Both mating types in the heterothallic fungus *Ophiostoma quercus* contain *MAT1-1* and *MAT1-2* genes

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ABSTRACT

In heterothallic Ascomycota, two opposite but distinct mating types control all sexual processes. Using mating crosses on agar plates, the heterothallic nature of the wood-inhabiting fungus *Ophiostoma quercus* was confirmed and mating types were assigned to 10 isolates. Primers were subsequently designed to target both the mating type 1 (*MAT1-1*) and 2 (*MAT1-2*) idiomorphs in these isolates. The results showed that all isolates contained sequence fragments representing both idiomorphs. This was unexpected, as each isolate from a heterothallic species would typically contain only one of the two possible *MAT* idiomorphs. An atypical mating system such as the one described in this study has not previously been encountered in any other Ascomycota mating locus described to date.

Keywords: fungal mating, *Ophiostoma*, *MAT1-1, MAT1-2*

1. INTRODUCTION

Ophiostoma represents a diverse genus in the Ascomycota with a worldwide distribution (Wingfield et al. 1993). Most species have a close association with tree-infesting bark beetles and some cause serious tree diseases (Hausner et al. 1993). This is true of species in the *O. piceae* complex (Chung et al. 2006; De Beer et al. 2003; Harrington et al. 2001; Kamgan et al. 2008; Uzunovic et al. 2000), which includes the Dutch elm disease pathogens (*O. ulmi* and *O. novo-ulmi*) that have been responsible for the death of millions of Elm trees in the Northern Hemisphere (Brasier 1990). Other species in the *O. piceae* complex result in blue-stain of timber and they degrade wood quality. For example, *O. quercus* is responsible for significant economic losses due to sapstain in hardwoods (De Beer et al. 2003; Harrington et al. 2001).

Species of *Ophiostoma* have various mating behaviours that range from strict homothallism through to strict heterothallism. For example, the Dutch elm disease pathogens and *O. quercus* are heterothallic (Brasier and Kirk 1993; Harrington et al. 2001; Solla et al. 2008) where sexual reproduction requires the interaction of two individuals of opposite mating type (Coppin et al. 1997). In contrast, individuals of homothallic species (e.g., *O. arduennense* and *O. minus*) are typically self-fertile and capable of completing the sexual cycle in the absence of a second individual (Carlier et al. 2006; Gorton and Webber 2000; Grobbelaar et al. 2009).

Sexual reproduction in the Ascomycota is controlled by the genes found at a single mating type locus (*MAT-1*) (Coppin et al. 1997; Turgeon 1998) with two idiomorph

alleles (Metzenberg and Glass 1990). In heterothallic species, individual isolates usually have either the *MAT1-1* or *MAT1-2* idiomorph, but they have never been found to contain both idiomorphs (Glass and Nelson 1994; Nelson 1996). In homothallic species, the genomes of all individuals harbour genes of both idiomorphs, frequently in different arrangements of the *MAT* locus (Elliott 1994; Nelson 1996).

Three genes are commonly located at the *MAT1-1* idiomorph, *MAT1-1-1*, *MAT1-1-2,* and *MAT1-1-3* (Coppin et al. 1997; Elliott 1994; Glass and Nelson 1994). Of these, the α-box protein encoding gene, *MAT1-1-1* (Coppin et al. 1997; Debuchy and Turgeon 2006), was first identified in *Saccharomyces cerevisiae* (Astell et al. 1981) and has subsequently been identified in all fungal *MAT1-1* idiomorphs (Glass et al. 1990; Kanematsu et al. 2007; Li et al. 2010). The *MAT1-1-2* gene encodes an amphipathic α-helix protein with a conserved Histidine, Proline, Glycine (HPG) domain (Debuchy and Turgeon 2006), while the *MAT1-1-3* gene encodes a protein with a High Mobility Group (HMG) domain (Coppin et al. 1997; Debuchy and Turgeon 2006). Another HMG domain protein, encoded by the *MAT1-2-1* gene, is characteristic of the *MAT1-2* idiomorph (Arie et al. 1997; Coppin et al. 1997; Nelson 1996). *MAT1-2-1* is generally the only gene located on the *MAT1-2* idiomorph and has been found in all *MAT1-2* idiomorphs that have been characterised (Arie et al. 1997; Coppin et al. 1997; Kanematsu et al. 2007), including those of the Dutch elm disease pathogens (Paoletti et al. 2005).

Recent studies of the *MAT* genes have revealed their importance in the biology and evolution of fungi (Bennett et al. 2003; Strandberg et al. 2010; Zaffarano et al. 2010).

For example, comparisons of *MAT* DNA sequences in different fungi have improved our understanding of the evolution of homothallic and heterothallic mating strategies (Arie et al. 1997; Bennett et al. 2003; Conde-Ferráez et al. 2007; Fraser and Heitman 2004; Li et al. 2010; Martin et al. 2011; Steenkamp et al. 2000; Turgeon 1998). Also, the availability of information on the mating idiomorphs allowed for the assessment of the presence of *MAT* genes in the genome of apparently asexual species (Foster and Fitt 2003; Mandel et al. 2007; Turgeon 1998). At the intra-species level, knowledge regarding the distribution of *MAT* genes has also shed light on the preferred reproduction mode (*i.e.* sexual versus asexual) of certain fungal populations (Britz et al. 1998; Linde et al. 2010; Zhan et al. 2002). Such information is particularly important for fungal pathogens, as sexual and asexual reproduction have markedly different effects on the population structures of the pathogens, which in practical situations require different disease management strategies (McDonald and Linde 2002).

