Meiotic Genes in Colpodean Ciliates Support Secretive Sexuality

Micah Dunthorn[1,*], Rebecca A. Zufall[2], Jingyun Chi[1], Konrad Paszkiewicz[3], Karen Moore[3], and Frédéric Mahé[1,4]

[1]Department of Ecology, University of Kaiserslautern, Kaiserslautern, Germany

[2]Department of Biology and Biochemistry, University of Houston, Houston, TX

[3]Biosciences, University of Exeter, Exeter, United Kingdom

[4]CIRAD, UMR LSTM, Montpellier, France

*Corresponding author: E-mail: dunthorn@rhrk.uni-kl.de.

Abstract

The putatively asexual Colpodean ciliates potentially pose a problem to macro-organismic theories of evolution. They are extremely ancient (although asexuality is thought to hasten extinction), and yet there is one apparently derived sexual species (implying an unlikely regain of a complex trait). If macro-organismic theories of evolution also broadly apply to microbial eukaryotes, though, then most or all of the colpodean ciliates should merely be secretively sexual. Here we show using de novo genome sequencing, that colpodean ciliates have the meiotic genes required for sex and these genes are under functional constraint. Along with these genomic data, we argue that these ciliates are sexual given the cytological observations of both micronuclei and macronuclei within their cells, and the behavioral observations of brief fusions as if the cells were mating. The challenge that colpodean ciliates pose is therefore not to evolutionary theory, but to our ability to induce microbial eukaryotic sex in the laboratory.

Key words: asexuality, Colpodea, genome sequencing, gene inventory.

Introduction

There are many costs to sex (Lehtonen et al. 2012; Maynard Smith 1978), where there is a fusion of meiotic products from different individuals (Lehtonen and Kokko 2014; Normark et al. 2003). Sex is thought to be maintained in animals and plants because it allows, for example, quicker escapes from parasites and quicker adaptations to changing environments (Bell 1982; Hamilton 2001; Maynard Smith 1978). Although the broad distribution of sex within eukaryotes has not been completely explained (Hartfield and Keightley 2012), putative asexual macro-organisms have long intrigued evolutionary biologists because they are theoretical anomalies (Normark et al. 2003; Schön et al. 2009). By contrast, these theories have often been ignored in microbial eukaryotes, where many species and higher clades are considered to be asexual (Fenchel and Finlay 2006; Foissner et al. 2011; Schlegel and Meisterfeld 2003; Sonneborn 1957). One of the largest putative asexual microbial eukaryotic groups are the Colpodea ciliates (Foissner 1993).

Ciliates have dimorphic nuclei within each cell: micronuclei, which are transcriptionally inactive during vegetative growth; and macronuclei, which produce all mRNA required for protein synthesis (Lynn 2008). Ciliate sex—called conjugation—occurs by brief cell fusion of complementary mating types and mutual exchange of haploid products of micronuclear meiotic division (Bell 1988; Phadke and Zufall 2009; Zufall 2016). Although sex has been widely observed in almost all ciliate groups, some ciliates are asexual because they lack micronuclei (Zufall 2016). Many of these amicronucleate strains are closely related to known sexual ciliates, but some are potentially old (Doerder 2014).

The putative asexual colpodean ciliates form a clade of over 200 described species that all have micronuclei and macronuclei. They have baroque morphologies in their somatic and oral regions, and are primarily found in terrestrial environments (Foissner 1993; Lynn 2008). Colpodeans have been consistent in their lack of conjugation in the laboratory even

Fig. 1.—Two *Bursaria truncatella* cells during conjugation (=ciliate sex). Out of over 200 described species of colpodean ciliates, only *B. truncatella* has directly been observed to have sex. Copyright © Charles Krebs.

after >40 years of observations, with the exception of only one species: *Bursaria truncatella* (Foissner 1993; fig. 1). Pseudoconjugation—where cells from clonal lines briefly fuse as if to mate—has been observed in other colpodeans while living in petri dishes, but there is no apparent exchange of haploid meiotic products (Foissner 1993).

