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Diverse members of the Xylariales lack canonical mating-type regions

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Abstract

A survey of genomes reported here for 10 isolates of *Monosporascus* species and an additional 25 genomes from other members of the Xylariales (representing 15 genera) available in public databases indicated that genes typically associated with *MAT1-1* (*mat A*) or *MAT1-2* (*mat a*) mating types are absent or have diverged greatly relative to counterparts in other Pezizomycotina. This was particularly surprising for isolates known to be homothallic, given that homothallic members of the Pezizomycotina typically possess a *MAT1-1-1* (*mat A-1*) gene and one or both of two other closely-linked mating-type genes, *MAT1-1-2* (*mat A-2*) and *MAT1-1-3* (*mat A-3*), in addition to *MAT1-2-1* (*mat a-1*). We failed to detect candidate genes for either *MAT1-1-1* or *MAT1-1-2* in any member of the Xylariales. Genes related to *MAT1-2-1* and *MAT1-1-3* are present in the genomes examined, but most appear to be orthologs of MATA_HMG (high-mobility group) genes with non-mating-type functions rather than orthologs of mating-type genes. Several MATA_HMG genes were found in genome positions that suggest they are derived from mating-type genes, but these genes are highly divergent relative to known *MAT1-2-1* and *MAT1-1-3* genes. The genomes examined represent substantial diversity within the order and include *M. cannonballus*, *M. ibericus*, *Xylaria hypoxylon*, *X. striata*, *Daldinia eschscholzii*, *Eutypa lata*, *Rosellinia necatrix*, *Microdochium bolleyi* and several others. We employed a number of avenues to search for homologs, including multiple BLAST approaches and examination of annotated genes adjacent to genes known to flank mating regions in other members of the Ascomycota. The results suggest that the mating regions have been lost from, or altered dramatically in, the Xylariales genomes examined and that mating and sexual development in these fungi are controlled differently than has been reported for members of the Pezizomycotina studied to date.

Keywords

Xylariales; *Monosporascus*; *Xylaria*; *Microdochium*; mating type; genomics

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1. Introduction

The mating-type genes that control sexual reproduction have been studied in diverse homothallic and heterothallic members of the Ascomycota. Within the Sordariales, Hypocreales, Ophiostomatales and other orders of Ascomycota, individuals from heterothallic species possess one of two mating idiomorphs. One of these idiomorphs (referred to as *mat A* in *Neurospora*, but *MAT1-1* in other groups) typically has three different linked genes: *MAT1-1-1* (*mat A-1*), *MAT1-1-2* (*mat A-2*), and *MAT1-1-3* (*mat A-3*). The *MAT1-1-1* gene shares a region of homology with the yeast *mat α 1* gene. The other idiomorph (designated *mat a* in *Neurospora*, *MAT1-2* in other groups) typically has one gene, designated *MAT1-2-1* (aka *mat a-1*) (reviewed by Martin et al. 2010).

The mating-gene arrangement in homothallic species varies, but when it is known, it usually falls within one of three types. In one type, both mating-type regions are present in the same haploid nucleus but are in different parts of the genome. A second type has *MAT1-1* and *MAT1-2* genes present within a single mating-type region. A third type has the *MAT1-1* (*mat α 1*) region but lacks an identifiable *MAT1-2* region. In the genus *Neurospora*, these three homothallic types are represented by *N. sublineolata*, *N. pannonica*, and *N. africana*, respectively (Gioti et al. 2012).