Analysis of the distribution of mating types within a population of a heterothallic fungus may be accomplished using either conventional mating studies or DNA-based approaches. Conventional mating tests are laborious and time-consuming as they involve mating all available isolates in every possible combination and subsequent assignment of mating specificities. This traditional approach has been used widely for heterothallic species of *Ophiostoma* (Brasier and Kirk 1993; De Beer et al. 2003; Grobbelaar et al. 2009; Harrington et al. 2001; Zhou et al. 2004). However, the mating type designations obtained under laboratory conditions do not always reflect the situation in natural environments (Marra et al. 2004; Marra and Milgroom 2001). Also, not all the individuals examined are necessarily equally fertile

under the conditions tested, and this can lead to erroneously assigned mating types. In contrast, DNA-based approaches are relatively inexpensive and usually provide reliable mating type assignments (Cherif et al. 2006; Dyer et al. 2001; Yokoyama et al. 2004). These DNA-based methods are, however, dependent on the availability of sequence information for the *MAT* locus, because *MAT* idiomorph-specific PCR assays exploit the inherent differences in the *MAT* genes (Dyer et al. 2001; Steenkamp et al. 2000).

For species of *Ophiostoma*, *MAT* sequence information is available only for the Dutch elm disease pathogens (Jacobi et al. 2010; Paoletti et al. 2005, 2006). For *O. quercus,* a number of mating tester strains is available from previous mating studies (Brasier and Kirk 1993; De Beer et al. 2003; Grobbelaar et al. 2009; Kamgan et al. 2008). The aim of this study was to characterise the *MAT* genes in some of these *O. quercus* isolates.

2. MATERIALS AND METHODS

2.1 Isolates and mating study

Ten *Ophiostoma quercus* isolates originating from single spores were used in this study (Table 1). These isolates were obtained from *Quercus*, *Acacia* and *Eucalyptus* hosts in Africa, Europe and North America. Their mating type specificities have been determined in previous studies (Brasier and Kirk 1993; De Beer et al. 2003; Kamgan et al. 2008). For routine cultivation of these isolates, malt-extract agar (MEA; 20 g/L malt extract [Biolab, Merck], 20 g/L agar [Biolab, Merck]) medium and an incubation temperature of 25°C were used.

To confirm the identity of all isolates used in this study, the ribosomal RNA (rRNA) internal transcribed spacer regions (ITS 1 and 2) and the 5.8S gene were amplified and sequenced using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Each 25 µl PCR reaction contained 1 U Roche FastStart *Taq* mixture and reaction buffer (Roche, Mannheim, Germany), 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.2 mM of each primer and 20-50 ng of template DNA. The latter was prepared for each isolate by scraping mycelium from the surface of 4-6 week old MEA cultures and subjecting the harvested mycelium to a salt-based DNA extraction method (Aljanabi and Martinez 1997). PCRs were performed on an Eppendorf thermocycler (Eppendorf AG, Mannheim, Germany) using the following conditions: one cycle of 5 min at 96°C, followed by 35 cycles of 30 s at 95°C, 30 s at 50°C and 1 min at 72°C, followed by one cycle of 7 min at 72°C. Products were visualized by agarose (LE Agarose, SeaKem, Rockland, USA) gel electrophoresis (Sambrook and Russell 2001), and purified using the High Pure PCR Product Purification Kit (Roche, Mannheim, Germany). Individual products were then sequenced using the original PCR primers, a Big Dye cycle sequencing kit with Amplitaq DNA polymerase (Perkin-Elmer, Warrington, UK) and an ABI PRISM 3300 Genetic Analyser (Applied Biosystems, Foster City, USA). After analysis of chromatograms with Chromas Lite v. 2.01 (Technelysium Pty. Ltd.; http://www.technelysium.com.au), the sequences of all isolates were compared to the ITS sequences of authentic *O. quercus* isolates (De Beer et al. 2003; Grobbelaar et al. 2009; Harrington et al. 2001).

To confirm the mating types of all ten isolates, mating tests were used as described previously (De Beer et al. 2003). Isolates were paired in all possible combinations on

sterilized *Quercus* twigs that were placed on 1.5% (w/v) agar (Biolab, Merck) medium. Control crosses were included where each isolate was paired with itself. A mating interaction was scored as negative if no ascomata were formed, if the ascomata contained no ascospores or if the ascospores were not viable when incubated on MEA medium. Positive mating responses were recorded only when ascomata containing viable ascospores were formed. The entire mating study was performed twice.

2.2 PCR, cloning and sequencing of the mating type genes

To study the *MAT* idiomorphs of *O. quercus*, published (Jacobi et al. 2010; Paoletti et al. 2005, 2006) and publicly available sequences (National Center for Biotechnology Information; http//:www.ncbi.nih.gov) for *O. novo-ulmi* were used. The software packages Primer3 v. 0.4.0 (Rozen and Skaletsky 2000) and CLC Main Workbench v. 5.5 (CLC Bio, Aarhus, Denmark) were used to design primers that would allow the amplification of *MAT* sequences (Table 2). To amplify the *MAT1-2* idiomorph gene *MAT1-2-1*, a set of primers was designed based on *O. novo-ulmi* sequence data. In an attempt to extend *MAT1-1* sequences we followed two strategies. In the first strategy, the *MAT1-1-1* and *MAT1-1-2* sequences were targeted by designing primers based on sequence information for *O. novo-ulmi*. In the second strategy, the *O. quercus MAT1-1-3* gene and 3' non-coding region was targeted using a primer set that was based on previously published sequence data (Table 2) (Paoletti et al. 2005). After a single round of PCR and sequencing, the resulting *O. quercus* sequence data were used to design another primer (Mt3cF), which was used together with primer Mt3cR, which was also based on unpublished *O. novo-ulmi* sequence data, to target the coding region of *MAT1-1-3*.