Because of macro-organismic theories about the maintenance of sex, two lines of evolutionary theory were previously used to argue that all, or almost all, of the colpodeans are secretively sexual (Dunthorn and Katz 2010). First, the colpodeans may have originated before the Phanerozoic (Wright and Lynn 1997), but ancient asexual lineages are extremely rare (Martens et al. 2003; Maynard Smith 1978; Normark et al. 2003). Second, *B. truncatella* is in a phylogenetically derived position (Dunthorn et al. 2011, 2008) implying a loss and then regain of sex, but reversing the loss of complex traits is thought unlikely to occur (Collin and Miglietta 2008; Gould 1970; Teotónio and Rose 2001).

In the absence of direct observations of sex in the Colpodea, the most powerful approach to evaluate secretive sexuality in this ciliate clade is to look for meiotic genes, as meiosis is the central aspect of eukaryotic sex (e.g., Chi et al. 2014b; Malik et al. 2008; Ramesh et al. 2005; Schurko and Logsdon 2008). We therefore sequenced the genomes of the known sexual colpodean *B. truncatella* and the putative asexual *Colpoda magna*. With these genomic data, we inventoried meiotic genes to evaluate their presence or absence, and we evaluated the rate of evolution of the inventoried genes relative to the same genes in known sexual ciliates from other clades (Chi et al. 2014a) to look for evidence of relaxed selection.

Materials and Methods

Cells of *B. truncatella* were obtained from Carolina Biological Supply Company (Burlington, NC, USA) and cells of *C. magna* were obtained from the American Type Culture Collection (#50128). Clonal cultures were established and grown in Volvic water with wheat grains and *Klebsiella* sp. *B. truncatella* cultures also included *Paramecium* sp. Individual cells were picked with a pipette and washed three times in sterilized Volvic water, then allowed to starve for 48 h. Ten starved cells from each species were individually whole genome amplified with REPLI-g Mini Kit (Hilden, Germany) following manufacturer's instructions. For each species, the ten whole-genome amplified products were combined in equal DNA concentrations.

Amplified DNA from *B. truncatella* was sequenced with Illumina MiSeq v2 chemistry (13,288,644 2 × 250 bp reads) and Illumina HiSeq v3 chemistry 113,546,269 2 × 150 bp reads). Amplified DNA from *C. magna* was sequenced with MiSeq v3 chemistry (18,770,554 2 × 300 bp reads) and HiSeq v3 chemistry (28,298,554 2 × 150 bp reads). As the genome sizes of these two species are unknown, we could not estimate sequencing coverage. The optimal k-mer length for genome assembly was searched within a 21–201 range with KmerGenie v1.6976 (Chikhi and Medvedev 2014), using the "diploid" parameter. Genomes were then assembled with Minia v2.0.7 (Chikhi and Rizk 2012), setting the kmer minimal abundance to 5. The obtained contigs were then analyzed with AUGUSTUS v2.7 (Stanke et al. 2004) for a structural annotation, using the following parameters: search on both strands; genome is partial; predict genes independently on each strand, allow overlapping genes on opposite strands; report transcripts with in-frame stop codons; species set to the ciliate *T. thermophila*. Reads were deposited in GenBank's Sequence Read Archive under BioProject numbers PRJNA381863 and PRJNA382551.

A query database of 11 meiosis-specific genes and 40 meiosis-related genes from ciliate and nonciliate eukaryotes was established using literature and keyword searches of the NCBI protein database was taken from Chi et al. (2014a). For REC8, we used canonical eukaryotic sequences and *T. thermophila*'s noncanonical *REC8* (Howard-Till et al. 2013).The ORFs of the two colpodeans were searched by the query database using BlastP (Altschul et al. 1990) and HMMER v3.0 (Eddy 2001). Hits with E-values $<10^{-4}$ for the full sequence were retained. Verification of candidate homologs used reciprocal BlastP search against the nonredundant protein sequence database of NCBI (supplementary Files 1 and 2, Supplementary Material online).