Relevant to the results presented below, several mating-type genes have domains that are characteristic of the high mobility group (HMG) of transcriptional activator genes. Among the mating-type genes with HMG domains, *MAT1-1-1* genes are the most distantly related. *MAT1-1-1* genes encode proteins that are part of a family referred to as MAT α _HMG, which includes the *Saccharomyces cerevisiae* MAT α 1 protein, whereas *MAT1-2-1* and *MAT1-1-3* genes encode proteins in the MATA_HMG family (Jackson et al. 2013, Martin et al. 2010). Although the *MAT1-2-1* and *MAT1-1-3* proteins possess clear regions of homology, phylogenetic analyses employing sordariomycete versions of these proteins suggested they form two distinct clades (Debuchy et al. 2010). The proteins encoded by the fourth group of mating-type genes present in diverse members of the Pezizomycotina, *MAT1-1-2* (*mat A-2*), has not been linked to the HMG superfamily, but homologs of this gene are conserved widely among members of the Sordariomycetes and have a conserved domain (PFAM PF17043). There is evidence that the *MAT1-1-1* protein interacts with other mating-type proteins and can be required for ascocarp development (Dyer et al. 2016).

Species of *Neurospora* and other Sordariomycetes possess other genes in the MATA_HMG transcription-factor family, including NCU03481 and *fmf-1* (NCU09387), that have been implicated in aspects of sexual development but which are not specifically mating-type genes. They are not located within the mating-type region and therefore in heterothallic species are found in both *MAT1-1* and *MAT1-2* strains. The *fmf-1* gene encodes the homolog of *Schizosaccharomyces pombe* Ste11p, a protein that activates multiple genes required for sexual development (Iyer et al. 2009). *N. crassa fmf-1* mutants are defective in ascocarp development, and the encoded protein appears to control other genes involved in sexual development (Johnson 1979, Wang et al. 2014). The *N. crassa* gene designated NCU03481 is the ortholog of the *Podospora anserina* gene *PaHMG8*. Deletion of *PaHMG8* in *P. anserina* results in failed ascocarp development, and similar to *fmf-1*, its gene product

has been implicated in the regulation of genes involved in sexual development (Ait Benkhali et al. 2013, Gautier et al. 2018).

The results reported here began with an attempt to identify mating-type genes in isolates of *Monosporascus*. The described species of *Monosporascus* include *M. cannonballus*, strains of which are important agricultural root pathogens on Cucurbitaceae (Edelstein et al. 1999), and *M. ibericus*, which was described as an endophyte occurring on diverse plants in Spain (Collado et al. 2002). Strains of a third described species, *M. eutypoides*, are also pathogenic on cucurbits and are closely related to *M. cannonballus* (Ben Salem et al. 2013). In the case of all three species, certain strains have been observed to produce ascocarps, and although publications do not specifically address the issue of homothallism versus heterothallism, it is possible to infer that many strains are homothallic. Direct evidence for homothallism among strains of *M. cannonballus* and *M. eutypoides* exists in the fact that hyphal-tip derived cultures (Ben Salem et al. 2013) and ascospore-derived progeny (Michael Stanghellini, personal communication) produce perithecia and ascospores. In the publication describing *M. ibericus* it was noted that two strains were observed to produce ascocarps in culture although eight others did not (Collado et al. 2002).

Our interest in species of *Monosporascus* derives in part from the fact that they are common root endophytes of diverse plants in arid ecosystems of the western United States (Dean et al., 2015; Porras-Alfaro et al. 2008, 2014). The identification of isolates from our fungal endophyte surveys have relied on molecular characterization, however, and these isolates have not been observed to produce ascocarps. Although as mentioned above homothallism has been documented for certain isolates in the genus, heterothallism has not been reported. Our failure to obtain ascocarps, along with reports of similar failures for certain strains in studies cited above, suggested the possibility that members of certain *Monosporascus* lineages might be heterothallic. We have obtained genome sequences from 10 isolates that include known pathogenic strains as well as endophytic strains that span the range of known diversity in the genus. We employed alignment-based searches with diverse mating-type genes to examine assembled genomes, including genomes from known homothallic strains, for the mating-type genes typically associated with *MAT1-1* and *MAT1-2* regions. The *Monosporascus* genomes contained genes distantly related to *MAT1-2-1* (*mat a-1*) and *MAT1-1-3* (*mat A-3*), but homologs of *MAT1-1-1* (*mat A-1*) and *MAT1-1-2* (*mat A-2*) were not found in either the agricultural or endophytic isolates.

