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Regular Articles

The heterothallic sugarbeet pathogen *Cercospora beticola* contains exon fragments of both *MAT* genes that are homogenized by concerted evolution

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ABSTRACT

Dothideomycetes is one of the most ecologically diverse and economically important classes of fungi. Sexual reproduction in this group is governed by mating type (*MAT*) genes at the *MAT1* locus. Self-sterile (heterothallic) species contain one of two genes at *MAT1* (*MAT1-1-1* or *MAT1-2-1*) and only isolates of opposite mating type are sexually compatible. In contrast, self-fertile (homothallic) species contain both *MAT* genes at *MAT1*. Knowledge of the reproductive capacities of plant pathogens are of particular interest because recombining populations tend to be more difficult to manage in agricultural settings. In this study, we sequenced *MAT1* in the heterothallic *Dothideomycete* fungus *Cercospora beticola* to gain insight into the reproductive capabilities of this important plant pathogen. In addition to the expected *MAT* gene at *MAT1*, each isolate contained fragments of both *MAT1-1-1* and *MAT1-2-1* at ostensibly random loci across the genome. When *MAT* fragments from each locus were manually assembled, they reconstituted *MAT1-1-1* and *MAT1-2-1* exons with high identity, suggesting a retroposition event occurred in a homothallic ancestor in which both *MAT* genes were fused. The genome sequences of related taxa revealed that *MAT* gene fragment pattern of *Cercospora zeae-maydis* was analogous to *C. beticola*. In contrast, the genome of more distantly related *Mycosphaerella graminicola* did not contain *MAT* fragments. Although fragments occurred in syntenic regions of the *C. beticola* and *C. zeae-maydis* genomes, each *MAT* fragment was more closely related to the intact *MAT* gene of the same species. Taken together, these data suggest *MAT* genes fragmented after divergence of *M. graminicola* from the remaining taxa, and concerted evolution functioned to homogenize *MAT* fragments and *MAT* genes in each species.

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1. Introduction

Sexual reproduction is a common phenomenon in eukaryotic organisms. Fungi that reproduce by sexual outcrossing generally have a superior ability to adapt to changing environments, are better able to purge mutations that threaten genome stability, and are more efficient at selecting beneficial mutations compared to asexual populations in which an individual inherits all genetic material from a single parent (Burt, 2000; Heitman et al., 2013; Lee et al., 2010). In terms of modern agriculture, genetic crop resistance and fungicides are significant forms of selection pressure imposed on fungal pathogen populations. Recombining pathogen populations often exhibit a high degree of genotype diversity and therefore pose a greater risk for defeating resistance genes or developing fungicide resistance than those that strictly rely on asexual reproduction (McDonald and Linde, 2002). Therefore, knowledge of the reproductive capacities of a given pathogen is critical for disease management strategies that aim to maximize the life expectancy of resistance genes and maintain fungicide efficacy.

Abbreviations: MAT, mating type genes; MAT1, mating type locus; MAT1-1, MAT1-2, mating type idiomorphs; MAT1-1-1, mating type gene encoding an alpha box (α1) protein; MAT1-2-1, mating type gene encoding a protein with a high mobility group domain.

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Mating type (*MAT*) genes are transcription factors that regulate global sexual reproduction in fungi (Heitman, 2010). In most ascomycetes, *MAT* genes are located at a single locus (*MAT1*) represented by two idiomorphs known as *MAT1-1* and *MAT1-2* (Turgeon and Yoder, 2000) that are required to control nonself-recognition and mating of compatible partners (Nelson, 1996). Rather than calling *MAT* genes alleles, the term 'idiomorph' has been adopted to emphasize that they are not obviously related by structure or common descent despite occupying the same locus (Metzenberg and Glass, 1990), although recent studies suggest that core motifs in some *MAT* genes may have a common evolutionary origin (Martin et al., 2010). Since sequence similarity between idiomorphs is very low, they do not normally recombine with each other despite occupying the same locus in the genome (Turgeon, 1998). In the *Dothideomycetes* (e.g., *Cercospora* and *Mycosphaerella* spp.), the *MAT1-1* idiomorph is represented by the *MAT1-1-1* gene encoding an alpha box (α1) protein. The *MAT1-2* idiomorph of most ascomycetes carries a single gene, *MAT1-2-1*, encoding a protein with a high mobility group (HMG) DNA-binding domain (Turgeon and Yoder, 2000). Little is known about the targets of these transcriptional regulators in filamentous fungi (Turgeon, 1998). In heterothallic species, haploid selfing is prevented because fusion between haploid cells (syngamy) can only occur between strains of the opposite mating type (Billiard et al., 2012). In contrast, homothallic fungi usually harbor a single *MAT1* locus with the α1 and HMG genes either fused or located in proximity allowing meiosis to occur without a partner (Yun et al., 2000, 1999). In addition, each haploid is theoretically universally compatible with any other haploid individual in a homothallic population (Billiard et al., 2012), although several lines of evidence suggest that some species, such as homothallic *Neurospora* taxa, still reproduce chiefly by sexual selfing (intra-haploid mating) in nature (Gioti et al., 2012; Nygren et al., 2011). Besides involvement in fungal mating, *MAT* genes have been shown to be involved with hyphal morphology, conidia formation, and production of secondary metabolites (Böhm et al., 2013).

