JOURNAL OF Evolutionary Biology

doi:10.1111/j.1420-9101.2010.02036.x

SHORT COMMUNICATION

Dynamic linkage relationships to the mating-type locus in automictic fungi of the genus *Microbotryum*

J. L. ABBATE* & M. E. HOOD†

*Department of Biology, University of Virginia, Charlottesville, VA, USA
†Department of Biology, Amherst College, Amherst, MA, USA

Keywords:

automixis;
intratetrad mating;
mating types;
meiotic parthenogenesis;
Microbotryum;
Neurospora tetrasperma;
recombination suppression;
sex chromosomes.

Abstract

Regions of the chromosomes determining mating compatibility in some fungi, including *Microbotryum lychnidis-dioicae* and *Neurospora tetrasperma*, exhibit suppressed recombination similar to sex chromosomes in plants and animals, and recent studies have sought to apply basic theories of sex chromosome evolution to fungi. A phylogeny of the MTL1 locus in *Microbotryum* indicates that it has become part of the nonrecombining regions of the mating-type chromosomes in multiple independent events, and that recombination may have been subsequently restored in some cases. This illustrates that fungal mating-type chromosomes can exhibit linkage relationship that are quite dynamic, adding to the list of similarities to animal or plant sex chromosomes. However, fungi such as *M. lychnidis-dioicae* and *N. tetrasperma* exhibit an automictic mating system, for which an alternate theoretical framework exists to explain the evolution of linkage with the mating-type locus. This study encourages further comparative studies among fungi to evaluate the role of mating systems in determining the evolution of fungal mating-type chromosomes.

Introduction

Suppression of recombination around genes that determine mating compatibility is fundamental to the evolution of sex chromosomes, contributing to the accumulation of mutations and eventually to their overall structural dimorphism (Bergero & Charlesowrth, 2009). In plants and animals, expansion of suppressed recombination to encompass large proportions of the sex chromosomes is thought to be driven by the recruitment of sexually antagonistic genes (Rice, 1987); i.e. genes that are not involved directly in achieving syngamy but that are beneficial in one sex and are costly in the other, typically with roles in mate choice or in determining other sex-specific ecological traits. The evolution of suppressed recombination in haploid-mating organisms, like fungi, is less well studied even though some species exhibit large structural differences between alternate mating-type chromosomes, and recent studies have suggested evolutionary parallels between mating-type chromosomes in fungi and sex chromosomes in plants and animals (e.g. Fraser & Heitman, 2003; Menkis *et al.*, 2008).

However, relatively few traits exist in fungi with the potential to be 'mating-type antagonistic' (in analogy to sexually antagonistic), which argues against a parallel process of recruiting chromosomal segments into linkage with fungal mating types. Fungi do not have separate sexes; instead, they determine mating compatibility as haploids, and the roles of mating types are largely limited to regulating gamete fusion and cytoplasmic inheritance. Except for these roles during the process of syngamy, haploids of alternate mating types typically have identical life histories, and both mating types invest equally in the provisioning of nutrients to offspring (reviewed in Billiard *et al.*, 2010). Thus, traits that distinguish fungal mating types do not to exist in a manner analogous to sexually antagonistic traits, and mating-type

Correspondence: Michael E. Hood, Biology Department, Amherst College, Rts 9 & 116, Amherst, MA 01002-5000, USA.
Tel.: 413 542 8538; fax: 413 542 7955; e-mail: MHood@amherst.edu
Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. GU453174–GU453235.

chromosomes should not be under similar evolutionary pressure to expand the region of suppressed recombination as are sex chromosomes in plants and animals. Indeed, regions of suppressed recombination around fungal mating types are sometimes small (e.g. Lee *et al.*, 1999; Fraser *et al.*, 2004), but some fungi have suppressed recombination over large chromosomal distances (e.g. 7 Mbp for ascomycete *Neurospora tetrasperma*, Menkis *et al.*, 2008). Mating-type chromosomes in the basidiomycete *Microbotryum lychnidis-dioicae* are size dimorphic (Hood, 2002) and exhibit fixed heterozygosity over much of their lengths; the mating-type chromosomes can range from 2.8 to 4.2 Mbp (Hood, 2002), and the proportions of random markers linked to mating type suggest the nonrecombining region is from 1 Mbp (Votintseva & Filatov, 2009) to 2.6 Mbp (Hood & Antonovics, 2004) in length. These fungal mating-type chromosomes can share other traits by convergence with sex chromosomes in plants and animals that are the consequences of suppressed recombination, such as the accumulation of transposable elements and the decrease in the density of genes relative to autosomes (Hood *et al.*, 2004).