To determine if our results were unique to the genus *Monosporascus*, we performed similar searches with diverse members of the Xylariales. In no instance were we able to identify genes typically associated with the *MAT1-1* region. Moreover, although homologs of the *MAT1-2-1* and *MAT1-1-3* genes were identified, these genes were not clear orthologs of MATA_HMG- encoding mating-type genes based on sequence similarity. Nevertheless, the positions of several of these genes relative to genes that commonly flank mating-type regions suggest they might well be derived from mating-type genes. Our results suggest that species of *Monosporascus* and other Xylariales lack typical *MAT1-1* and *MAT1-2* regions, and although our sample size is limited by available Xylariales genomes, the results imply that the genetic mechanisms that control sexual reproduction in the Xylariales are different from those of other members of the Pezizomycotina.

2. Materials and methods

2.1. Genomes examined

Genome assemblies were available from public databases for the Xylariales members presented in Table 1 with the exception of 10 isolates from the genus *Monosporascus*. The results presented here for *Monosporascus* isolates were obtained using genome assemblies created from Illumina sequences. Six of the isolates examined came from root endophyte surveys conducted in New Mexico (eg. Dean et al. 2015). *Monosporascus cannonballus* strains CBS 609.92 and 586.93, as well as *Monosporascus ibericus* strain CBS 110550, were obtained from the CBS-KNAW fungal collection (<http://www.westerdijkinstituut.nl/Collections/>). *Monosporascus cannonballus* MC13–8B was a gift from Michael Stanghellini, University of California, Riverside. Genomic DNA was purified using a CTAB extraction protocol (Hutchinson et al. 2015). Genomic libraries for each isolate were prepared using the KAPA Hyper Prep Kit (Kapa Biosystems, Wilmington, Massachusetts). Paired-end sequencing was conducted using an Illumina NextSeq 500 platform configured for mid-output (130 million reads) and 150 base-pair read lengths. The resulting Illumina reads were paired, trimmed and filtered using Trimmomatic (Bolger et al. 2014). The quality control filtered reads from each isolate were then assembled using three separate software packages designed for short read de-novo microbial genome assemblies: Velvet (Zerbino et al. 2008), SPAdes (Bankevich et al. 2012) and SOAPdenovo2 (Luo et al. 2012). The parameters for each assembly software package were optimized on a per-sample basis by generating multiple assemblies then comparing common assembly metrics and the overall quality of the assembly. Assembly statistics were generated using Quast (Gurevich et al. 2013), and assemblies that contained a small total contig number while maintaining a large N50 value were selected as representative assemblies to be used in subsequent steps. Quality assessment of these representative assemblies was performed using the BUSCO software package (Simão et al. 2015), which checks for the presence of fungal specific-single copy orthologs. Assemblies produced in SPAdes showed the highest consistent results in both categories and were annotated using the gene prediction program AUGUSTUS (Stanke, and Morgenstern 2005, Hoff and Stanke 2013). AUGUSTUS was run using *Neurospora crassa* for the species parameter, limited to few alternative transcripts and only complete gene predictions. These annotated genomes were used in the analysis described below. They have been deposited at GenBank with accession numbers QJNS000000000, QJNT000000000, QJNU000000000, QJNV000000000, QJNW000000000, QJNX000000000, QJNY000000000, QJNZ000000000, QJOA000000000, and QJOB000000000 (Table 1).

2.2. Mating-type gene searches

Given that genes required for mating and ascocarp development in the Xylariales have not been reported, we relied on genes from other orders in the Sordariomycetes to serve as references for identifying potential MAT homologs in members of the order. The NCBI database contains annotations for genes of both mating types for several members of the Sordariales, Hypocreales, Ophiostomatales and Magnaporthales. The protein sequences in Table 2 were selected based on sequence quality and completeness, and they included representatives from homothallic and heterothallic strains. These sequences were used as queries in alignment-based methods (BLAST) to identify potential homologs in the

Xylariales. When nucleotide-based searches failed to find Xylariales mating-type gene homologs, we employed protein amino-acid sequences to search protein databases and translated nucleotide sequences. Those results are presented here.