Amplification reactions of the mating type regions and purification of PCR products were carried out as described above. Purified products were cloned using the pGem®-T Easy cloning kit (Promega, Madison, USA) after which cloned inserts were amplified directly from colonies using the vector-specific primers T7 and SP6 (Butler and Chamberlin 1982; Dunn et al. 1983). The latter PCRs utilized the same PCR reaction and cycling conditions as before, with the only exception that 30 amplification cycles instead of 35 were used. These PCR products were also purified and sequenced as before, except that primers T7 and SP6 were used.

To confirm the identity of sequenced fragments of the *MAT* idiomorphs, comparisons were made with the available sequences for the Dutch elm disease pathogens (Jacobi et al. 2010; Paoletti et al. 2005, 2006) by making use of the NCBI nucleotide database and BLASTn (Zhang et al. 2000). Predicted protein sequences for *O. quercus* were obtained by using the online version of the *de novo* prediction program Augustus (Stanke et al. 2006) as well as by comparison to the predicted protein sequences for *O. novo-ulmi* (Jacobi et al. 2010; Paoletti et al. 2005). For analysis of *MAT1-1* fragments, the produced *O. quercus* sequences were compared with the same region of the previously determined *MAT1-1* sequences for *O. novo-ulmi*. These included two representative sequences for *O. novo-ulmi* isolate H327 (accession numbers FJ858801 and EU163846) and sequences from previous studies (Jacobi et al. 2010; Paoletti et al. 2006). For analyses of the *MAT1-2* fragments, we included only the ORF and intron sequences of the *MAT1-2-1* gene determined previously for *O. novo-ulmi* subsp. *novo-ulmi* (accession numbers AY887028 and AY887029) and *O. novo-ulmi* subsp. a*mericana* (accession numbers

AY887025 and AY887026). These comparisons were facilitated by constructing multiple alignments with the online interface of the alignment program MAFFT v. 6 using the G-INS-i strategy (Katoh et al. 2002). All sequence analysis and visualisation was done using the CLC Main Workbench v. 6.1 (CLC Bio, Aarhus, Denmark).

TABLE 1. Ophiostoma quercus isolates used in this study. Mating groups were arbitrarily assigned to indicate the mating specificity of the 10 isolates

TABLE 2. Primers used in this study.

3. RESULTS

3.1 Isolates and mating study

The ITS sequences of the 10 *O. quercus* isolates used in this study were similar to those reported previously for isolates of this species (Grobbelaar et al. 2009), confirming their identity. The heterothallic behaviour of these isolates were also confirmed (Table 1) and agreed with that previously determined (Brasier and Kirk 1993; De Beer et al. 2003; Kamgan et al. 2008). Of the 55 mating tests performed, none of the 10 self-pairings produced ascomata or ascospores (Fig. 1b). In contrast, nine positive matings (Fig. 1a) were observed among the 45 remaining mating combinations. In all these cases, the ascomata produced abundant ascospores that were viable on MEA medium. Similar results were obtained when the trial was

replicated. Based on these results, the isolates were separated into two groups of five isolates, and respectively assigned “+” and “-” mating types (Table 1).

Figure 1: Mating in *Ophiostoma quercus*.

3.2 PCR, cloning and sequencing of the mating type genes

Using the *MAT1-1* idiomorph-specific primer pair OqMt1F+OqMt1R (Table 2), it was possible to amplify and sequence a fragment of 181 bp from the genome of the 10 *O. quercus* isolates (Table 1). The BLASTn results confirmed that the sequence of this fragment was similar to those previously determined for *MAT-1* isolates of *O. novo-ulmi* (Fig. 2) (Paoletti et al. 2006). None of these amplicons showed any sequence similarity to fragments amplified from the *MAT1-2* idiomorph of *Ophiostoma* spp. examined in the present or previous studies (Paoletti et al. 2005). The sequence of this 181 bp fragment overlapped with the last 37 nucleotides of the *MAT1-1-3* gene of *O. novo-ulmi* (Jacobi et al. 2010), while the remainder of the fragment shared similarity with 144 nucleotides of the 3’ non-coding region immediately following the *MAT1-1-3* gene (Jacobi et al. 2010; Paoletti et al. 2006). The nucleotide sequence of the *MAT1-1* fragments for the 10 *O. quercus* isolates were identical. In two of the isolates (CMW 1034 and CMW 2521), a second fragment of approximately 600 bp was co-amplified, but its sequence showed no similarity to any *MAT* gene or to any other sequence in the NCBI database and was thus excluded from subsequent analyses.

