Extensive Divergence Between Mating-Type Chromosomes of the Anther-Smut Fungus

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ABSTRACT Genomic regions that determine mating compatibility are subject to distinct evolutionary forces that can lead to a cessation of meiotic recombination and the accumulation of structural changes between members of the homologous chromosome pair. The relatively recent discovery of dimorphic mating-type chromosomes in fungi can aid the understanding of sex chromosome evolution that is common to dioecious plants and animals. For the anther-smut fungus, *Microbotryum lychnidis-dioicae* (= *M. violaceum* isolated from *Silene latifolia*), the extent of recombination cessation on the dimorphic mating-type chromosomes has been conflictingly reported. Comparison of restriction digest optical maps for the two mating-type chromosomes shows that divergence extends over 90% of the chromosome lengths, flanked at either end by two pseudoautosomal regions. Evidence to support the expansion of recombination cessation in stages from the mating-type locus toward the pseudoautosomal regions was not found, but evidence of such expansion could be obscured by ongoing processes that affect genome structure. This study encourages the comparison of forces that may drive large-scale recombination suppression in fungi and other eukaryotes characterized by dimorphic chromosome pairs associated with sexual life cycles.

The cessation of meiotic recombination is a hallmark of sex chromosome evolution, and recent studies on mating-type chromosomes in fungi have broadened our understanding of this phenomenon (Fraser *et al.* 2004; Fraser and Heitman 2004; Hood *et al.* 2004; Whittle and Johannesson 2011). As in plants and animals, the chromosomes determining reproductive compatibility in fungi are derived from an autosome pair. Through processes of recombination suppression and a suite of associated evolutionary forces (*e.g.*, sheltering genetic load, reduced effective population size, and the accumulation of repetitive DNA), a pair of sex or mating-type chromosomes becomes differentiated from the autosomes and from each other (Bergero and Charlesworth 2009). While still relatively poorly understood compared to plant and animal systems, further studies of mating-type chromosomes in fungi can help to illuminate the evolution of dimorphic chromosome pairs, identified as "allosomes" following Montgomery (1911) (also "heterosomes"), as being a fundamental genomic feature of many sexual eukaryotes.

The first size dimorphic fungal mating-type chromosomes were described in the anther-smut fungus, *Microbotryum lychnidis-dioicae* (= *M. violaceum* isolated from *Silene latifolia*) (Hood 2002), but there have been recent conflicting reports regarding the extent of recombination suppression between complementary mating types (referred to as a_1 and a_2). Data provided by Hood and Antonovics (2004) suggested that regions of allelic heterozygosity in linkage to mating type is sufficiently great to comprise the majority of the mating-type chromosome lengths. In brief, 11–12% of random genomic markers was heterozygous and linked to mating type, while the mating-type chromosomes themselves represent only ~13% of the total genome size. More recently, a study provided contradictory evidence based on allelic divergence and segregation analysis using artificial crosses, suggesting that the mating-type-specific region was small and roughly one quarter the length of the mating-type chromosomes (Votintseva and Filatov 2009). This latter study added evidence that the mating-type locus is flanked by two pseudoautosomal regions, and the authors suggested that there was a gradient of divergence between the a_1 and a_2 chromosomes that was greatest at the mating-

type locus and decreased toward these pseudoautosomal regions.

Across fungal species, the size and degree of dimorphism for mating-type chromosomes can be evolutionarily dynamic traits. In some fungal species of the genera *Neurospora*, *Ustilago*, and *Cryptococcus*, regions of recombination suppression linked to mating type have been shown to extend from a few thousand base pairs to >75% of the chromosome length (Fraser *et al.* 2004; Menkis *et al.* 2008; Bakkeren *et al.* 2008; Ellison *et al.* 2011; Whittle *et al.* 2011). Similar to mammalian X and Y chromosomes, mating-type chromosomes can exhibit overall length polymorphism and have frequent localized rearrangements, possibly mediated by the build up of transposable elements in nonrecombining regions (Fraser *et al.* 2004; Whittle *et al.* 2011).

