasites say 'no'. Proc. Natl. Acad. Sci. USA *95*, 3346–3348.
8. Mark Welch, D., and Meselson, M. (2000). Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. Science *288*, 1211–1215.
9. Mark Welch, J.L., Mark Welch, D.B., and Meselson, M. (2004). Cytogenetic evidence for asexual evolution of bdelloid rotifers. Proc. Nat. Acad. Sci. USA *101,* 1618-1621.
10. Mark Welch, D.B., Cummings, M.P., Hillis, D.M., and Meselson, M. (2005). When did gene copies in the asexual class Bdelloidea diverge? Proc. Natl. Acad. Sci. USA, in press.
11. Birky, C.W., Jr. (2004). Bdelloid rotifers revisited. Proc. Natl. Acad. Sci. USA *101*, 2651–2652.
12. Baruch, A.C., Isaac-Renton, J., and Adam, R.D. (1996). The molecular epidemiology of *Giardia lamblia*: a sequence-based approach. J. Infect. Dis. *174*, 233–236.
13. Birky, C.W., Jr. (1996). Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. Genetics *144*, 427–437.
14. Hull, C.M., and Johnson, A.D. (1999). Identification of a mating type-like locus in the asexual pathogenic yeast *Candida albicans.* Science *285*, 1271–1275.
15. Soll, D.R. (2004). Mating-type locus homozygosis, phenotypic switching and mating: a unique sequence of dependencies in *Candida albicans.* Bioessays *26*, 10–20.
16. Ramesh, M.A., Malik, S.-B., and Logsdon, J.M., Jr. (2004). A phylogenomic inventory of meiotic genes: evidence for sex in Giardia and an early eukaryotic origin of meiosis. Curr. Biol. this issue.
17. McArthur, A.G., Morrison, H.G., Nixon, J.E., Passamaneck, N.Q., Kim, U., Hinkle, G., Crocker, M.K., Holder, M.E., Farr, R., Reich, C.I., et al. (2000). The *Giardia* genome project database. FEMS Microbiol. Lett. *189*, 271–273.
18. Ragitsch, K.P., Petronczki, M., Javerzat, J.-P., Genier, S., Chwalla, B., Schleiffer, A., Tanaka, T.U., and Nasmyth, K. (2003). Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis I. Dev. Cell *4*, 535–548.
19. Gibson, W. (2001). Sex and evolution in trypanosomes. Int. J. Parasitol. *31*, 643–647.
20. MacLeod, A., Tweedie, A., Welburn, S.C., Maudlin, I., Turner, C.M.R., and Tait, A. (2000). Minisatellite marker analysis of *Trypanosoma brucei*: Reconciliation of clonal, panmictic, and epidemic population genetic structures. Proc. Natl. Acad. Sci. USA *97*, 13442–13447.

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Skin Cancer: Lights on Genome Lesions

Sunlight generates skin damage mainly by inducing DNA lesions in epidermal cells. The recent development of transgenic mice expressing specific photolyases has identified cyclobutane pyrimidine dimers as the major player in ultraviolet-induced damage, including skin cancer.

Keronninn M. Lima-Bessa and Carlos F.M. Menck

Dermatologists always recommend we should take care to avoid too much exposure to sunlight. Besides sunburn, which rapidly causes a red skin response and skin peeling, there is a clear risk of a late and more devastating effect: skin cancer, including malignant squamous cell carcinoma and melanoma. This advice is especially valuable because depletion of the stratospheric ozone layer has increased the incidence of ultraviolet (UV) wavelength,