In order to determine the strength of purifying selection acting on the inventoried meiotic genes, we measured $\omega = dN/dS$, the number of nonsynonymous substitutions per nonsynonymous site divided by the number of synonymous substitutions per synonymous site. Sequences generated from

this study were aligned with homologous sequences identified from *T. thermophila*, *P. tetraurelia*, *Ichthyophthirius multifiliis*, and *Oxytricha trifallax* by Chi et al. (2014a). Sequences were aligned in Geneious v4.8.3 (Kearse et al. 2012) using Translation Align with ClustalW v2 (Larkin et al. 2007). Sequences that did not have sufficient overlap with the other genes, were unalignable, or were determined to be paralogous to the genes from these other species were excluded from further analysis (supplementary table 1, Supplementary Material online). Maximum Likelihood genealogies of each gene were inferred with MEGA7 (Kumar et al. 2016), and synonymous and nonsynonymous substation rates were estimated with PAML v4.8 (Yang 2007). ω was first calculated between *B. truncatella* and *C. magna*. Then all species were included to test whether the lineage leading to *C. magna* exhibited higher values of ω, which would be expected if these genes were no longer functional and thus experiencing relaxed selection. Using codeml, we compared a model of evolution with one value of ω for the whole tree (model = 0) to a model where the *C. magna* lineage had a separate value of ω (model = 2). A log-likelihood ratio test was used to determine whether the second model provided a significantly better fit to the data.

Meiotic Gene Inventory and Evolutionary Rates

To uncover the genes involved in meiosis in *B. truncatella* and *C. magna*, clonal cell lines were *de novo* genome sequenced. The aim of the sequencing was to produce open reading frames, not to resolve issues of chromosomal scaffolding or differences between micronuclei and macronuclei. From these open reading frames, we evaluated the presence or absence of 11 meiosis-specific genes, and 40 meiosis-related genes that are also involved in mitosis.

The complement of meiotic genes in the two colpodeans generally matched those from sexual ciliates (table 1). Both *B. truncatella* and *C. magna* had *SPO11*, which causes the double-strand DNA breaks that initiate meiosis (Keeney 2001). Six other meiosis-specific genes that are involved in crossover regulation were uncovered: *DMC1*, *HOP2* (but not in *C. magna*), *MER3* (which was not found in other ciliates), *MND1*, *MSH4*, and *MSH5*. Like other ciliates, *HOP1*, *RED1*, and *ZIP1* were not found in the colpodeans, supporting the view of Chi et al. (2014a) that ciliates in general have a slimmed crossover pathway 1 that lacks a synaptonemal complex. *B. truncatella* and *C. magna* mostly had the same complement of meiosis-related genes as found in the other ciliates, including *MUS81*; except that *CDC2*, *MPH1/FANCM*, *MPS3/SUN-1/SAD1*, *SGS1*, and *SLX1* were missing in one or both of them.

To look for evidence of relaxed selection in the uncovered meiotic genes from the colpodeans, we evaluated the rates of nonsynonymous relative to synonymous substitutions (ω; table 1). Genes showing evidence of relaxed selection (that is,

elevated ω) would indicate the loss of functional constraints due to loss of use in sexual reproduction (Lahti et al. 2009). Evolutionary rates were first measured in the genes contained in both *B. truncatella* and *C. magna*. In this pairwise comparison, all estimated values of ω were much <1, indicating strong purifying selection in the meiotic genes. A second comparison was made of the two colpodeans with homologs from other ciliate species, where estimated values of ω were also very low. For this comparison between all of the colpodeans and other ciliates, two models were evaluated: M0, where all species were modeled to evolve at the same rate; and M2, where all evolve at the same rate except *C. magna*. If the genes in *C. magna* were experiencing relaxed selection, in contrast to purifying selection in the other taxa, we would expect to find evidence of larger values of ω in the *C. magna* lineage. However, only three genes show a significant difference in evolutionary rates in *C. magna* (that is, the *P* value for the log-likelihood ratio test was ≤0.05), and of those three only one, *MUS81*, is in the predicted direction.