Evolutionary explanations for the origin of dimorphic mating-type chromosomes remain elusive, as do the connections to the evolution of dimorphic sex chromosomes in plants and animals (Abbate and Hood 2010). A very satisfying conceptual model, involving the repeated recruitment of genes determining sexually antagonistic traits, has gained acceptance for the formation of age-structured "evolutionary strata" of recombination cessation in sex chromosomes (Rice 1987; Lahn and Page 1999; Bergero and Charlesworth 2009). However, in the haploid determination of mating compatibility of fungi, the absence of sexes (*i.e.*, the lack of an association between anisogamy and mating types, see Billiard *et al.* 2011) and the absence of mating-type-specific ecological traits aside from processes involved in syngamy, suggests that forces aside from sexual antagonism have given rise to convergence between fungi and other sexual eukaryotes (Fraser *et al.* 2004; Abbate and Hood 2010). There has also been the recent suggestion that the importance of sexually antagonistic traits in the evolution of sex chromosomes in plants and animals has been overstated relative to the available data (Ironside 2010). Then, for fungi the reporting of evolutionary strata in *Cryptococcus neoformans*, *Neurospora tetrasperma*, and *M. lychnidis-dioicae* (Metin *et al.* 2010; Menkis *et al.* 2008; and Votintseva and Filatov 2009, respectively), adds strength to this call for reevaluating the theories and empirical support on sex chromosome evolution.

In particular, a stronger empirical understanding of recombination suppression in fungi is needed so that generalities can be established and compared to the predominating concepts in the sex chromosome literature. Toward this goal, we describe the nonrecombining region of mating-type chromosomes in the anther-smut fungus *M. lychnidis-dioicae* using full-length assemblies of restriction digest optical maps for integration with prior studies. *M. lychnidis-dioicae* is a basidiomycete fungal pathogen in the Pucciniomycotina subphylum; this fungus has been previously reported under the epithet *M. violaceum* when isolated from the host *Silene latifolia*. It is an exceptionally well-studied model for the evolutionary ecology of infectious disease (Bernasconi *et al.* 2009), fungal speciation, and genetics (Giraud *et al.* 2008). It is

among the fungi first used to demonstrate bipolar segregation of mating compatibility factors (Goldschmidt 1928). Sexual reproduction is an obligate component of the life cycle, with meiosis and syngamy occurring prior to infection of each new host plant. The size-dimorphic mating-type chromosomes in *M. lychnidis-dioicae* are known to be rich in transposable elements and poor in other genic content relative to the autosomes (Hood *et al.* 2004). By establishing the extensive divergence between the alternate mating-type chromosomes and support for the presence of two pseudoautosomal regions, this study better resolves our understanding of fungi and the characteristics of genome evolution across sexual eukaryotes.

Materials and Methods

The genotype of *M. lychnidis-dioicae* used in this study was isolated from *S. latifolia* in Lamole, Italy, and haploid products of a meiosis were isolated by micromanipulation and cultured on growth medium (as in Hood and Antonovics 2004). This is the same strain used in the majority of previous studies by Hood and colleagues (Hood 2002; Hood and Antonovics 2004; Hood *et al.* 2004) cited herein, but not in the study by Votintseva and Filatov (2009). The mating types of haploid cultures were identified by pairing with cultures of known mating types and examining the conjugation response that is elicited by the alternate mating pheromone (Day 1979). In addition, PCR primers that discriminate between a_1 and a_2 pheromone receptors (Devier *et al.* 2009) were used to test for mating type following DNA extraction with the DNeasy Plant Mini kit (Qiagen, Valencia, CA).