An alternate explanation for recombination suppression in fungi like *M. lychnidis-dioicae* and *N. tetrasperma* relates to the particular mating system of automixis (= intratetrad mating), where mating type is linked to the centromere (Zakharov, 2005; Giraud *et al.*, 2008). Automixis appears to be the predominant mating system in *Microbotryum* species (Thomas *et al.*, 2003; Giraud *et al.*, 2008; Granberg *et al.*, 2008), but whether rates vary among species has not been determined. Theoretical studies predict that such fungi will experience selection for the spread of modifiers that suppress recombination with mating type, and an influx of load loci (i.e. deleterious recessive mutations) or overdominant loci (i.e. heterozygote advantage mutations) can cause feedbacks that accelerate this evolution of recombination suppression (e.g. Antonovics & Abrams, 2004; Johnson *et al.*, 2005). This theory is based upon the expectation that linkage disequilibrium between a self-incompatibility locus and a load or overdominant locus is required for the spread of modifiers that decrease recombination between these loci (Fisher, 1935; Leach *et al.*, 1986). Outside of strong fitness effects of epistasis between the self-incompatibility locus and a load or overdominant locus, even rare recombination between mating type and a load or overdominant locus would prevent the spread of linkage modifiers because of population-level linkage equilibrium. However, when automixis occurs, linkage disequilibrium between mating type and even weakly linked loci arises within the meiotic tetrad itself (i.e. where the tetrad represents a highly reduced 'mating population'), allowing selection to favour modifiers for stronger linkage and thus encompassing larger regions of the mating-type chromosome (Antonovics & Abrams, 2004). The discrepancy between linkage disequilibrium within the meiotic tetrad vs. equilibrium in the population as a whole can further select for modifiers to increase rates of automixis that prevent the exposure of costly genetic load (Johnson *et al.*, 2005).

Recent studies in mammals, fish, birds and plants have shown that linkage relationships on sex chromosomes can be highly variable, with the same gene becoming linked to the sex-determining loci independently in closely related species (Ellegren & Carmichael, 2001; Marais & Galtier, 2003; Handley *et al.*, 2004), consistent with model of selection for sex-linkage and expansion of recombination suppression based on sexually antagonistic genes. To assess whether such dynamics of gene linkage represent an additional similarity between mating-type chromosomes of fungi and sex chromosomes of plants and animals, we reconstructed the phylogeny for MTL1, a locus linked to mating type in *M. lychnidis-dioicae*, from which MTL1 was first obtained (Hood, 2002). We observed that MTL1 has become completely linked to mating type in multiple independent events since the divergence of extant *Microbotryum* species clades. This result is presented in a context that is consistent with the evolution of recombination suppression under an automictic mating system, with the understanding that convergent evolutionary patterns between mating-type chromosomes and sex chromosomes have likely arisen because of different selective forces.

Materials and methods

Study system and assessment of linkage dynamics

Microbotryum is a basidiomycete genus, consisting of fungal pathogens that cause anther-smut disease on plants in the Caryophyllaceae. The life cycle of *Microbotryum* has been recently reviewed (Giraud *et al.*, 2008). Fungi in the genus *Microbotryum* have two intercompatible haploid mating types, called A1 and A2 (i.e. these fungi exhibit a bipolar heterothallic breeding system), and these fungi mate often among the products of the same meiotic tetrad (i.e. an automictic mating system; Giraud *et al.*, 2008). Our goal was to determine whether divergence of MTL1 alleles indicated the ancestral complete linkage with mating type or ongoing recombination in some lineages. Ancestral complete linkage to mating type would produce a MTL1 phylogeny with two major clades, one clade containing alleles derived from all A1 haploid cells and the other clade containing alleles from all A2 haploid cells, as was seen for the phylogeny of the *Microbotryum*-mating pheromone receptor gene (Devier *et al.*, 2009). Ongoing recombination would allow the MTL1 locus to become homozygous under the highly selfing mating system, resulting in no differences between alleles from A1 or A2 haploid cells, or on-going recombination would produce inconsistent cosegregation with mating type across meioses if heterozygosity at the MTL1 locus was present.