To extend our *MAT1-1-3* sequence, the *O. quercus*-based primer Mt3cF was used with Mt3cR (Table 2) in PCRs with DNA from two isolates of opposite mating type, *i.e.*, isolates CMW 2520 (*MAT*+) and CMW 2521 (*MAT*-) (Table 1). This primer pair

allowed amplification and sequencing of a 645 bp portion of the *MAT1-1-3* gene in isolate CMW 2520 (*MAT*+) only (Fig. 2). Combination of this 645 bp fragment with the one obtained using primers OqMt1F+1R resulted in a sequence fragment of 834 bp in length. Sequence comparisons showed that this fragment is homologous to the 3'-end of the 728 bp *MAT1-1-3* ORF and a region downstream to it in *O. novo-ulmi* isolate H327 (accession number FJ858801), with 675 bp of the *O. quercus* sequence overlapping with the ORF and the remainder corresponding to the downstream region. *O. quercus* isolate CMW 2520 and *O. novo-ulmi* isolate H327 shared 78% nucleotide similarity in this 675 bp portion with the majority of the variation situated in the three predicted introns (20 nucleotide substitutions and three nucleotide deletions in intron 1, 31 substitutions and two deletions in intron 2 and 26 substitutions and three deletions in intron 3). An AUGUSTUS prediction of the *O. quercus* sequence yielded a peptide containing an HMG box-conserved domain identical to that predicted for the *O. novo-ulmi MAT1-1-3* gene (accession number ABX10185) and other fungal *MAT1-1-3* genes.

For the primers based on the *O. novo-ulmi MAT1-1-1* and *MAT1-1-2* sequences (Table 2), those that target *MAT1-1-2* did not yield any amplicons in either isolate CMW 2520 (*MAT*+) or CMW 2521 (*MAT*-). However, with the *MAT1-1-1* primer pair Mt1aF+Mt1aR (Table 2), a 715 bp fragment was amplified and sequenced from the *MAT*- isolate CMW 2521, but not from the *MAT*+ isolate CMW 2520 (Table 1, Fig. 2). Comparison of this sequence with that of the *O. novo-ulmi MAT1-1-1* sequence (accession number FJ858801) revealed only two nucleotide differences (a single insertion/deletion and a single point mutation all within the exon). Peptide prediction with AUGUSTUS and BLASTp analysis showed that this sequence also harbours the

expected conserved α-box motif predicted for *O. novo-ulmi* (accession number ACZ53927) and other Ascomycota.

The sequence for the full *MAT1-2-1* gene (666 bp) was obtained for all 10 *O. quercus* isolates. The AUGUSTUS software predicted that it encodes a protein with 202 amino acid residues and that the gene is interrupted by a single intron of 57 bp. The intron was predicted at a conserved serine position, which is similar to what has been reported for the *O. novo-ulmi MAT1-2-1* gene (Paoletti et al. 2005). All the *O. quercus MAT1-2-1* sequences were identical to each other, but 21 polymorphic sites were observed when compared to *O. novo-ulmi* isolate US411. Of these only two occurred in the intron. Seven of the remaining 19 polymorphic sites represented synonymous substitutions, while 12 represented non-synonymous substitutions in the exon of this ORF. Nevertheless, BLASTp analysis with the inferred amino acid sequence against the NCBI database showed similarity to the predicted MAT1-2-1 protein from *Ophiostoma* species (accession number AAX83065) and other fungal MAT1-2-1 proteins.

Figure 2: Diagrammatic representation of the gene information currently available for *Ophiostoma*.

4. DISCUSSION

Results of this study showed that the *MAT* locus of *O. quercus* has a unique structure that has not previously been encountered in any other Ascomycota. Previous work has shown that the *MAT1-2* idiomorph of *Ophiostoma* species such as *O. ulmi*, *O. novo-ulmi* and *O. himal-ulmi* encodes the *MAT1-2-1* gene (Paoletti et

al. 2005), while the *MAT1-1* idiomorph harbours only the *MAT1-1-3* gene (Jacobi et al. 2010). Unpublished, but publicly available nucleotide data indicate that the *Ophiostoma MAT1-1* idiomorph also contains the *MAT1-1-2* and *MAT1-1-1* genes (Figure 1). Although this gene organisation is quite common among the Ascomycota (Coppin et al. 1997; Debuchy and Turgeon 2006; Glass and Nelson 1994; Nelson 1996), elements thought to be exclusively associated with either the *MAT1-1* or *MAT1-2* idiomorphs were found in all the *O. quercus* isolates examined. Despite the fact that it was possible to assign "+" and "-" mating specificities to the strictly heterothallic isolates of this fungus, isolates of both mating types harboured *MAT1-1-3* and *MAT1-2-1* sequences.

The full HMG box containing *MAT1-2-1* gene was amplified and sequenced for the 10 *O. quercus* isolates included in this study (Fig 2). Although the *MAT1-1-3* gene associated with the *MAT1-1* idiomorph also encodes an HMG-box motif, a detailed analysis of the MAT1-2-1 and MAT1-1-3 HMG box domains from *O. novo-ulmi* indicated that the MAT1-2-1 domain is specific to the *MAT1-2-1* idiomorph (Jacobi et al. 2010). Also, the *MAT1-2-1* ORF and intron encoded by the 666 bp fragment characterized in this study, shows very high similarity to the *MAT1-2-1* sequences reported for the Dutch elm disease pathogens (Paoletti et al. 2005) and other Ascomycota such as *Cordyceps militaris* (accession BAC66500), *Isaria tenuipes* (Yokoyama et al. 2003) (BAC67543) and *Glomerella lindemuthiana* (García-Serrano et al. 2008) (ABY84976). This provides confidence that the *MAT1-2-1* gene characterized in the present study corresponds to the typical HMG domain-encoding gene associated with the typical Ascomycota *MAT1-2* idiomorph.