Chromosomes were separated by pulsed-field electrophoresis (as in Hood 2002) using low melt agarose. Regions of the gels containing bands representing the a_1 and a_2 mating-type chromosomes identified by segregating size dimorphism (Hood 2002) were excised and supplied to OpGen (Gaithersburg, MD) for production of optical maps. High molecular weight DNA was isolated from the agarose and loaded into microfluidic channels for stretching and immobilization. The restriction endonucleases *Nhe*I and *Afl*II were each used as replicate treatments to separately digest immobilized DNA molecules, followed by image analysis of cut site distributions. Overlapping fragment patterns of separate DNA molecules were assembled into high-resolution consensus maps of the full-length chromosomes. Maps of the a_1 and a_2 mating-type chromosomes were compared to each other with the MapSolver software (OpGen) to calculate alignment scores based on a cumulative scoring function that rewards matching cut sites and penalizes mismatch or missing sites. The software's default settings were used to calculate alignment scores. As described in Latreille *et al.* (2007) (see also Tang and Waterman 2001), alignment in MapSolver are generated by a searching algorithm that compares two optical maps in forward and inverse orientations, and alignment scores are positively scaled to the log of an alignment's length and the number of matching restriction cut sites within

them, but missing or mismatched cut sites penalize the score value.

For the enzyme *Afl*II, which produced fewer cut sites overall, the map alignments were also analyzed by relaxing two parameters that contribute to the stringency of the score. Specifically, for this secondary *Afl*II analysis, the number of standard deviations in sizing error allowed in an alignment between fragments was doubled from the default of 2 to 4, and the parameter determining the degree to which mismatched cut sites contribute to the score was adjusted to apply a lesser penalty by doubling the value from the default of 0.2 to 0.4.

Scores of alignments between a_1 and a_2 chromosomes were analyzed separately for the *Nhe*I and *Alf*II restriction site optical maps for the presence of extreme values based on mean and standard error calculations among observed alignments; this was used to assess confidence in describing the pseudoautosomal regions.

Position data for alignments were used to assess whether more or stronger matches between regions of the a_1 and a_2 chromosomes were distributed closer to the pseudoautosomal regions as suggested by the results of Votintseva and Filatov (2009); note that distribution alignments nearer to the pseudoautosomal regions would be expected from the evolutionary strata hypothesis, which assumes additions to an ancestral area of suppressed recombination extending up to the pseudoautosomal boundary where recombination continues (*e.g.*, Lahn and Page 1999). The relationship of proximity to the pseudoautosomal regions and strengths of alignment scores was tested using Pearson bivariate correlation analysis in PSAW Statistics v18 (SPSS, Chicago). The distributional proximity of alignments was tested against random expectations by simulating random positions for a_1 and a_2 sites involved in alignments for an equal number of alignments as in the observed data, with 1000 replicated randomizations for comparison to the observed mean distances to the pseudoautosomal regions.

Results

Optical maps of restriction site distributions show that alignments of the a_1 and a_2 mating-type chromosomes in the anther-smut pathogen, *M. lychnidis-dioicae*, were poor over ~90% of their lengths, indicating the greatest extent of nonrecombination yet shown in a fungal species (Figure 1). The replicate restriction optical maps generated with the enzymes *Nhe*I and *Afl*II gave the same result in this regard (Supporting Information, Figure S1). Regions located at each end of the chromosome arms showed the highest alignment scores and should therefore correspond to pseudoautosomal regions, each being ~4–6% of the chromosome length and extending to each end of the chromosomes. For the optical map generated from *Nhe*I restriction sites, only the pseudoautosomal regions had alignment scores >1.96 standard errors from the mean of observed alignment scores (*i.e.*, exceeding the 95% confidence interval) (Table S1). For the optical map

generated from *Afl*II restriction sites, only the alignments of two pseudoautosomal regions were obtained under the alignment's default parameter settings (Table S1). Under the relaxed parameter setting described above, necessitated by the lower density of cut sites overall with this enzyme, again only the pseudoautosomal regions had alignment scores >1.96 standard errors from the mean of observed alignment scores. Also, only the pseudoautosomal regions represented alignments in common to both *Nhe*I and *Afl*II restriction optical maps. In agreement with pulsed-field electrophoretic gels published previously (Hood 2002), the a_1 chromosome is shorter, estimated from the optical maps to be 3.3 Mbp. Thus the nonrecombining region of the a_1 chromosome represents a slightly smaller proportion than for the larger 4.0 Mbp a_2 chromosome; nonrecombining regions being 89 *vs.* 91% of the a_1 and a_2 chromosomes, respectively.