Isolation of haploid meiotic products

Microbotryum specimens were collected from natural populations, with multiple samples included for some species where possible (Table 1). *Microbotryum* species names are given where available or otherwise indicated by their host-of-origin and locality. Diploid spores of the fungi were allowed to germinate on potato dextrose agar for 24 h at room temperature, at which time they had completed meiosis and produced yeast-like sporidia as the post-meiotic cells. Meiotic tetrads were isolated by micromanipulation, and yeast-like cultures derived from single cells of opposite mating type were used for DNA extraction. Mating type of each culture was determined by performing crosses with cultures of known mating type and examining for conjugating cells.

Phylogenetic reconstruction of the MTL1 locus

A neighbour-joining phylogeny for *Microbotryum* species was reconstructed based upon DNA sequences of the MTL1 locus obtained from haploid cells of the alternative mating types. PCR primers were 5′-CTTCGGAATAAC-GAGAAGGC-3′ and 5′-AGGTATGAGCAGTGGATCGG-3′, and sequences are available in GenBank with accessions numbers GU453174–GU453235. Phylogenetic analysis was conducted in MEGA version 3.1 (Kumar *et al.*, 2004), where all insertion/deletion mutations were removed (53/311 positions), leaving 62 positions with nucleotide polymorphisms within the 311-bp alignment.

Results and discussion

Nucleotide sequence differences (i.e. heterozygosities) were found between MTL1 alleles from A1 vs. A2 meiotic products for specimens of *M. lychnidis-dioicae*, *Microbotryum silenes-dioicae*, *Microbotryum violaceum sensu stricto*, *Microbotryum dianthorum* and *Microbotryum shykoffianum*. Across meioses from multiple individuals within each of these species, there was consistent meiotic cosegregation of MTL1 allelic variants with the alternate mating types, further indicating complete linkage. In contrast, *Microbotryum lagerheimii*, *Microbotryum saponariae* and the yet unnamed *Microbotryum* species from *Silene caroliniana*,

Table 1 *Microbotryum* lineages and GenBank (NCBI) accession numbers for sequences used to investigate linkage of MTL1 to mating type.

Microbotryum species	Host species	Sample location	MTL1 accession numbers
Microbotryum dianthorum	*Dianthus monspessulanus*	Chambery, France	GU453206, GU453205
M. dianthorum	*Dianthus neglectus*	Valle Pesio, Italy	GU453189, GU453190
Microbotryum lagerheimii	*Silene uniflora*	Somerset, UK	GU453188, GU453187
M. lagerheimii	*Silene vulgaris*	Chambery, France	GU453176, GU453175
Microbotryum silenes-dioicae	*Silene dioica*	Lac de Puy Vachier, France	GU453201, GU453202
M. silenes-dioicae	*S. dioica*	Somerset, UK	GU453203, GU453204
Microbotryum saponariae	*Saponaria officinalis*	Serramonacesca, Italy	GU453215, GU453216
M. saponariae	*Saponaria officinalis*	Borro, Italy	GU453209, GU453210
M. saponariae	*Saponaria ocymoides*	Borgata Sestriere, Italy	GU453211, GU453212
M. saponariae	*Saponaria ocymoides*	Cesana Tor, Italy	GU453213, GU453214
Microbotryum shykoffianum	*Dianthus carthusianorum*	Castelina, Italy	GU453208, GU453207
M. shykoffianum	*D. carthusianorum*	Sestriere, Italy	GU453197, GU453199
M. shykoffianum	*Dianthus sylvestris*	La Grave, France	GU453191, GU453186
M. shykoffianum	*D. sylvestris*	Cesana Tor, Italy	GU453219, GU453220
Microbotryum lychnidis-dioicae	*Silene latifolia*	Broadway Field, Virginia, USA	GU453224, GU453225
M. lychnidis-dioicae	*S. latifolia*	Olomouc, Czech Republic	GU453223, GU453226
M. lychnidis-dioicae	*S. latifolia*	Orsay, France	GU453194, GU453196
M. lychnidis-dioicae	*S. latifolia*	Oxfordshire, UK	GU453221, GU453222
M. lychnidis-dioicae	*S. latifolia*	San Gimignano, Italy	GU453228, GU453229
M. lychnidis-dioicae	*S. latifolia*	Lamole, Italy	GU453200, GU453198
Microbotryum violaceum	*Silene nutans*	Guarda, Switzerland	GU453177, GU453179
M. violaceum	*S. nutans*	Lac du Puy Vachier, France	GU453178, GU453180
N/A	*Atocion rupestre*	Chambery, France	GU453217, GU453218
N/A (*MvLfc*)	*Lychnis flos-cuculi*	Sheffield, UK	GU453231, GU453230
N/A (*MvSa*)	*Silene acaulis*	Sestriere, Italy	GU453193, GU453192
N/A (*MvSa*)	*S. acaulis*	Valle de Pesio, Italy	GU453235, GU453234
N/A (*MvSa*)	*S. acaulis*	Peyrou d'Amont, France	GU453227, GU453195
N/A (*MvSa*)	*S. acaulis*	Lac de Puy Vachier, France	GU453233, GU453232
N/A (*MvSspA*)	*Silene caroliniana*	Blue Ridge Parkway, Virginia, USA	GU453184, GU453183
N/A (*MvSspA*)	*Silene virginica*	Charlottesville, Virginia, USA	GU453182, GU453185
N/A (*MvSspA*)	*S. virginica*	Floyd, Virginia, USA	GU453181, GU453174