In this study, the sequences for two genes usually associated with the typical Ascomycota *MAT1-1* idiomorph (Fig. 2) were determined. From the *MAT*- isolate CMW 2521 (Table 1), a large portion of the *MAT1-1-1* gene was amplified, which encodes the typical *MAT1-1* α-domain known from other Ascomycota (Coppin et al. 1997; Debuchy and Turgeon 2006; Glass and Nelson 1994; Nelson 1996). However, a 180-bp portion of the *MAT1-1-3* gene was also found in all 10 *O. quercus* isolates. It was possible to obtain the near-complete sequence for this gene in the *MAT*+ isolate CMW 2520 (Table 1) but not in isolate CMW 2521. The predicted protein sequence for this region showed high similarity to the *MAT1-1-3* sequences for other species, e.g. *O. novo-ulmi* (accession ACZ53925), *Claviceps purpurea* (BAD72602) (Yokoyama et al. 2005) and *Epichloe* species (BAD72610, BAD72606) (Yokoyama et al. 2005).

In the typical heterothallic *MAT* locus arrangement, the *MAT1-1* idiomorph contains at least the α-domain *MAT1-1-1* gene in addition to the *MAT1-1-3* and *MAT1-1-2* genes (Coppin et al. 1997; Nelson 1996; Turgeon and Yoder 2000), while the *MAT1-2* idiomorph always contains the *MAT1-2-1* gene (Arie et al. 1997; Coppin et al. 1997). Nothing is known regarding the mating type loci of homothallic *Ophiostoma* species, but previous research has shown that homothallic mating idiomorphs share similarity with that of heterothallic species. For example, the single *MAT* locus of the homothallic *Gibberella zeae,* harbour all four *MAT* genes, *MAT1-1-1*, *MAT1-1-2*, *MAT1-1-3* and *MAT1-2-1* (Yun et al. 2000). In another example, the *Cochliobolus MAT* locus is characterized by different organisations ranging from fused single genes to only two *MAT* genes located in opposite orientation within a single *MAT* idiomorph (Yun et al. 1999). In this respect, the *MAT* locus of *O. quercus* might seem

more similar to those of homothallic species because *MAT1-1* and *MAT1-2* idiomorph-specific sequences were present in single isolates originating from single spores.

The occurrence of an atypical *MAT* locus in an apparently strictly heterothallic species is not unique to *O. quercus*. Two heterothallic *Diaporthe* species were recently shown to harbour unusual *MAT1-2* idiomorph structures while having a normal *MAT1-1* idiomorph structure with the three expected genes (Kanematsu et al. 2007). The *Diaporthe MAT1-2* idiomorph contained three genes, one which represents the *MAT1-2* idiomorph-specific gene *MAT1-2-1*. The other two apparently represent homologues of the *MAT1-1* idiomorph genes *MAT1-1-2* and *MAT1-1-3*. The authors suggested that this arrangement might have come about after a duplication event and that the ancestral type contained a *MAT* locus with three genes, *MAT1-1-2*, *MAT1-1-3* and another gene similar to either *MAT1-1-1* or *MAT1-2-1* (Kanematsu et al. 2007). In the same manner, gene duplications could potentially explain the existence of the *MAT1-1-3* (and potentially even *MAT1-1-2,* although we did not manage to detect it with our primers) in a single isolate of *O. quercus*.

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TABLE 1. *Ophiostoma quercus* isolates used in this study. Mating groups were arbitrarily assigned to indicate the mating specificity of the 10 isolates.

Isolate[a]		Host	Country	Collector	Mating group	Genbank acc. Nr.			Reference
CMW	Other					ITS	MAT1-1	MAT1-2	
2520	CBS 116321	*Eucalyptus* chips	South Africa	ZW de Beer	+	AF493241[c]	FJ865416[d] JN225450[e]	FJ865421	(De Beer et al. 2003)
2521		*Eucalyptus* chips	South Africa	ZW de Beer	-	FJ441283[c]	FJ865417[d] JN225451[f]	FJ865420	(De Beer et al. 2003)
14307		*Acacia mearnsii*	Uganda	J Roux	-	FJ959044	FJ865415[d]	FJ865425	(Kamgan et al. 2008)
17256[b]		*A. mearnsii*	Uganda	J Roux	+	FJ959042	FJ865411[d]	FJ865422	(Kamgan et al. 2008)
17257[b]		*A. mearnsii*	Uganda	J Roux	+	FJ959045	FJ865414[d]	FJ865424	(Kamgan et al. 2008)
17258[b]		*A. mearnsii*	Uganda	J Roux	-	FJ959043	FJ865418[d]	FJ865423	(Kamgan et al. 2008)
27845	H 2190	*Quercus* sp.	Canada	K Seifert	+	AF211840[c]	FJ865419[d]	FJ865426	(Brasier and Kirk 1993)
27846	H 1039	*Quercus* sp.	UK	PT Scard	+	AF211838[c]	FJ865410[d]	FJ865427	(Brasier and Kirk 1993; De Beer et al. 2003)

27847	H 920	*Quercus* sp.	UK	JN Gibbs	-	AF081134[c]	FJ865413[d]	FJ865429	(Brasier and Kirk 1993)
27848	H 1042	*Quercus* sp.	UK	PT Scard	-	EF429089[c]	FJ865412[d]	FJ865428	(Brasier and Kirk 1993; De Beer et al. 2003)

[a] CMW = Culture collection of the Forestry and Biotechnology Institute (FABI), University of Pretoria, South Africa; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; H = From the collection of Brasier & Kirk (Brasier and Kirk 1993).

[b] All three these single spore isolates were obtained from one isolate, CMW 5826, as tester strains for *O. quercus* (Kamgan et al. 2008).

[c] Sequences already in NCBI database from previous studies.

[d] Sequence produced using primer pair OqMt1F/OqMt1R.

[e] Sequence produced using primer pair Mt3cF/R.