Genomic Data Support Secretive Sex in Colpodeans

The meiotic genes found in this inventory of the two colpodeans were the same as those found in known sexual ciliates. These meiotic genes would have been lost in *C. magna* if the colpodeans were asexual. In addition, there was no evidence of relaxed selection on these genes in *C. magna*, suggesting that they are under functional constraint just as in the sexual species. If these genomic data apply equally to the unsampled species, then, as predicted (Dunthorn and Katz 2010), the colpodean ciliates are likely sexual.

It should be noted, however, that having functional meiotic genes can also allow for activities other than what we defined here as sex (where there is a requirement of recombination between two individuals). These meiotic genes could be used in canonical genetic pathways such as automixis (=selfing), which could allow for the purging of deleterious alleles, but would not provide the benefit of genetic exchange between individuals. They can also be used in noncanonical genetic pathways such as: diplomixis in the protist *Giardia intestinalis*, where there is homologous recombination between the two nuclei within each cell but no meiotic reduction in ploidy (Carpenter et al. 2012; Poxleitner et al. 2008); parasexuality in the fungus *Candida albicans*, where tetraploidy caused by cell fusion is nonmeiotically reduced to diploidy (Bennett and Johnson 2003; Forche et al. 2008); and DNA repair in bdelloid rotifers from damages induced by desiccation and UV radiation that likely has allowed this large and ancient metazoan clade to be asexual (Bininda-Emonds et al. 2016; Gladyshev and Meselson 2008; Hespeels et al. 2014).

Beyond these genomic data, there are two additional lines of support for sexuality in *C. magna* and rest of the colpodean ciliates. One is cytological. The other is behavioral.

Table 1

Meiosis genes inventoried in two colpodean ciliates: *Bursaria truncatella* and *Colpoda magna*

Gene	Ciliate species						dN/dS				
	Bursaria truncatella	*Colpoda magna*	*Tetrahymena thermophila*	*Paramecium tetraurelia*	*Ichthyophthirius multifiliis*	*Oxytricha trifallax*	ω(B+C)	ω(M0)	*P* Value	ω(all)	ω(Col)
DOUBLE STRAND BREAK FORMATION											
REC114/REC7	−	−	−	−	−	−					
SPO11/REC12	+	+	+	+	+	+	0.348	0.028	0.790		
CROSSOVER REGULATION											
DMC1	+	+	+	−	+	+	0.001	0.003	0.730		
HOP1	−	−	−	−	−	−					
HOP2	+	−	+	+	+	+					
MER3	+	+	−	−	−	−	0.008				
MND1	+	+	+	+	+	+					
MSH4	+	+	+	+	−	+	0.005	0.044	0.296		
MSH5	+	+	+	+	−	+	0.150	0.076	0.902		
RED1/ASY3/REC10	−	−	−	−	−	−					
ZIP1	−	−	−	−	−	−					
DOUBLE STRAND BREAK REPAIR AND MEIOTIC DIVISIONS											
REC8	−	−	+	−	−	−					
BOUQUET FORMATION											
MPS3/SUN-1/SAD1	−	+	+	+	+	+					
DNA DAMAGE SENSING/RESPONSE											
MEC1/ATR	+	+	+	+	+	+	0.002	0.004	0.925		
TEL1/ATM	−	−	−	−	−	−					
MRE11	+	+	+	+	+	+	0.002	0.005	0.293		
RAD17	+	+	+	+	+	−	0.061	0.037	0.176		
RAD23	+	+	+	+	+	+	0.007	0.080	0.238		
RAD24	+	+	+	+	+	+	0.004	0.064	0.170		
RAD50	+	+	+	+	+	+	0.003	0.019	0.687		
XRS2/NBS1	−	−	−	−	−	−					
DOUBLE STRAND BREAK REPAIR (non-homology end join)											
KU70	+	+	+	+	+	+	0.007	0.008	0.656		
KU80	+	+	+	+	+	+	0.133	0.031	0.393		
LIG4/DNL1	+	+	+	+	+	+	0.007	0.018	0.687		
XRCC4/LIF1	−		−	−	−	−					
RECOMBINATIONAL REPAIR											
BRCA1	+	+	+	−	−	−	0.008	0.014	0.649		
BRCA2	+	+	+	+	+	+	0.003	0.029	0.025	0.038	0.004
DNA2	+	+	+	+	+	+	0.008	0.072	0.647		
MMS4/EME1	+	+	+	+	−	−	0.011	0.004	0.727		
EXO1	+	+	+	+	+	+	0.003	0.035	0.004	0.044	0.003
FEN1	+	+	+	+	+	+	0.202	0.009	0.434		
MLH1	+	+	+	+	+	+	0.032	0.102	0.193		
MLH3	+	+	+	−	+	−	0.039	0.017	0.782		
MPH1/FANCM	−	+	+	+	+	+					
MSH2	+	+	+	+	+	+	0.003	0.018	0.473		
MSH3	−	−	−	−	−	−					
MSH6	+	+	+	+	+	+	0.012	0.029	0.295		
MUS81	+	+	+	+	+	+	0.017	0.002	0.002	0.017	0.126
PMS1	+	+	+	+	+	+	0.004	0.010	0.992		
RAD51	+	+	+	+	+	+	0.078	0.020	0.894		
RAD52	−	−	−	−	−	−					
RAD54	+	+	+	+	+	−	0.005	0.038	0.223		
RTEL1	+	+	+	+	+	+	0.011	0.032	0.206		
SAE2/COM1/CTIP	−	−	+	+	−	−					