Alignment scores for sections in the nonrecombining regions of the mating-type chromosome were weaker and covered shorter lengths than for the pseudoautosomal regions (Figure 1). Using position data based upon the distance in kilobase pairs to pseudoautosomal regions, alignments between the a_1 and a_2 chromosomes along the length of the nonrecombining regions were not nearer to pseudoautosomal regions than expected by chance alone. In particular, the mean distance of alignments to the pseudoautosomal regions was not closer than 95% ($n = 1000$ replications) of simulated randomized alignment positions (Figure 2). These results were similar for both mating-type chromosomes, but with the a_1 chromosome approaching significance with the mean distance to pseudoautosomal regions being closer than 91% ($n = 1000$ replications) of simulated randomized alignment positions. Moreover, there was no apparent positive relationship between the strength of the alignment scores and proximity to the pseudoautosomal regions as would also have been expected by more recent cessation of recombination in those regions (a_1 correlation coefficient -0.171, $P = 0.4$; a2 correlation coefficient 0.060, $P = 0.8$).

This distribution of the alignments in the nonrecombining region could potentially be interpreted to suggest a large scale inversion involving the majority of the mating-type chromosomes, followed by further localized rearrangements that obscure the inverse co-linearity of alignment order that is expected to result from such an inversion event. Alternatively, the distribution of alignments may suggest "hot spots" (see Richard *et al.* 2008) of segmental duplications where sites in the nonrecombining region of one chromosome match multiple sites on the complementary chromosome.

Discussion

The region of the genome containing the mating-type locus in *M. lychnidis-dioicae* has likely been shaped by long-term evolutionary processes, in contrast to fungi suggested to serve as models for the early evolution of sex chromosomes of plants and animals (Fraser and Heitman 2004; Menkis *et al.* 2008). Alternate alleles at the mating pheromone
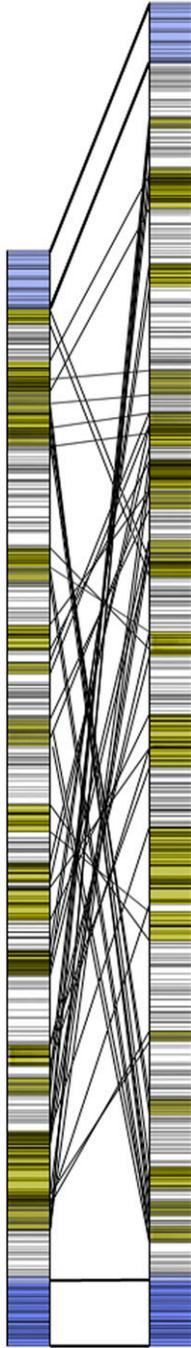
Figure 1 Alignment of restriction digest optical maps for the a$_1$ and a$_2$ mating-type chromosomes of *Microbotryum lychnidis-dioicae* (= *M. violaceum* isolated from *Silene latifolia*). Horizontal lines within each chromosome represent the distribution of sites cut by the restriction endonucleases *Nhe*I and *Afl*II; separate restriction enzyme maps are shown in Figure S1. Blue shading indicates the two pseudoautosomal regions, connected by bold lines. Yellow shading indicates areas of weaker alignment, connected by lines, in the nonrecombining region. The alignments for the *Afl*II optical map are included for the less stringent alignment parameters, as described in the text, where the default parameters produced alignments only for the pseudoautosomal regions. The a$_1$ and a$_2$ chromosomes are estimated to be ~3.3 and 4.0 Mbp, respectively.

receptor locus in *Microbotryum* actually exhibit the most ancient pattern of balancing selection and maintenance across speciation events for a locus in any known group of organisms (Devier *et al.* 2009). The observation that optical maps could not be aligned over most of the mating-type chromosome lengths indicates that the recombination suppression is sufficiently ancient to allow the extensive accumulation of differentiating mutations, noting that non-recombining regions may be subject to higher substitution rates than recombining regions (Charlesworth 1994).