Local BLAST protein and nucleotide databases were created using the annotation files for *Eutypa lata*, *Daldinia eschscholzii*, *Microdochium bolleyi*, *Pestalotiopsis fici*, *Rosellinia necatrix*, *Monosporascus cannonballus*, *Xylaria striata*, *Hypoxylon* sp., *Pseudomassariella vexata*, and several *Monosporascus* sp. isolates from our southwestern United States collections (Table 1). Diverse MAT protein sequences (Table 2) were used in BLASTp, tBLASTn and tBLASTx queries against the Xylariales databases. Potential homologs of MAT1-2-1 (Mat a-1) and MAT1-1-3 (Mat A-3) were identified as amino-acid and translated-nucleotide sequences based on best BLAST scores in the annotated Xylariales databases. Regions of putative homology were identified using the coordinates obtained from BLAST alignments. In cases where a BLAST alignment was shorter than the original query, sequence ranges were expanded to ensure that full regions of potential homology were captured. In cases where functional annotations were available they were also examined for hits to MAT-associated domains and superfamilies. Because proteins encoded by *MAT1-2-1* and *MAT1-1-3* genes belong to the MATA_HMG family and filamentous fungi typically possess distant homologs of these proteins not associated with the mating-type region (see, for example, Hutchinson et al. 2015), potential Xylariales MAT1-2-1 and MAT1-1-3 homologs were used in reciprocal searches against NCBI databases to assess the likelihood that these proteins were true orthologs of mating-type proteins.

2.3. Molecular alignments and phylogenetic analyses.

Amino-acid sequences for HMG homologs employed in phylogenetic analyses were aligned with Clustal Omega (Sievers et al. 2011). Trees were constructed using RaxML (Stamatakis 2006) with the PROTCATDAYHOFF substitution model and 1000 bootstrap replicates.

In addition to BLAST searches against whole Xylariales genomes and protein databases, we carefully examined regions between and adjacent to homologs of genes that typically flank mating regions in other members of the Ascomycota (Butler et al. 2004, see below). These flanking genes were identified in all members of the Xylariales by employing searches with the Sla2 (XP_964240.1) and Apn2 (ESA43843.1) predicted-protein sequences from *Neurospora crassa*. Regions between and flanking these genes in the Xylariales genomes were employed in BLAST searches against GenBank databases to search for homologs of genes associated with mating type.

3. Results and Discussion

We examined the genomes of 35 strains across 15 different Xylariales genera. Our searches employed known MAT1-1-1, 2, 3 (Mat A-1, 2, 3) and MAT1-2-1 (Mat a-1) amino-acid sequences from members of the Sordariales, Hypocreales, Magnaporthales and Ophiostomatales (Table 2). In no instance were we able to identify likely Xylariales homologs (orthologs) for either MAT1-1-1 or MAT1-1-2. While we detected homologs of the *MAT1-2-1* (*mat a-1*) and *MAT1-1-3* (*mat A-3*) mating-type genes, the interpretation of these results is complicated to by the fact that proteins encoded by these genes are in the

MATA_HMG family of transcriptional activators, and members of the Pezizomycotina typically have multiple genes for this family (discussed below).