(continued)

Table 1 Continued

Gene	Ciliate species						dN/dS				
	Bursaria truncatella	Colpoda magna	Tetrahymena thermophila	Paramecium tetraurelia	Ichthyophthirius multifiliis	Oxytricha trifallax	ω(B+C)	ω(M0)	P Value	ω(all)	ω(Col)
SGS1	–	–	+	+	+	+					
SLX1	+	–	–	–	–	–					
SLX4/HIM-18/MUS312	–	–	–	–	–	–					
SMC5	–	–	–	–	–	–					
SMC6	–	–	–	–	–	–					
YEN1/GEN1	–	–	–	–	–	–					
MEIOTIC ENTRY											
CDC2	+	–	+	+	+	+					

NOTE.— Genes are grouped according to functions. Meiosis-specific genes are underlined. Data from *Tetrahymena*, *Paramecium*, *Ichthyophthirius*, and *Oxytricha* are from Chi et al. (2014a). ω(B+C) indicates the value of dN/dS between *B. truncatella* and *C. magna*. ω(M0) indicates the value of dN/dS for all taxa. The significance of the difference between a model with one parameter for ω versus a model with an addition parameter for ω on the *C. magna* branch is shown by the P value from a chi-squared test with one degree of freedom. In cases where this test is significant, ω(all) indicates the background rate and ω(Col) is the rate for *C. magna*.

All colpodean species that have been described in enough detail have micronuclei (e.g., Bourland et al. 2013; Dunthorn et al. 2009; Foissner 1993; Foissner et al. 2014; Quintela-Alonso et al. 2011). As micronuclei are only involved in sex (as far as we know), we propose that this cytological feature would have been lost over evolutionary time if the colpodeans were asexual. While the colpodeans have micronuclei, at least six species from two different subclades have micronuclei and macronuclei with shared outer nuclear membranes (Dunthorn et al. 2008; Foissner 1993). This shared outer membrane could possibly chain the micronucleus to the macronucleus, and thereby prevent it from participating in sex. That is, having micronuclei chained to macronuclei could be analogous to having no micronuclei at all. However, it is unknown if this shared outer-nuclear membrane is present in most individuals within those six species or if the micronuclei can break free at some point during the cell cycle (Dunthorn et al. 2008).