Species designations are as given in Denchev (2007), Lutz *et al.* (2005) and Denchev *et al.* (2009), and when not available (N/A) further identified species are indicated in parentheses according to Le Gac *et al.* (2007).

Silene virginica and *Atocion rupestre* contained alleles that were identical between A1 and A2 haploid cells, indicating MTL1 homozygosity that is consistent with recombination between MTL1 and mating type and the highly selfing mating system of *Microbotryum*. For the species of *Microbotryum* from *Silene acaulis*, some individuals were homozygous for the MTL1 locus, whereas others were heterozygous, thus suggesting less than complete linkage of MTL1 with mating type (Fig. 1).

Aside from mating-type linked differences, most sister species relationships in the MTL1 phylogeny were the same as previously published nuclear gene phylogenies (Freeman *et al*., 2002; Le Gac *et al*., 2007); e.g. the species pairs of *M. lycnhidis dioicae* with *M. silenes-dioicae*, *M. lagerheimii* with *Microbotryum* from *A. rupestre*, *M. violaceum* with *Microbotryum* from *Lychnis flos-cuculi* and *M. saponariae* with *M. dianthorum* and *M. shykoffianum*. For *M. lychnidis-dioicae* and *M. silenes-dioicae*, where MTL1 appears completely linked to mating type, the species pair relationship was seen within each of the clades containing either MTL1 alleles derived from A1 haploid cells or the clade containing MTL1 alleles derived from