Considering the possibility that genes of the mating-type region have undergone rapid change that would confound BLAST searches, we paid special attention to regions that possess homologs of two genes, *sla2* and *apn2*, that flank the mating-gene region in species of *Neurospora* and diverse Ascomycota (Butler et al. 2004, Gioti et al. 2012, Hutchinson et al. 2016). We used the *N. crassa* Sla2 and Apn2 predicted proteins to identify homologs in members of the Sordariomycetes, Leotiomycetes, Eurotiomycetes and Dothidiomycetes (Supplemental Table S1), as well as in the Xylariales (Supplemental Table S2). We then examined these regions for *MAT1-1-1* (*mat A-1*) and *MAT1-2-1* (*mat a-1*) genes. With the exception of the Xylariales, members of all groups examined possessed either one or both of these mating-type genes either between or adjacent to *apn2* and *sla2* genes. Again, none of the Xylariales genomes possessed genes with apparent homology to *MAT1-1-1*.

Our results with respect to MATA_HMG genes are more difficult to interpret. While *sla2* and *apn2* homologous genes were found to be linked in most members of the Xylariales, in most cases, homologs of *MAT1-2-1* and *MAT1-1-3* were absent from *sla2/apn2* regions, further suggesting a loss or displacement of these genes. In some instances, however, MATA_HMG genes were found to be between or adjacent to *sla2/apn2* genes (Supplemental Tables S2 and S3). In cases of close linkage, the orientation and order of the three genes (*sla2*, *apn2*, MATA_HMG) varied among the genomes examined (Fig. 1).

In efforts to evaluate whether MATA_HMG-encoding genes were true orthologs of mating-type genes, we performed reciprocal BLAST searches against genome and protein sequences from species of *Neurospora* using the predicted Xylariales proteins identified in searches with known MAT1-2-1 and MAT1-1-3 sequences. We targeted sequences from species and strains of *Neurospora* because the mating-type and non-mating-type HMG genes in this genus have been characterized, and their chromosomal locations are known. The genome assemblies for *Monosporascus* strains and several strains from other Xylariales species each possessed multiple HMG genes. Almost invariably the Xylariales HMG proteins did not have top hits to either Mat a-1 (MAT1-2-1) or Mat A-3 (MAT1-1-3) proteins or their respective genes in *Neurospora* genomes. Instead, the top hits for the Xylariales sequences typically corresponded to one of the non-mating-type MATA_HMG genes discussed above, either the gene designated NCU03481 (GenBank accession XM_951277) or the gene designated *fmf-1* (GenBank accession 958740), both of which have been implicated in sexual development (Iyer et al. 2009).

Acknowledging that selection, differential rates of divergence or long divergence times could obscure true relationships and likewise make BLAST scores unreliable as indicators of relationships, we employed diverse Xylariales MATA_HMG-predicted proteins and those from other Pezizomycotina in phylogenetic analyses using alignments based on full-length proteins as well as alignments based on only the HMG core (residues 122–233 in the predicted *N. crassa* Mat a-1 protein, GenBank accession AAA33598; Supplemental Fig. S1). In these analyses, known MAT1-2-1 proteins from diverse Sordariomycetes and a member of the Leotiomycetes (*Sclerotinia sclerotiorum*) consistently formed a distinct group. In

contrast, similar to results obtained with BLAST results, several Xylariales proteins consistently grouped with either the NCU03481 gene product or FMF-1. Many Xylariales sequences, however, did not show clear affinities with any of these previous groups (Supplemental Tables S2 and S3, Supplemental Fig. S1, TreeBase submission ID 23036).

[Reviewer access to the HMG alignment can be found at TreeBase: <http://purl.org/phylo/treebase/phyloids/study/TB2:S23036?x-access-code=544f6f4a1a7b17fa03ce8b474c559cf0&format=html>]

Although we failed to identify obvious *MAT1-2-1* orthologs based on sequence similarity, it is safe to assume that some, if not most, of the MATA_HMG-family genes observed in these genomes serve directly or indirectly in sexual development, whether or not mating is involved. It is possible that members of Xylariales possess a mode of sexual reproduction similar to the “unisexual” reproduction reported for *Huntia moniliformis*, which appears to rely on *MAT1-2-1* in the absence of the *MAT1-1* region (Wilson et al. 2015). Alternatively, given that the *P. anserina* orthologs of *fmf-1* (*PaHMG5*) and NCU03481 (*PaHMG8*) gene products have been proposed as upstream regulators of mating-type genes (Benkhali et al. 2013), it seems plausible that regulatory pathways have been modified to bring mating and other aspects of sexual development under the control of these upstream regulators.