Pseudoconjugation has been observed in at least four colpodean ciliates while living in petri dishes (Foissner 1993). As conjugation is only involved in sex (as far as we know), we propose that this behavioral feature would have been lost over evolutionary time if the colpodeans were asexual. It should be noted, however, that pseudoconjugation can also allow for activities other than what we defined here as sex. For example, pseudocopulation occurs in the all female *Aspidoscelis uniparens* (desert grassland whiptail lizards), where females need to be mounted by other females to induce parthenogenesis (Crews and Fitzgerald 1980).

Whiles these genomic data and observations support sexuality in *C. magna* and other colpodean ciliates, additional analyzes can be performed. For example, population genomics methods can be used to test for the presence and rates of outcrossing and recombination within and between populations in nature (Halkett et al. 2005; Ruderfer et al. 2006). Decisive evidence of sex in the colpodeans will have to come from direct observations of successful conjugation in the laboratory, but as with *Aspergillus fumigatus* (O'Gorman et al. 2009), finding the correct conditions to induce sex may be difficult.

Conclusions

Our genomic analyses show that ciliates do not violate the macro-organismic theories against ancient asexuals and the loss-and-regain of complex characters: the Colpodea are sexual. This finding supports the increasingly accepted view that sex in microbial eukaryotes is ubiquitous although often secretive (Dunthorn and Katz 2010; Speijer et al. 2015). Such secretive sex may result in long periods of mitotic division without meiosis, which could lead to the buildup of high mutational loads in the quiescent germline genomes of the colpodeans. However, rare sex could be tolerated if either the colpodeans have extremely low base-substitution mutation rates as found in the ciliates *Paramecium tetraurelia* and *Tetrahymena thermophila* (Long et al. 2016; Sung et al. 2012), or if the rare sex provides the same benefits as does more frequent sex (D'Souza and Michiels 2010; Green and Noakes 1995).

Supplementary Material

Supplementary data are available at Genome Biology and Evolution online.

Literature Cited

Altschul SF, Fish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215:403–410.

Bell G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. Berkeley: University of California Press.

Bell G. 1988. Sex and death in protozoa: the history of an obsession. Cambridge: Cambridge University Press.

Bennett RJ, Johnson AD. 2003. Completion of a parasexual cycle in Candida albicans by induced chromosome loss in tetraploid strains. EMBO J. 22:2505–2515.

Bininda-Emonds ORP, Hinz C, Ahlrichs WH. 2016. Evidence supporting the uptake and genomic incorporation of environmental DNA in the "ancient asexual" bdelloid rotifer Philodina roseola. Life 6:38.

Bourland WA, Wendell L, Hampikian G, Vd'ačný P. 2013. Morphology and phylogeny of Bryophryoides ocellatus n. g., n. sp. (Ciliophora, Colpodea) from in situ soil percolates of Idaho, U.S.A. Eur J Protistol. 50:47–67.

Carpenter ML, Assaf ZJ, Gourguechon S, Cande WZ. 2012. Nuclear inheritance and genetic exchange without meiosis in the binucleate parasite Giardia intestinalis. J Cell Sci. 125:2523–2532.

Chi J, Mahé F, Loidl J, Logsdon J, Dunthorn M. 2014a. Meiosis gene inventory of four ciliates reveals the prevalence of a synaptonemal complex-independent crossover pathway. Mol Biol Evol. 31:660–672.

Chi J, Parrow MW, Dunthorn M. 2014b. Cryptic sex in Symbiodinium (Alveolata, Dinoflagellata) is supported by an inventory of meiotic genes. J Eukaryot Microbiol. 61:322–327.

Chikhi R, Medvedev P. 2014. Informed and automated k-mer size selection for genome assembly. Bioinformatics 30:31–37.

Chikhi R, Rizk G. 2012. Space-efficient and exact de Bruijn graph representation based on a Bloom filter. Workshop on Algorithms in Bioinformatics (WABI).