There are two technical issues that likely contribute to disagreement between the prior study suggesting the non-recombining region of the mating-type chromosomes in *M. lychnidis-dioicae* was small (Votintseva and Filatov 2009) and the two studies indicating extensive divergence of the chromosome pair [*i.e.*, these optical maps and the previous measure of allelic heterozygosity in linkage to mating type by Hood and Antonovics (2004)]. The smaller size estimate of the nonrecombining region was based upon a set of shot-gun DNA fragments that were originally isolated from the mating-type chromosome bands in electrophoretic karyo-types by Hood *et al.* (2004). Allelic identity *vs.* divergence at these loci between haploid a$_1$ and a$_2$ cultures was used to assign these loci to the pseudoautosomal *vs.* nonrecombining regions of the mating-type chromosomes, respectively. The first issue relates to background autosomal contamination that may be present in the gel-isolated electrophoretic bands from which the shotgun fragments originated. Auto-somal contamination would not create a false positive in assessing differences between the mating-type chromosomes and autosomes in Hood *et al.* (2004), but mistakenly assign-ing homozygous autosomal loci to mating-type chromosomes would inflate the estimated size of the recombining pseu-doautosomal regions in Votintseva and Filatov (2009). Quan-tification of such autosomal contamination may be achieved when a well-assembled genome sequence is produced. The second issue is related to the nature of the 61 loci found by Votintseva and Filatov (2009) that have allelic identity be-tween sequenced PCR products from haploid a$_1$ and a$_2$ cul-tures and were thus assigned to pseudoautosomal regions. Among these sequences, ~20% were identified as probable transposable elements in Hood *et al.* (2004). As repetitive elements, they are expected to be present as sequences in multiple genomic locations, at nonsyntenic positions on both mating-type chromosomes as well as on autosomes. There-fore, including transposable elements sequences with the 0% divergence between PCR products from a$_1$ and a$_2$ cultures would cause the proportion of loci assigned to the pseudoau-tosomal regions to be substantially overestimated in Votintseva and Filatov (2009).

The conclusions from the optical maps are in strong agreement with the estimate of heterozygosity in the majority of the mating-type chromosomes from the genome-wide survey of marker variation and linkage to mating type (Hood and Antonovics 2004). In general, recent studies have cited the smaller size estimate of the nonrecombining region of
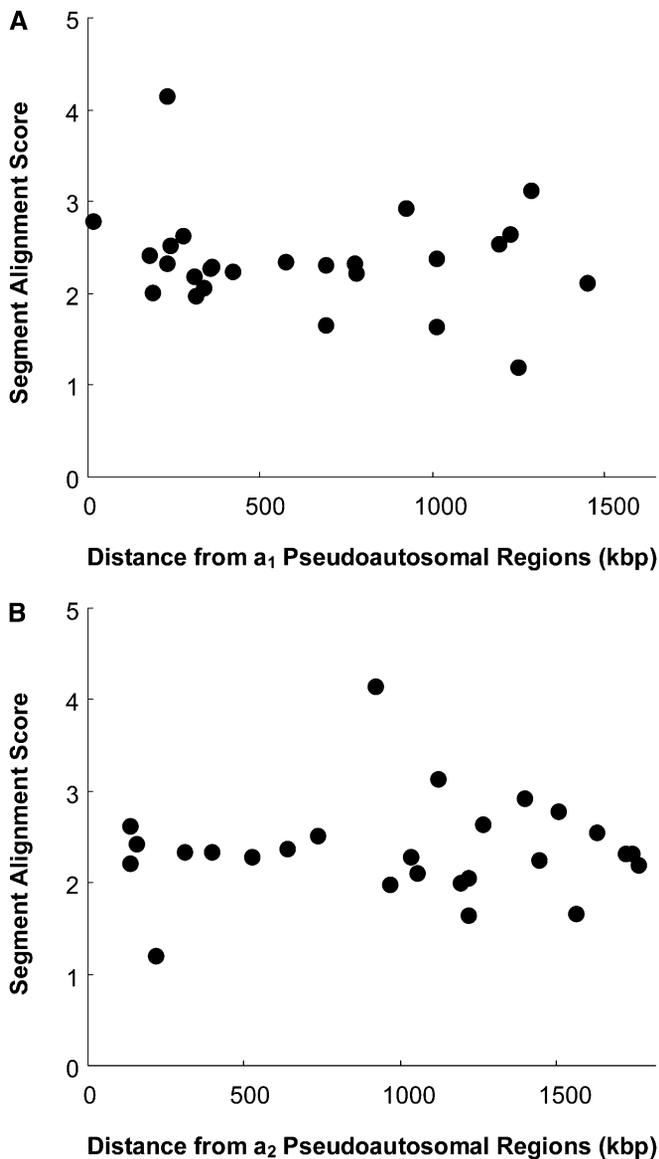
Figure 2 Relationship between alignment scores for regions of the *Nhe*I and *Afl*II optical maps and alignment positions on the mating-type chromosomes of *Microbotryum lychnidis-dioicae*. Positions of aligned regions for the a_1 (A) and a_2 (B) chromosomes were plotted relative to the distance in kilobase pairs from the closest pseudoautosomal region; thus, the x-axis for each chromosome measures roughly half the chromosome length.