While none of the predicted Xylariales MATA_HMG proteins grouped with known MAT1-2-1 proteins in tree building analyses (Supplemental Fig. S1), the proximity of several of the MATA_HMG Xylariales genes to genes that typically flank mating genes in Sordariomycetes (Fig. 1) suggests they are highly divergent *MAT1-2-1* genes. Evidence has been presented for the direct physical interaction of MAT1-2-1 proteins with either MAT1-1-1 (Jacobsen et al. 2002) or other MAT transcription factors (Zheng et al. 2013). Our observations suggest the possibility that Xylariales genomes have lost the *MAT1-1* mating-type region. It is therefore possible that the loss of *MAT1-1* genes in the Xylariales could have resulted in reduced or altered constraint on *MAT1-2-1* genes, in turn resulting in rapid divergence.

Although the presence of MATA_HMG genes and their locations leave open the possibility that *MAT1-2* mating-type regions remain, if this is the case, genes and gene arrangements have been dramatically altered relative to other members of the Pezizomycotina. The order Xylariales represents a monophyletic group (Zhang et al. 2006) with a large number of species with diverse ecological roles across numerous genera and several families, as well as well-known iconic species such as *Xylaria polymorpha* (“dead man’s fingers”) and *Daldinia concentrica* (“coal fungus”). Despite the many studies that have focused on members of the order, information regarding life cycles is nearly non-existent in the literature. While homothallism appears to be the rule among those species for which single-ascospore cultures have been derived (Yu-Ming Ju, personal communication), circumstantial evidence for heterothallism has been presented for two species of *Hypoxylon*. Using RAPD markers, Vannini et al. (1999) presented evidence for genetic assortment among ascospore-derived progeny from single stromata of *H. mediterraneum*. Griffin et al. (1992) inferred heterothallism for *H. mammatum* after observing different genetically-based types of hyphal

interactions, resembling compatible and incompatible somatic interactions, among ascospore progeny derived from the same ascus as well as from different stroma. Although these studies have not shed light on the genetics of mating or confirmed heterothallic mating in these species, they do suggest that some form of genetic assortment accompanies sexual reproduction.

Our results are consistent with a previous failed attempt to find mating-type genes in *Eutypa lata* with PCR using degenerate primers. In a report posted by the American Vineyard Foundation, Long and Bradshaw (2002) detailed efforts employing more than 100 different PCR experiments with multiple primer sets and 34 different *E. lata* isolates, targeting but failing to identify *MAT1-1-1 (mat A-1)* and *MAT1-2-1 (mat a-1)* genes.

We are therefore left with the possibility that diverse members of the Xylariales produce ascocarps and ascospores without the genes required for mating in other Pezizomycotina. Alternatively, it is possible that relative to other members of the Pezizomycotina mating-type genes in the Xylariales have diverged substantially in terms of both sequence and genome arrangement, with the result that inferences regarding function are not possible based on sequence analysis alone. This situation is made all the more puzzling by the fact that although the Xylariales are basal to the groups (particularly the Sordariales) within the Sordariomycetes for which mating-type genes have been best studied (Zhang et al. 2006), even more distantly related fungi, for example members of the Dothidiomycetes and Leotiomyces, possess mating-type gene systems similar to those of the Sordariomycetes (Dyer et al. 2016, Martin et al. 2010).