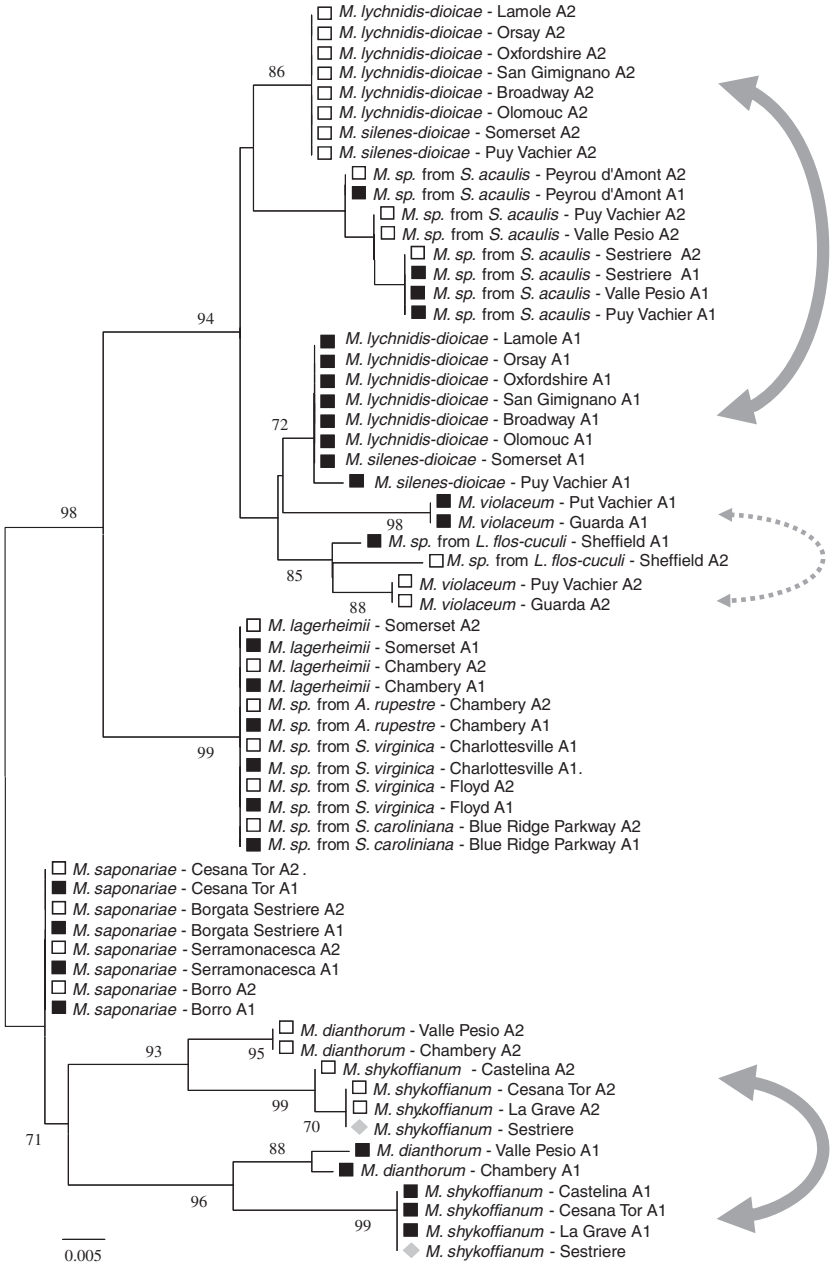

Fig. 1 Neighbour-joining phylogeny for *Microbotryum* species based upon DNA sequences of the MTL1 locus that were obtained from haploid cells of the alternative mating types, termed A1 (filled boxes) and A2 (open boxes). For meiotic products of *Microbotryum shykoffianum* from the Sestriere population, mating types could not be determined by conjugation assays (grey diamonds). *Microbotryum* species names are given where available or otherwise indicated by their host-of-origin and locality (see Table 1). Node support based on 500 bootstrap psuedoreplications, and scale bar shows number of nucleotide substitutions per base pair. Two major (large arrows) and one suspected (dashed arrow) cases of cessation of recombination between MTL1 and mating type are indicated.

A2 haploid cells; the same pattern was observed for the species pair of *M. dianthorum* and *M. shykoffianum*. One major incongruence was the MTL1 identity of the North American species (*Microbotryum* from *S. caroliniana* and *S. virginica*) to some European species (*M. lagerheimii* and *Microbotryum* from *A. rupestre*), which are known to be among the most phylogenetically divergent species within the *Microbotryum* genus (Freeman *et al.*, 2002; Le Gac *et al.*, 2007). Internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA genes were sequenced for all specimens to confirm they matched the *Microbotryum* species described previously (data not shown), and multiple DNA extractions and amplification of MTL1 and ITS confirmed these results. Further studies are needed to ascertain possible reasons for sequence identity between these distant lineages.

These results suggest that the MTL1 locus has become completely linked to the nonrecombining mating-type region at least twice since the differentiation of *Microbotryum* species on the Caryophyllaceae: one instance was in the ancestor of *M. lychnidis-dioicae* and *M. silenes-dioicae*, and the other in the ancestor of *M. dianthorum* and *M. shykoffianum*. The observation that MTL1 sequences from *M. lychnidis-dioicae* and *M. silenes-dioicae* did not group together with sequences from *M. dianthorum* and *M. shykoffianum* according to mating-type supports the phylogenetic independence of the cessation of recombination and that there was not complete linkage of MTL1 with mating type ancestral to this fungal genus. Moreover, prior studies have shown that the lineage of *Microbotryum* from *S. caroliniana* and *S. virginica* is sister to a clade containing the rest of the *Microbotryum* species examined here (Le Gac *et al.*, 2007), and the lack of mating-type linkage of MTL1 in both of these clades further supports the conclusion that cessation of recombination is a derived trait.

Each cessation of recombination predated speciation events in *Microbotryum*, and variation at MTL1 thus represents trans-species polymorphisms. However, recombination appears to have been restored for *Microbotryum* from *S. acaulis* and also for *M. violaceum sensu stricto* and *Microbotryum* from *L. flos-cuculi*, resetting homozygosity sufficiently long ago that some mutations have accumulated to differentiate the alternate mating types. Votintseva & Filatov (2009) recently showed that *M. lychnidis-dioicae* contains DNA sequences with varying ages of cessation of recombination with the mating pheromone receptor; however, the MTL1-containing sequence (A2-441) was amplified from only A1 mating-type cells in their study, and its genetic map distance from mating type was not estimated.