[f] Sequence produced using primer pair Mt1aF/R

TABLE 2. Primers used in this study.

Primer name	Sequence (5' to 3')	Primer binding region in *O. novo-ulmi MAT1-1* and *MAT1-2* idiomorphs	Region amplified
OqMt1F	TGGCAAGAAAGGAAGACTGG	1653..1672[b]	*MAT1-1* idiomorph
OqMt1R	GCGTTATTGGGAGACAGGAA	1493..1512[b]	
OqMt2	GCACACAACTTTGCCAGGTA	119..138[c]	*MAT1-2* idiomorph
Seq9[a]	GGGGATGTAAAAGGAAC	1188..1204[c]	
Mt1aF	CCCAGGTCCTCAAATAATAA	4622..4641[b]	*MAT1-1-1* gene
Mt1aR	GAAACTCCCCACCGATAA	5324..5341[b]	
Mt2aF	GAGTCATCTTACCGAAACA	2961..2934[b]	*MAT1-1-2* gene
Mt2aR	CGGCGGATCATAGTACTTA	2916..2934[b]	
Mt2bF	AATGCGAGTCATCTTACC	2911..2928[b]	
Mt2bR	TGTGTTTCTAGGTGGCTG	3597..3614[b]	
Mt3cF	CTCCCAGTCTTCCTTTCT	1650..1667[b]	*MAT1-1-3* gene
Mt3cR	GAAATTCATTGTCGTCATCC	2291..2310[b]	

[a]From Paoletti *et al.* (2005).

[b]Sequence positions corresponding to *O. novo-ulmi* isolate H327 - Accession number FJ858801

[c]Sequence positions corresponding to *O. novo-ulmi* isolate R66 - Accession number AY887028

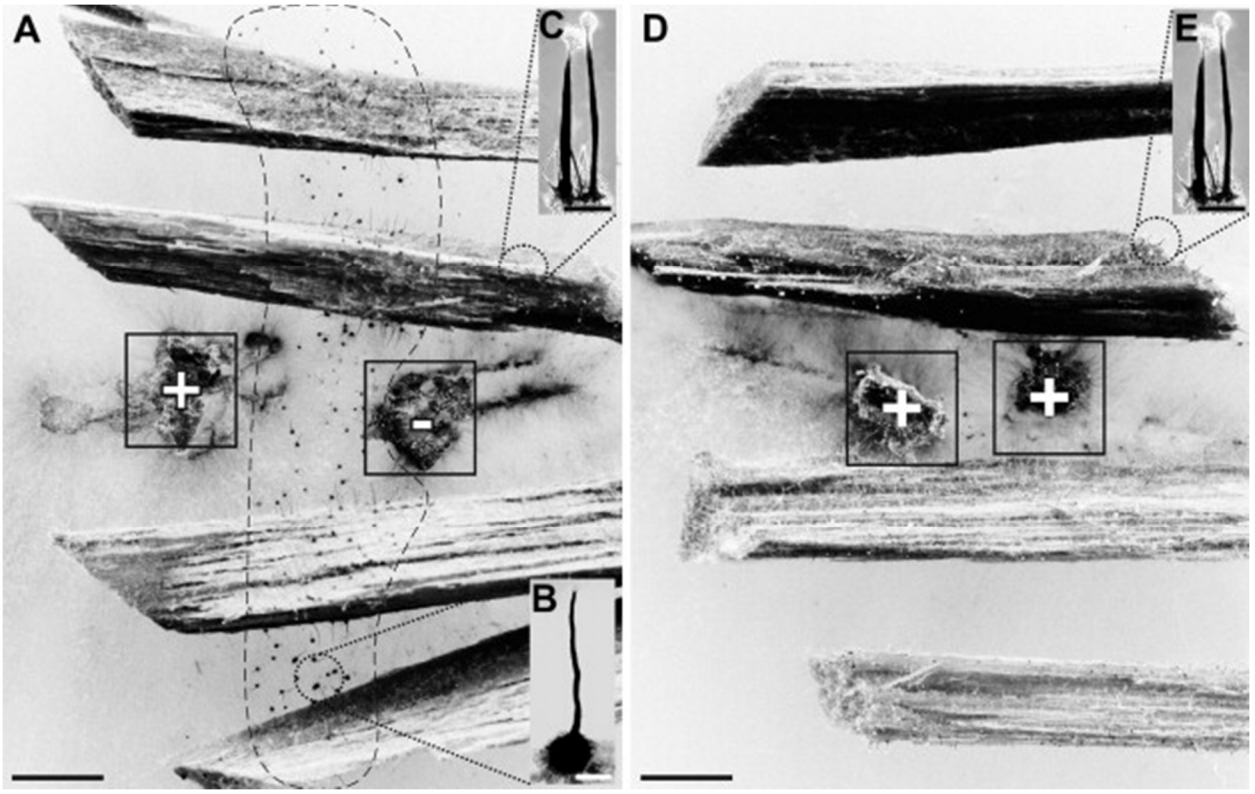
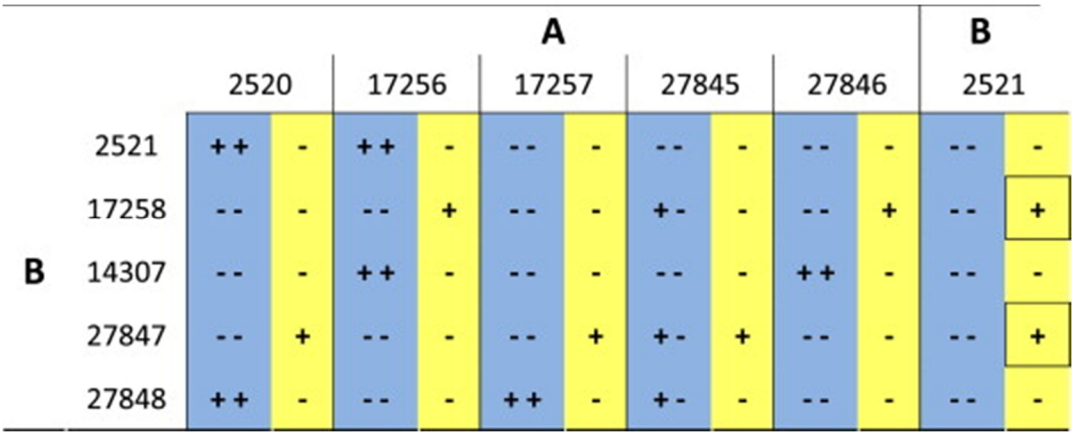