We note that distantly-related members of the Ascomycota, notably the Saccharomycotina for which mating has been studied extensively, have mating systems that differ substantially from those of the Pezizomycotina discussed here. In the context of our results, it is interesting that a homothallic member of the Saccharomycetales, *Lodderomyces elongisporus* (a close relative of *Candida* species), appears to lack all four mating-type genes typically associated with members of this group (Butler et al. 2009). This result reinforces the many observations that despite the general themes that exist for mating systems across broad fungal groups evolution has resulted in a myriad of variations (Butler 2010, Dyer et al. 2016, Gioti et al. 2012).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Members of the fungal order Xylariales lack canonical mating-type regions
- Homology searches failed to find homologs for *MAT1-1-1* or *MAT1-1-2* genes
- Distant homologs were observed for *MAT1-2-1* and *MAT1-1-3* genes, but it is unknown if any are functional mating-type genes

Fig. 1.

Comparison of *sla2/apn2* gene regions from three Xylariales genomes with the mating-type regions from *Neurospora crassa*. Homologs of *sla2* and *apn2* are commonly found flanking mating-type genes in other Ascomycota and gene orientations are highly conserved (discussed in text). Note that the orientations of *sla2*, *apn2* and *MATA_HMG* genes in *Microdochium bolleyi* J235TASD1 are consistent with the orientations of *sla2*, *apn2* and *MATA_HMG* mating-type genes (*mat a-1* and *mat A-3*) in *N. crassa*. On the other hand, the orientation of the *MATA_HMG* gene in two strains of *Monosporascus sp.*, MG133 and MG162, is reversed relative to homologs in *M. bolleyi* and *N. crassa*, and the order of the three genes is different in *Xylaria hypoxylon* OSC100004. The arrangement of these genes in other members of the Xylariales varies substantially. In contrast with the results shown here, in most cases *MATA_HMG* genes in members of the Xylariales were not observed to be linked with *sla2* and *apn2* homologs (Supplemental Table S2). Accession numbers for *N. crassa* mating-type proteins and gene regions are given in Table 2. Accession numbers for the Xylariales genomes are given in Table 1, and specific gene locations and protein IDs are presented in Supplemental Table S2.

Table 1.Xylariales genomes examined for mating-type genes¹

Species (strain)	NCBI accession or JGI ² ID
<i>Daldinia eschscholtzii</i> (UM1400)	CCED00000000.1
<i>Xylaria striata</i> (RK1–1)	LOBO00000000.1
<i>Xylaria</i> sp. (MSU SB201401)	NPFG00000000.1
<i>Xylaria hypoxylon</i> (OSC100004) v1.0	JGI Project ID: 1050987
<i>Xylaria</i> sp. (JS573)	JWIU00000000.1
<i>Pestalotiopsis fici</i> (W106–1)	ARNU00000000.1
<i>Pestalotiopsis</i> sp. (JCM 9685)	BCGF00000000.1
<i>Microdochium bolleyi</i> (J235TASD1)	LSSP00000000.1
<i>Rosellinia necatrix</i> (W97)	BBSO00000000.2
<i>Anthostoma avocetta</i> (NRRL 3190) v1.0	JGI Project ID: 1006061
<i>Apiospora montagnei</i> (NRRL 25634) v1.0	JGI Project ID: 1006423
<i>Biscogniauxia nummularia</i> (BnCUCC2015) v1.0	JGI Project ID: 1106943
<i>Daldinia eschscholtzii</i> (EC12)	MDGZ00000000.1
<i>Entoleuca mammata</i> (CFL468) v1.0	JGI Project ID: 1117716
<i>Eutypa lata</i> (UCREL1)	AORF00000000.1
<i>Hypoxylon</i> sp. (EC38)	MDCK00000000.1
<i>Hypoxylon</i> sp. (CO27–5)	MDCL00000000.1
<i>Hypoxylon</i> sp. (CI-4A)	MDGY00000000.1