M. lychnidis-dioicae in Votintseva and Filatov (2009), but they have not used that estimate in additional quantitative analyses that would necessitate reassessment (*e.g.*, Ellison *et al.* 2011; Kües *et al.* 2011). Further studies are needed to ascertain whether there is sufficient variation among strains of *M. lychnidis-dioicae* that might contribute to the contradictory results, particularly as Votintseva and Filatov (2009) used different strains for the segregation analysis *vs.* the source of loci that they characterized. However, at least the presence and direction of the mating-type chromosome size dimorphism has been consistent among multiple field-collected specimens of this fungal species (Hood 2002).

To advance the analogies between mating-type chromosomes and sex chromosomes, greater consideration is needed regarding the nature of the nonrecombining regions. Relative to dioecious plants and animals, few traits have the potential to be harmful when recombined between the mating types in fungi. The exception might be traits involved directly in the process of syngamy (Billiard *et al.* 2011), but these are not normally considered under the concept of sexual antagonism in plants and animals. Consequently, the most accepted adaptive model for expansion of recombination suppression in sex chromosome evolution does not likely apply to fungi (Bergero and Charlesworth 2009). Moreover, even among plants and animals, broad empirical support for sexually antagonistic traits as the driving force for recombination suppression has been suggested to be lacking (Ironside 2010); nonadaptive processes that fix recombination-blocking mutations (*e.g.*, inversions) in linkage to regions of permanent heterozygosity by drift or under the fitness effects of genetic load were proposed as alternative models (Ironside 2010), which may just as well be applicable to fungi.

Linkage to mating types in fungi has been the subject of considerable study, and reports of large regions of recombination suppression in diverse species prompts the need for mechanistic explanations (*e.g.*, Menkis *et al.* 2008; Votintseva and Filatov 2009; Metin *et al.* 2010). Particular to basidiomycete fungi, recombination suppression has been suggested to allow linkage between the two loci determining pre- and postsyngamy viability (Bakkeren and Kronstad 1994). Also, linkage of mating type with the centromere is frequently associated with the mating system of automixis in fungi, as in some insects (*i.e.*, intratetrad selfing) (Lewis and John 1963; Mogie 1986; Zakharov 2005). Under these scenarios, further expansion of a nonrecombining region is not necessarily expected because a single step of recombination cessation is sufficient, as might have achieved centromere linkage of mating type in *N. tetrasperma* (Ellison *et al.* 2011). However, theoretical studies regarding the automictic mating system, in particular, have described the spread of modifiers that suppress recombination with mating type, and the accumulation of load loci (*i.e.*, deleterious recessive mutations) or overdominant loci (*i.e.*, heterozygote advantage mutations) can accelerate this evolution (Antonovics and Abrams 2004; Johnson *et al.* 2005). Lending strength to this theoretical scenario, both *M. lychnidis-dioicae* and *N. tetrasperma*, two fungi with cessation of recombination across the majority of the mating-type chromosomes, are in fact strongly automictic (Giraud *et al.* 2008; Ellison *et al.* 2011).