For the remaining *Microbotryum* species, homozygosity for MTL1 or inconsistent co-segregation with mating type indicates ongoing recombination, although sequencing flanking regions to MTL1 could potentially reveal additional heterozygosities. For these species, it is unknown whether the MTL1 locus is on an autosome or a recombining region of the mating-type chromosomes. Thus, the overall patterns of differentiation could reflect expanding or contracting regions of nonrecombination or translocations involving other genomic regions. Sequences of the MTL1 region did not contain start or stop codons or align with any published fungal protein in the NCBI database (BLASTx network service functions as of 27 January 2010). However, there were six length variants over two indel regions, all involving multiples of three nucleotides and suggesting that MTL1 may code for a protein.

Characterization of A1 and A2 mating pheromone receptor alleles from *Microbotryum* revealed the oldest trans-specific polymorphism known in any organism (> 370 million years old; Devier *et al.*, 2009), more than twice the estimated age for mammalian XY sex chromosome systems (< 166 million years old; Veyrunes *et al.*, 2008). The ancient nature of this trans-specific polymorphism suggests that regions associated with fungal mating-type chromosomes represent the very long-term product of genome evolution, rather than early stages of a process similar to sex chromosome differentiation. Despite the continued discovery of shared characteristics between fungal mating-type chromosomes and sex chromosomes of plants and animals, the selective forces responsible for the evolution of recombination suppression are likely to differ between these groups of organisms because the identity in life history for alternate mating types in fungi provides little opportunity for the mating-type (or sexually) antagonistic traits to drive linkage relationships. In *N. tetrasperma*, Menkis *et al.* (2008) recently reported that two different loci became linked to the fungal mating type at different times. Such similar results in distant groups of automictic fungi should encourage more integrated studies on whether the mating system has had a primary influence on the evolution of recombination suppression in accordance with the model by Antonovics & Abrams (2004). Forthcoming genome sequences of other automictic fungi, such as the ascomycete yeast *Saccharomyces ludwigii* (M. Knop, personal communication) and the basidiomycete mushroom *Agaricus bisporus* var. *bisporus* (DOE Joint Genomes Institute), may facilitate broader comparative studies on mating systems and the evolution of mating-type chromosome in fungi.

Acknowledgments

We thank Janis Antonovics for helpful comments on the manuscript. The work was supported under the award NSF-DEB 0747222 to MEH.

References

Antonovics, J. & Abrams, J.Y. 2004. Intra-tetrad mating and the evolution of linkage relationships. *Evolution* **58**: 702–709.

Bergero, R. & Charlesowrth, D. 2009. The evolution of restricted recombination in sex chromosomes. *Trends Ecol. Evol.* **24**: 94–102.

Billiard, S., Lopez-Villavicencio, M., Devier, B., Hood, M.E., Fairhead, C. & Giraud, T. 2010. Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. *Biol. Rev.* (in press).

Denchev, C.M. 2007. *Microbotryum lagerheimii* sp. nov. (Microbotryaceae). *Mycol. Balc.* **4**: 61–67.

Denchev, C.M., Giraud, T. & Hood, M.E. 2009. Three new species of anthericolous smut fungi on Caryophyllaceae. *Mycol. Balc.* **6**: 79–84.

Devier, B., Aguileta, G., Hood, M.E. & Giraud, T. 2009. Ancient trans-specific polymorphism at pheromone receptor genes in basidiomycetes. *Genetics* **181**: 209–223.

Ellegren, H. & Carmichael, A. 2001. Multiple and independent cessation of recombination between avian sex chromosomes. *Genetics* **158**: 325–331.

Fisher, R.A. 1935. The sheltering of lethals. *Am. Nat.* **69**: 446–455.

Fraser, J.A. & Heitman, J. 2003. Fungal mating-type loci. *Curr. Biol.* **13**: R792–R795.

Fraser, J.A., Diezmann, S., Subaran, R.L., Allen, A., Lengeler, K.B., Dietrich, F.S. & Heitman, J. 2004. Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. *PLoS Biol.* **2**: e384.