Species (strain)	NCBI accession or JGI ² ID
<i>Hypoxylon</i> sp. (E7406B)	JYCQ00000000.1
<i>Hypoxylon pulicicidum</i> (MF5954)	PDUJ00000000.1
<i>Annulohypoxylon stygium</i> (MG137)	QLPL00000000.1
<i>Kretzschmaria deusta</i> (DSM 104547)	MLHU00000000.3
<i>Microdochium trichocladiopsis</i> (MPI-CAGE-CH-0230) v1.0	JGI Project ID: 1103673
<i>Pseudomassariella vexata</i> (CBS 129021)	MCFJ00000000.1
<i>Truncatella angustata</i> (HP017) v1.0	JGI Project ID: 1103645
<i>Monosporascus cannonballus</i> (CBS609.92)	QJNS00000000
<i>Monosporascus cannonballus</i> (CBS586.93)	QJNT00000000
<i>Monosporascus</i> sp. (GIB2)	QJNV00000000
<i>Monosporascus</i> sp. (MC13–8B)	QJNW00000000
<i>Monosporascus</i> sp. (MG133)	QJNX00000000
<i>Monosporascus</i> sp. (MG162)	QJNY00000000
<i>Monosporascus</i> sp. (CRB-8-3)	QJNZ00000000
<i>Monosporascus</i> sp. (CRB-9-2)	QJOA00000000
<i>Monosporascus</i> sp. (5C6A)	QJOB00000000
<i>Monosporascus ibericus</i> (CBS110550)	QJNU00000000

¹Life cycle information: Strains listed as *Monosporascus cannonballus* and *M. ibericus* are known to be homothallic. As discussed in the text, ascocarps have not been observed for the strains listed as *Monosporascus* sp. Other strains listed in this table are assumed to be homothallic or are anamorphic stages of unknown life cycle.

²JGI = US Department of Energy Joint Genome Institute

Table 2.

Sordariomycete mating-type sequences used as queries in alignment-based searches.

Species (strain)	Reproductive Strategy & Mating Type	Protein Accession #	Genomic Region Accession #
Hypocreales			
<i>Fusarium graminearum</i> (CBS 139514)	Homothallic		
	MAT1-1-1	AMP43945.1	
	MAT1-1-2	AMP43944.1	KT855220.1
	MAT1-1-3	AMP43943.1	
	MAT1-2-1	AMP43946.1	
Ophiostomatales			
<i>Ophiostoma himalulmi</i> (HP25)	Heterothallic		
	MAT1-1-1	AHL24887.1	KF961046.1
	MAT1-1-2	AHL24886.1	
	MAT1-1-3	AHL24885.1	
<i>Ophiostoma himalulmi</i> (HP62)	Heterothallic		AY887030.1
	MAT1-2-1	AAX83073.1	
Sordariales			
<i>Neurospora crassa</i> (74-ORS-A)	Heterothallic		
	MAT1-1-1	AAC37478.1	M33876.1
	MAT1-1-2	AAC37477.1	
	MAT1-1-3	AAC37476.1	
<i>Neurospora crassa</i>	Heterothallic		M54787.1
	MAT1-2-1	AAA33598.2	
<i>Myceliophthora heterothallica</i> (ThNM 146)	Heterothallic		
	MAT1-1-1	ALD16238.1	KR119057.1
	MAT1-1-2	ALD16239.1	
	MAT1-1-3	ALD16240.1	
<i>Myceliophthora heterothallica</i> (ThNM 053)	Heterothallic		KR119058.1
	MAT1-2-1	ALD16244.1	
Magnaporthales			
<i>Magnaporthe grisea</i> (Y93-164g-1)	Heterothallic		
	MAT1-1-1	BAC65091.1	
	MAT1-1-2	BAC65092.1	AB080672.2
	MAT1-1-3a	BAC65093.2	
	MAT1-1-3b	BAE66612.1	

Species (strain)	Reproductive Strategy & Mating Type	Protein Accession #	Genomic Region Accession #
<i>Magnaporthe grisea</i> (70–14)	Heterothallic		
	MAT1-2-1	BAC65090.1	AB080671.2
	MAT1-2-2a	BAE66610.1	
	MAT1-2-2b	BAE66611.1	

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