Collin R, Miglietta MP. 2008. Reversing opinions on Dollo's Law. Trends Ecol Evol. 23:602–609.

Crews D, Fitzgerald KT. 1980. "Sexual" behavior in parthenogenetic lizards (Cnemidophorus). Proc Natl Acad Sci U S A. 77:499–502.

D'Souza TG, Michiels NK. 2010. The costs and benefits of occasional sex: theoretical predictions and a case study. J Heredity 101:S34–S41.

Doerder FP. 2014. Abandoning sex: multiple origins of asexuality in the ciliate Tetrahymena. BMC Evol Biol. 14:112.

Dunthorn M, et al. 2009. Phylogenetic placement of the Cyrtolophosididae Stokes, 1888 (Ciliophora; Colpodea) and neotypification of Aristerostoma marinum Kahl, 1931. Int J Syst Evol Microbiol. 59:167–180.

Dunthorn M, Foissner W, Katz LA. 2011. Expanding character sampling in ciliate phylogenetic inference using mitochondrial SSU-rDNA as a molecular marker. Protist 162:85–99.

Dunthorn M, Foissner W, Katz LA. 2008. Molecular phylogenetic analysis of class Colpodea (phylum Ciliophora) using broad taxon sampling. Mol Phylogenet Evol. 46:316–327.

Dunthorn M, Katz LA. 2010. Secretive ciliates and putative asexuality in microbial eukaryotes. Trends Microbiol. 18:183–188.

Eddy SR. 2001. HMMER: Profile hidden markov models for biological sequence analysis. Available from: http://hmmer.wustl.edu.

Fenchel T, Finlay BJ. 2006. The diversity of microbes: resurgence of the phenotype. Philos Trans R Soc B Biol Sci. 361:1965–1973.

Foissner W. 1993. Colpodea (Ciliophora). Protozoenfauna 4:1–798.

Foissner W, Bourland WA, Wolf K, Stoeck T, Dunthorn M. 2014. New SSU-rDNA sequences for eleven colpodeans (Ciliophora, Colpodea) and description of Apocyrtolophosis nov. gen. Eur J Protistol. 50:40–46.

Foissner W, Stoeck T, Agatha S, Dunthorn M. 2011. Intraclass evolution and classification of the Colpodea (Ciliophora). J Eukaryot Microbiol. 58:397–415.

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:e110.

Gladyshev E, Meselson M. 2008. Extreme resistance of bdelloid rotifers to ionizing radiation. Proc Natl Acad Sci U S A. 105:5139–5144.

Gould SJ. 1970. Dollo on Dollo's Law: irreversibility and the status of evolutionary laws. J Hist Biol. 3:189–212.

Green RF, Noakes DLG. 1995. Is a little bit of sex as good as a lot? J Theor Biol. 174:87–96.

Halkett F, Simon J-C, Balloux F. 2005. Tackling the population genetics of clonal and partially clonal organisms. Trends Ecol Evol. 20:194–201.

Hamilton WD. 2001. Narrow roads of gene land, Vol. 2: the evolution of sex. Oxford: Oxford University Press.

Hartfield M, Keightley PD. 2012. Current hypotheses for the evolution of sex and recombination. Integr Zool. 7:192–209.

Hespeels B, et al. 2014. Gateway to genetic exchange? DNA double-strand breaks in the bdelloid rotifer Adineta vaga submitted to desiccation. J Evol Biol. 27:1334–1345.

Howard-Till RA, Lukaszewicz A, Novatchkova M, Loidl J. 2013. A single cohesin complex performs mitotic and meiotic functions in the protist Tetrahymena. PLoS Genet. 9:e1003418.

Kearse M, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Keeney S. 2001. Mechanism and control of meiotic recombination initiation. Curr Topics Dev Biol. 52:1–53.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 33:1870–1874.

Lahti DC, et al. 2009. Relaxed selection in the wild. Trends Ecol Evol. 24:487–496.