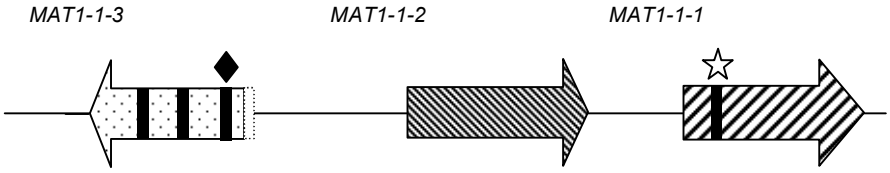
Fig 1. Mating in *Ophiostoma quercus*.(A) Cross between two *O. quercus* isolates of opposite mating types [CMW 2520 (MAT A) × CMW 2521 (MAT B)] inoculated onto agar with wood pieces. Inocula indicated with squares. Abundant sexual ascomata (B) are produced all along the interaction zone (dashed line) between the two isolates. Some asexual conidiophores (C) were also produced. (D) Control cross of two identical isolates [CMW 2520 (A) × CMW 2520 (A)] forming no ascomata, but only some asexual conidiophores (E). Scale bars *a*, *d* = 5 mm; *b*, *c*, *e* = 100 μm.



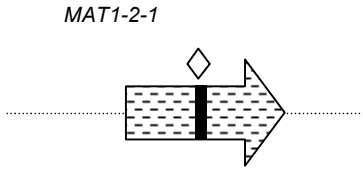
		A										B	
		2520		17256		17257		27845		27846		2521	
B	2521	++	-	++	-	--	-	--	-	--	-	--	-
	17258	--	-	--	+	--	-	+-	-	--	+	--	+
	14307	--	-	++	-	--	-	--	-	++	-	--	-
	27847	--	+	--	-	--	+	+-	+	--	-	--	+
	27848	++	-	--	-	++	-	+-	-	--	-	--	-

Fig 2. Condensed results of the mating studies.CMW numbers and assigned mating type are shown for all isolates. Self-matings and matings between isolates of the same mating type that produced no perithecia are excluded. The two columns for each mating interaction indicate the results of the two repeats for the agar block (two blue columns) and liquid broth (yellow column) mating test. Mating interactions between opposite mating types were expected to be positive for a strict heterothallic fungus, and 15 positive matings were observed. Matings between isolates of the same mating type (e.g. CMW2521 *versus* CMW17258) were expected to be negative, but two positive reactions were seen in the liquid broth mating test (shown in blocks). A**+** indicates a positive mating reaction with the formation of perithecia, while A**–** indicates the absence of perithecia and was scored as a negative result.

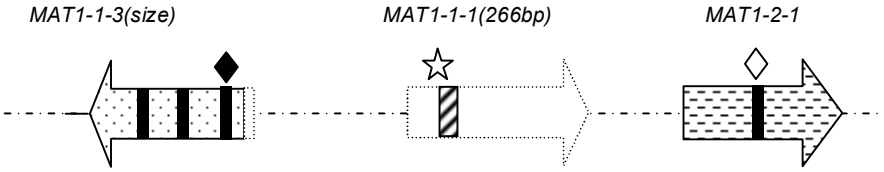
a)*O. novo-ulmiMAT1-1*isolate



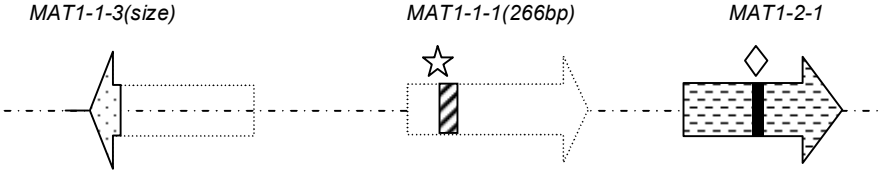
b)*O. novo-ulmi MAT1-2* isolate



c)*O. quercus* MAT A isolate



d)*O. quercus* MATB isolate



e) *O. quercus* isolate CMW 2521 (Pseudoselfing)