The test of whether alignments of restriction digest optical maps supported the presence of evolutionary strata in *M. lychnidis-dioicae* provided a negative but nonconclusive result. Divergence of mating-type chromosomes by evolutionary strata would predict that the most recent cessation of recombination, and thus the least divergence between the a_1 and a_2 mating-type chromosomes, would be nearest the

pseudoautosomal regions. However, neither the strengths nor the distributions of alignments in the nonrecombining region were greater nearer to the pseudoautosomal regions. Importantly, these results do not prove that a history involving evolutionary strata is absent in this species, but the results indicate that perhaps other types of data, including physical mapping of DNA sequences, should be pursued to help resolve the issue. At present, genome sequencing of the strain of *M. lychnidis-dioicae* by the Broad Institute (www. broadinstitute.org) has not provided long enough sequence assemblies for the placement on the optical maps, perhaps challenged by elevated densities of transposable elements on the mating-type chromosomes of *M. lychnidis-dioicae* (Hood *et al.* 2004).

The confirmation and characterization of two pseudoautosomal regions in *M. lychnidis-dioicae*, representing just ~10% of the mating-type chromosomes, draw additional similarity between sex-related allosomes in fungi and other eukaryotes. Like the evolution of nonrecombining regions of sex chromosomes, the pseudoautosomal regions have received recent attention (reviewed by Otto *et al.* 2011). Pseudoautosomal regions at either side of the sex-determining locus are found in the majority of dioecious plants and about half of animals. The role of pseudoautosomal regions is often assumed to be to facilitate the proper alignment and segregation of sex chromosomes during meiosis in the heterogametic sex, although other selective forces that favor recombination may be important (Otto *et al.* 2011). In fungi, however, the haploid determination of mating compatibility creates the distinction that pseudoautosomal regions would be under more frequent selection to maintain this homolog-pairing role during every meiosis, rather than in just half of meioses, because there is no homogametic diploid. Similarly, the role of partial sex linkage in plants and animals as an influence on the evolution of pseudoautosomal regions (Otto *et al.* 2011) would be contrasted by the absence of sexes in fungi. Thus, the distribution and structure of fungal pseudoautosomal regions may contribute substantially to the understanding of their occurrence in dioecious plants and animals by the contrasting expectation about the relevant evolutionary forces.

Recent work has shown that the evolution of linkage to mating type occurs independently in various species of *Microbotryum* (Abbate and Hood 2010; Petit *et al.* 2012). The results presented here reflect the mating-type chromosome characteristics of the best-studied species in this genus, including the a_1 genotype that is the subject of a current genome sequencing project (Broad Institute). However, variation in the overall structure of mating-type chromosomes is known to occur in both distantly and very closely related fungi. With indications that variation in reproductive mode is a major influence on these genomic structures (*e.g.*, Whittle *et al.* 2011), fungi provide opportunities to address the role of sexual life cycles for the origins of allosomes in a broad phylogenetic context and for making illuminating comparisons across eukaryotic kingdoms.