Larkin MA, et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.

Lehtonen J, Jennions MD, Kokko H. 2012. The many costs of sex. Trends Ecol Evol. 27:172–178.

Lehtonen J, Kokko H. 2014. Sex. Curr Biol. 24:R305–R306.

Long H, et al. 2016. Low base-substitution mutation rate in the germline genome of the ciliate Tetrahymena thermophila. Genome Biol Evol. 8:3629–3639.

Lynn DH. 2008. The ciliated protozoa: characterization, classification, and guide to the literature, 3rd edn. Dordrecht: Springer.

Malik S-B, Pightling AW, Stefaniak LM, Schurko AM, Logsdon JM. 2008. An expanded inventory of conserved meiotic genes provides evidence for sex in Trichomonas vaginalis. PLoS One 3:e2879.

Martens K, Rossetti G, Horne DJ. 2003. How ancient are ancient asexuals? Proc R Soc Lond B 270:723–729.

Maynard Smith J. 1978. The evolution of sex. Cambridge: Cambridge University Press.

Normark BB, Judson OP, Moran NA. 2003. Genomic signatures of ancient asexual lineages. Biol J Linn Soc. 79:69–84.

O'Gorman CM, Fuller HT, Dyer PS. 2009. Discovery of a sexual cycle in the opportunistic fungal pathogen Aspergillus fumigatus. Nature 457:471–474.

Phadke SS, Zufall RA. 2009. Rapid diversification of mating systems in ciliates. Biol J Linn Soc. 98:187–197.

Poxleitner MK, et al. 2008. Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite Giardia intestinalis. Science 319:1530–1533.

Quintela-Alonso P, Nitsche F, Arndt H. 2011 Molecular characterization and revised systematics of Microdiaphanosoma arcuatum (Ciliophora, Colpodea). J Eukaryot Microbiol. 58:114–119.

Ramesh MA, Malik S-B, Longsdon JM. 2005. A phylogenomic inventory of meiotic genes: evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. Curr Biol. 15:185–191.

Ruderfer DM, Pratt SC, Seidel HS, Kruglyak L. 2006. Population genomic analysis of outcrossing and recombination in yeast. Nat Genet. 38:1077–1081.

Schlegel M, Meisterfeld R. 2003. The species problem in protozoa revisited. Eur J Protistol. 39:349–355.

Schön I, Martens K, van Dijk P. 2009. Lost sex: the evolutionary biology of parthenogenesis. Dordrecht: Springer.

Schurko AM, Logsdon JM. 2008. Using a meiosis detection toolkit to investigate ancient asexual "scandals" and the evolution of sex. BioEssays 30:579–589.

Sonneborn TM. 1957. Breeding systems, reproductive methods, and species problems in protozoa. In: Mayr E, editor. The species problem. Washington DC: American Association for the Advancement of Science. p. 155–324.

Speijer D, Lukeš J, Eliáš M. 2015. Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. Proc Natl Acad Sci U S A. 112:8827–8834.

Stanke M, Steinkamp R, Waack S, Morgenstern B. 2004. AUGUSTUS: a web server for gene finding in eukaryotes. Nucleic Acids Res. 32:W309–W312.

Sung W, et al. 2012. Extraordinary genome stability in the ciliate *Paramecium tetraurelia*. Proc Natl Acad Sci U S A. 109:19339–19344.

Teotónio H, Rose MR. 2001. Perspective: reverse evolution. Evolution 55:653–660.

Wright A-DG, Lynn DH. 1997. Maximum ages of ciliate lineages estimated using a small subunit rRNA molecular clock: crown eukaryotes date back to the paleoprotoerozoic. Arch Protistenk. 148:329–341.

Yang Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol. 24:1586–1591.

Zufall R. 2016. Mating systems and reproductive strategies in *Tetrahymena*. In: Witzany G, Nowacki M, editors. Biocommunication of ciliates. Switzerland: Springer International Publishing.

Associate editor: John Archibald