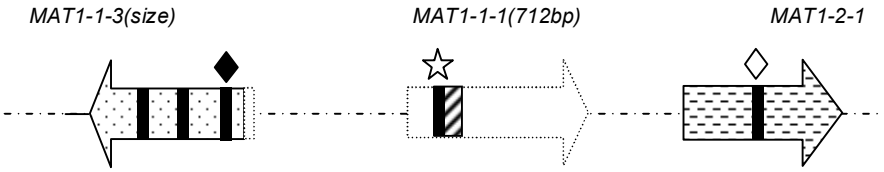


Fig 3. Diagrammatic representation of the gene information currently available for *Ophiostoma*.For *O. novo-ulmi*, three *MAT* genes are predicted for a MAT1-1 isolate (NCBI accession number FJ858801) (A), while only a single gene is present in a MAT1-2 isolate. (B) In this study, the *MAT1-2-1* gene was amplified from both MAT A and MAT B isolates of *O. quercus* (C, D). In addition, all five MAT A isolates contain a partial *MAT1-1-1* (266 bp) and a large fragment of the *MAT1-1-3* (766 bp) gene (C), while four of the MAT B isolates also encode fragments of the *MAT1-1-3* (180 bp) and *MAT1-1-1* (266 bp) genes. (D) For the MAT B isolate CMW2521, a large fragment of the *MAT1-1-3* (766 bp) and *MAT1-1-1* (712 bp) genes was amplified in addition to the *MAT1-2-1* gene. (E) The structure and gene order of the *O. quercus* idiomorphs are implied from that of *O. novo-ulmi*. Dark bars indicate the presence of an intron. Stars indicate the α-box conserved domain. Diamond shapes represent HMG-boxes for the *MAT1-1-3* (filled) and *MAT1-2-1* (clear) genes. Dashed lines and boxes indicate sections of the idiomorph and coding regions for which sequence is not available. The diagrams are not drawn to scale.

Fig 4. Alignment of the HMG-box domain of the MAT1-1-3 protein.An alignment of the HMG-box conserved domain characteristic of the MAT1-1-3 protein was done. A plot showing the conservation across the protein fragment is presented at the bottom of the alignment. The *O. quercus* sequence is representative of the MAT1-1-3 protein sequences produced for all but isolate CMW 27845. Accession numbers: *O. quercus* sequences – Table 1, *O. novo-ulmi* subsp. *novo-ulmi* – ACZ53925; *Chaetomium globosum* – EAQ89965; *Cryphonectria parasitica* – AF380365_1; *Gibberella fujikuroi* – AAC71053; *G. zeae* – AAG42812; *Magnaporthe grisea* – BAC65085; *Neurospora crassa* – AAC37476; *Podospora anserina* – CAA52051.

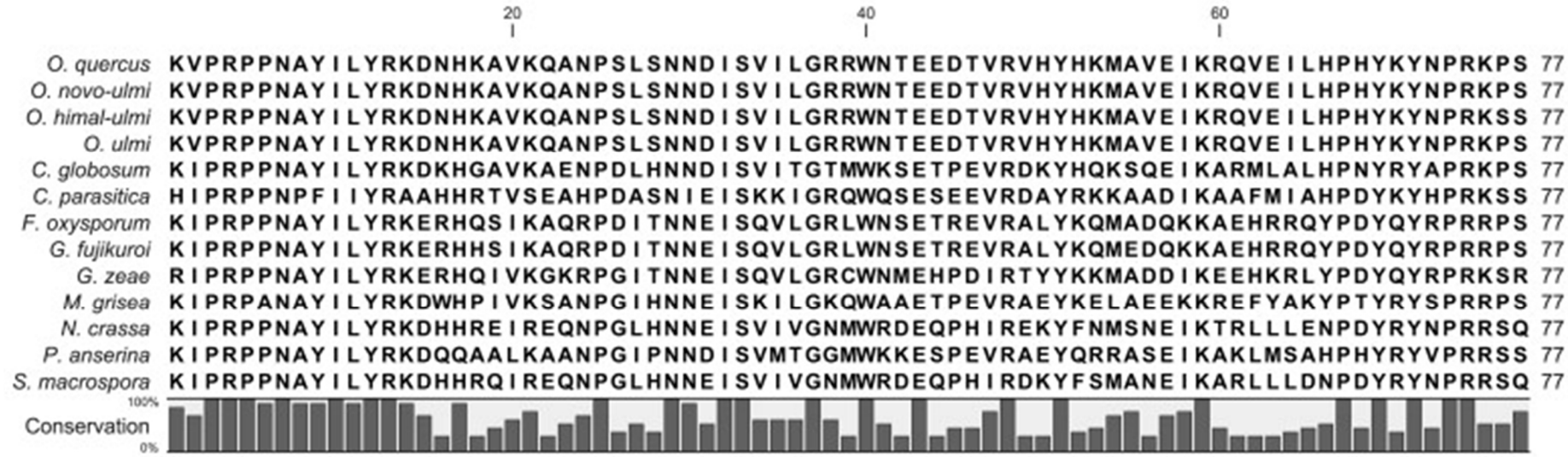


Fig 5. Alignment of the HMG-box domain of the MAT2-1 protein.An alignment showing the conservation of amino acids across the HMG-box domain of the mating type protein MAT1-2-1 (MAT2-1). The last row included a conservation plot indicating the conservation in amino acid sequence across the fragment. The listed *O. quercus* sequence is representative of the MAT1-2-1 HMG region for all isolates used in this study. Accession numbers: *O. quercus* sequences – Table 1, *O. novo-ulmi* – AAX83067; *O. himal-ulmi* – AAX83073; *O. ulmi* – AAX83065; *Chaetomium globosum* – EAQ91645; *Cryphonectria parasitica* – AF380364_1; *Fusarium oxysporum* – BAA28611; *Gibberella fujikuroi* – AAC71056; *G. zeae* – AAG42810; *Magnaporthe grisea* – BAC65090; *Neurospora crassa* – AAA33598; *Podospora anserina* – CAA45520; *Sordaria macrospora* – CAA71624.