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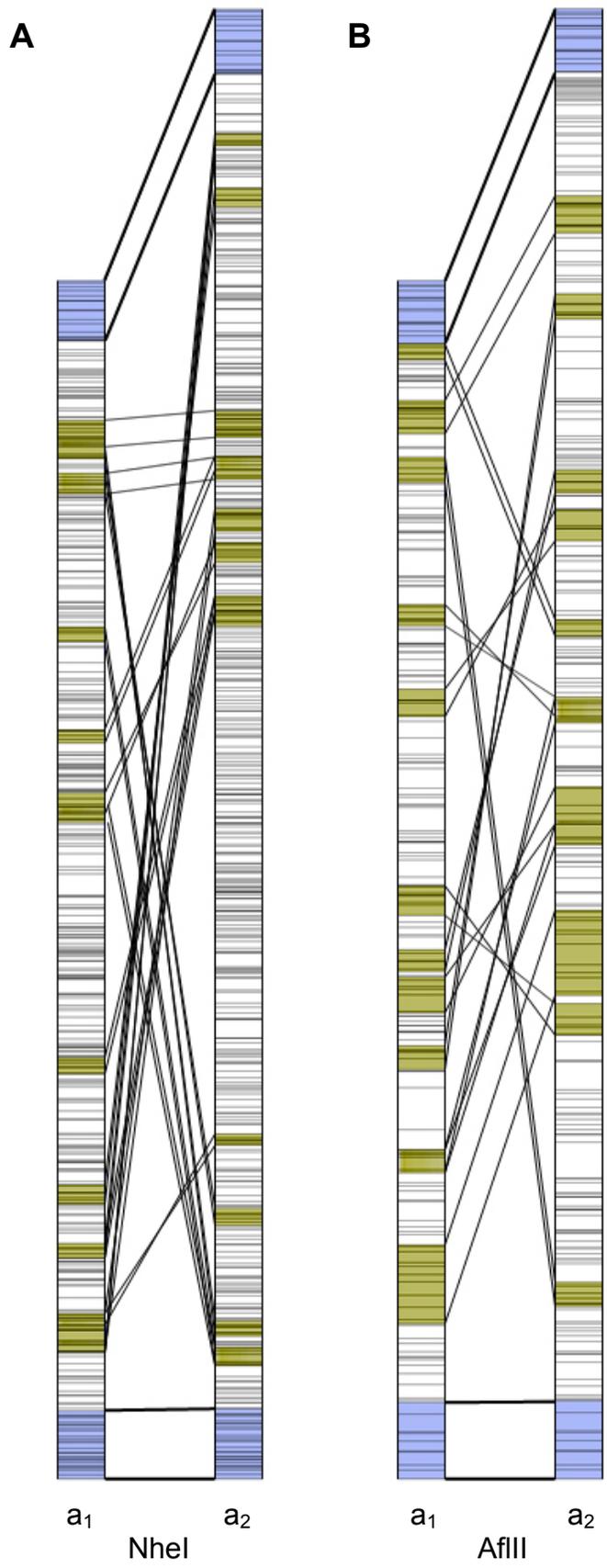
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Extensive Divergence Between Mating-Type Chromosomes of the Anther-Smut Fungus

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A   B

a₁   a₂   a₁   a₂
   NheI   AflII

Figure S1   Alignment of restriction digest optical maps for the a1 and a2 mating type chromosomes of Microbotryum lychnidis-dioicae (=M. violaceum isolated from Silene latifolia). Horizontal lines within each chromosome represent the distribution of sites cut by the restriction endonucleases NheI and AflII. Panel A and B show optical maps for the separate restriction enzymes. Blue shading indicates the two pseudoautosomal regions, connected by bold lines. Yellow shading indicates areas of weaker alignment, connected by lines, in the non-recombining region. The alignments for the AflII optical map are included for the less stringent alignment parameters, as described in the text, where the default parameters produced alignments only for the pseudoautosomal regions. The a1 and a2 chromosome are estimated to be ca. 3.3 and 4.0 Mbp, respectively.

Table S1 Data on alignments of restriction optical maps of mating type chromosomes in *Microbotryum lychnidis-dioicae*.

Restriction Enzyme Optical Map	MapSolver Alignment Parameters	Alignment Score: Pseudoautosomal Regions (PAR)*	Alignment Score: Non-Recombining Regions	Position on a1 Chromosome**	Position on a2 Chromosome**
NheI					
	Default				
		19.871 (bottom)			
		6.261 (top)			
			2.508	428	915
			2.278	2683	2725
			4.135	2814	2839
			2.181	2736	1995
			2.33	760	3451
			2.626	1815	2495
			2.405	367	3607
			1.989	374	2567
			2.226	608	2316
			2.915	1113	2360
			2.608	2766	317
			2.2002	2262	318
			2.262	2686	704
			1.182	1794	396
AflII					
	Default				
		7.145 (bottom)			
		9.776 (top)			
			n/a	n/a	n/a
	Less-Stringent***				
		7.868 (bottom)			
		11.799 (top)			
			2.047	515	1402
			1.96	492	2857
			2.098	1630	1238
			1.648	872	1749
			2.301	872	2081

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2.534	1373	1813
2.322	2989	3427
2.308	2441	2097
2.771	3201	2314
1.633	2203	2602
3.115	1466	2700
2.362	1193	3186

* position relative to Fig 1

** positions in Kbp

*** as described in text