Freeman, A.B., Kellye, D.K., Shi, T.-L., Hughes, C.F. & Perlin, M.H. 2002. Isolates of *Microbotryum violaceum* from North American host species are phylogenetically distinct from their European host-derived counterparts. *Mol. Phylogenet. Evol.* **23**: 158–170.

Giraud, T., Yockteng, R., López-Villavicencio, M., Refrégier, G. & Hood, M.E. 2008. The mating system of the anther smut fungus, *Microbotryum violaceum*: selfing under heterothallism. *Euk. Cell* **7**: 765–775.

Granberg, A., Carlsson-Graner, U., Arnqvist, P. & Giles, B. 2008. Variation in breeding system traits within and among populations of *Microbotryum violaceum* on *Silene dioica*. *Int. J. Plant Sci.* **169**: 293–303.

Handley, L.L., Ceplitis, H. & Ellegren, H. 2004. Evolutionary strata on the chicken Z chromosome: implications for sex chromosome evolution. *Genetics* **167**: 367–376.

Hood, M.E. 2002. Dimorphic mating-type chromosomes in the fungus *Microbotryum violaceum*. *Genetics* **160**: 457–461.

Hood, M.E. & Antonovics, J. 2004. Mating within the meiotic tetrad and the maintenance of genomic heterozygosity. *Genetics* **166**: 1751–1759.

Hood, M.E., Antonovics, J. & Koskella, B. 2004. Shared forces of sex chromosome evolution in haploid-mating and diploid-mating organisms: *Microbotryum violaceum* and other model organisms. *Genetics* **168**: 141–146.

Johnson, L.J., Antonovics, J. & Hood, M.E. 2005. The evolution of intratetrad mating rates. *Evolution* **59**: 2525–2532.

Kumar, S., Tamura, K. & Nei, M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* **5**: 150–163.

Le Gac, M., Hood, M.E., Fournier, E. & Giraud, T. 2007. Multiple gene phylogenies reveal that several host races of the anther smut fungus *Microbotryum violaceum* belong to different species. *Evolution* **61**: 15–26.

Leach, C.R., Mayo, O. & Morris, M.M. 1986. Linkage disequilibrium and gametophytic self-incompatibility. *Theor. Appl. Genet.* **73**: 102–112.

Lee, N., Bakkeren, G., Wong, K., Sherwood, J.E. & Kronstad, J.W. 1999. The mating-type and pathogenicity locus of the fungus Ustilago hordei spans a 500-kb region. *Proc. Natl. Acad. Sci. USA* **96**: 15026–15031.

Lutz, M., Goker, M., Piatek, M., Kemler, M., Begerow, D. & Oberwinkler, F. 2005. Anther smuts of Caryophyllaceae: molecular characters indicate host-dependent species delimitation. *Mycol. Prog.* **4**: 225–238.

Marais, G. & Galtier, N. 2003. Sex chromosomes: how X-Y recombination stops. *Curr. Biol.* **13**: R641–R643.

Menkis, A., Jacobson, D.J., Gustafsson, T. & Johannesson, H. 2008. The mating-type chromosome in the filamentous ascomycete *Neurospora tetrasperma* Represents a model for early evolution of sex chromosomes. *PLoS Genet.* **4**: e1000030.

Rice, W.R. 1987. The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. *Evolution* **41**: 911–914.

Thomas, A., Shykoff, J., Jonot, O. & Giraud, T. 2003. Mating-type ratio bias in populations of the phytopathogenic fungus *Microbotryum violaceum* from several host species. *Int. J. Plant Sci.* **164**: 641–647.

Veyrunes, F., Waters, P.D., Miethke, P., Rens, W., McMillan, D., Alsop, A.E., Grützner, F., Deakin, J.E., Whittington, C.M., Schatzkamer, K., Kremitzki, C.L., Graves, T., Ferguson-Smith, M.A., Warren, W., Marshall Graves, J.A. 2008. Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. *Genome Res.* **18**: 965–973.

Votintseva, A.A. & Filatov, D.A. 2009. 'Evolutionary strata' in a small mating type-specific region of the smut fungus *Microbotryum violaceum*. *Genetics* **182**: 1391–1396.

Zakharov, I.A. 2005. Intratetrad mating and its genetic and evolutionary consequences. *Russ. J. Genet.* **41**: 402–411.

Received 11 March 2010; revised 29 April 2010; accepted 10 May 2010