Cercospora leaf spot, caused by the fungal pathogen *Cercospora beticola* Sacc., is the most destructive foliar disease of sugarbeet (*Beta vulgaris* L.) worldwide (Weiland and Koch, 2004). The fungus has a heterothallic arrangement of *MAT* genes at *MAT1* (Groenewald et al., 2006). The species is well-known for high levels of variability, particularly with regard to fungicide resistance (Birla et al., 2012; Bolton et al., 2012a,b, 2013). Population studies have shown that *C. beticola* displays a high degree of genetic variation (Groenewald et al., 2008; Moretti et al., 2006, 2004). In addition, both mating type idiomorphs are present in approximate 1:1 ratios in most *C. beticola* populations worldwide (Bolton et al., 2012c; Groenewald et al., 2006, 2008) and *MAT* genes are expressed *in planta* (Bolton et al., 2012c). High levels of genotypic diversity and an equal distribution of *MAT* genes suggests that sexual recombination may be occurring within a population (Linde et al., 2003; Milgroom, 1996). Although *C. beticola* populations exhibit several hallmarks of sex, however, no sexual reproduction in the field or in the laboratory has been confirmed to our knowledge.

During past investigations of *MAT* gene distribution in *C. beticola*, a segment of *MAT1-2-1* coding sequence was discovered in *MAT1-1* isolates (Bolton et al., 2012c). These results were unexpected since each isolate from a filamentous heterothallic fungus typically contains only one of the two *MAT* genes. In the present study, we investigated this phenomenon further to show that in addition to the intact *MAT1-1-1* or *MAT1-2-1* genes, all *C. beticola* isolates contained nearly the entire exons for *MAT1-1-1* and *MAT1-2-1* in fragments spread throughout the genome. The same pattern of *MAT* gene fragmentation was also found in the closely-related pathogen *Cercospora zeae-maydis*. Our data suggest that retroposition of a fused *MAT* gene transcript occurred prior to speciation, but the finding that *MAT* gene fragments were more closely related to the intact *MAT* genes within each species demonstrates that concerted evolution acted on *MAT* fragments and *MAT* genes after speciation. This finding sheds light on a lineage in the *Dothideomycetes* that likely evolved from a homothallic ancestor.

2. Materials and methods

2.1. Southern analysis of C. beticola MAT1

Sixty-eight *C. beticola* isolates were used in this study (Supplementary Table 1). All isolates were single spore derived and characterized for mating type as described earlier (Bolton et al., 2012c). Six *C. beticola* isolates (*MAT1-1*: EU10-73, 10-260, 10-261; *MAT1-2*: 98-23, 10-287, and 10-431) were used to investigate *MAT* gene copy number. Fungal DNA isolation was carried out as previously described (Bolton et al., 2013). A total of 5 μg of genomic DNA of each isolate was completely digested with the restriction enzyme *Eco*RI or *Hin*dIII at 37 °C. DNA blotting, Southern probe preparation, hybridization, and signal detection were performed as described by Faris et al. (2000). Primers 43/44 and 45/899 (Supplementary Table 2) were used in PCR to generate *MAT1-1-1* and *MAT1-2-1* gene sequences to be used as probes, respectively.

2.2. BAC library screening and sequencing

A *C. beticola* bacterial artificial chromosome (BAC) library was created by Amplicon Express (Pullman, WA, USA) using the *MAT1-2* isolate 98-23 that was estimated to have ~6X genome coverage. BAC clones were spotted onto Hybond N+ membranes (GE Healthcare, Pittsburgh, USA) using a Q-bot (Genetix Inc. UK). Probe preparation, hybridization, and signal detection were performed using standard techniques (Sambrook and Russell, 2001). BAC clones containing a *MAT* gene or gene fragments were identified using *MAT* probes used for Southern analyses described above. To confirm that each BAC clone harbored a unique *MAT* gene or gene fragment, PCRs with primer combinations 432/433, 458/459, 230/79, 45/99, or 275/276 (Supplementary Table 2) were chosen because they amplified small regions of each *MAT* gene. Differential amplification of each primer pair was used to "code" BACs in order to identify BACs with a unique *MAT* gene signature and ensure no BAC sequencing redundancy. PCR amplifications were carried out using the GoTaq Flexi DNA Polymerase kit (Promega, Madison, WI, USA) in a total volume of 25 μl containing 1× Green GoTaq Flexi buffer, 2.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.4 μM of each primer, 1.25 U of GoTaq DNA polymerase, and ~10 ng of genomic DNA. The PCR protocol was an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C (30 s), 55 °C (30 s), and 72 °C (30 s). A final elongation step at 72 °C (5 min) was included. PCR products were visualized in 1.5% (w/v) agarose gels. Four BACs were chosen for sequencing. Fragment library construction, 454 Titanium pyrosequencing, and assembly were carried out by Beckman Coulter Genomics (Danvers, MA, USA).

2.3. MAT1 locus and MAT gene fragment sequencing

The full-length *MAT1-1-1* and *MAT1-2-1 C. beticola* gene sequences (GenBank accession numbers JN863084 and KC960680, respectively) were aligned to each BAC sequence using the BLAST 2 Sequences (bl2seq) tool at NCBI (http://blast.ncbi.nlm.nih.gov/) to identify regions of interest (ROI) that contained *MAT* gene sequence homology. Since the BAC library was derived from a *MAT1-2* isolate, a series of primers designed on the derived BAC sequences were used to verify that each ROI was not unique to the