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Genetic basis for high population diversity in *Protea*-associated *Knoxdaviesia*

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ABSTRACT

Sexual reproduction is necessary to generate genetic diversity and, in ascomycete fungi, this process is controlled by a mating type (*MAT*) locus with two complementary idiomorphs. *Knoxdaviesia capensis* and *K. proteae* (Sordariomycetes; Microascales; Gondwanamycetaceae) are host-specific saprophytic fungi that show high population diversity within their *Protea* plant hosts in the Cape Floristic Region of South Africa. We hypothesise that this diversity is the result of outcrossing driven by a heterothallic mating system and sought to describe the *MAT1* loci of both species. The available genome assembly of each isolate contained only one of the *MAT1* idiomorphs necessary for sexual reproduction, implying that both species are heterothallic. Idiomorph segregation during meiosis, a 1:1 ratio of idiomorphs in natural populations and mating experiments also supported heterothallism as a sexual strategy. Long-range PCR and shot-gun sequencing to identify the opposite idiomorph in each species revealed no sequence similarity between *MAT1-1* and *MAT1-2* idiomorphs, but the homologous idiomorphs between the species were almost identical. The *MAT1-1* idiomorph contained the characteristic *MAT1-1-1* and *MAT1-1-2* genes, whereas the *MAT1-2* idiomorph consisted of the genes *MAT1-2-7* and *MAT1-2-1*. This gene content was similar to that of the three species in the Ceratocystidaceae (Microascales) with characterized *MAT* loci. The *Knoxdaviesia MAT1-2-7* protein contained an alpha domain and predicted intron, which suggests that this gene arose from *MAT1-1-1* during a recombination event. In contrast to the Ceratocystidaceae species, *Knoxdaviesia* conformed to the ancestral Sordariomycete arrangement of flanking genes and is, therefore, a closer reflection of the structure of this locus in the Microascalean ancestor.

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1. Introduction

Sexual reproduction is universal across eukaryotic life, despite being more biologically costly than asexual propagation (Lehtonen et al., 2012; Ni et al., 2011; Otto, 2009; Stearns, 1987). Most fungi maintain both of these reproductive strategies that are controlled by diverse genetic mechanisms (Billiard et al., 2012; Ni et al., 2011). Evidence suggests that many fungi exploit the low cost of clonal reproduction during favourable environmental conditions, but switch to sexual reproduction under stress when adaptation becomes necessary (Ni et al., 2010; Nielsen and

Heitman, 2007; Seymour et al., 2005). Novel allele combinations are essential for adaptation and the re-shuffling of genetic material enables selection against harmful or unfavourable genotypes that may be propagated through clonal reproduction (Lynch et al., 1993).

The mating type (*MAT*) genes of fungi control the recognition between sexual partners and the subsequent development of sexual progeny (Coppin et al., 1997; Perkins, 1987). In ascomycetes, mating type is determined by a single locus, *MAT1*, and two mating type idiomorphs (dissimilar alleles), *MAT1-1* and *MAT1-2* (Kronstad and Staben, 1997; Nelson, 1996; Turgeon and Yoder, 2000). Homothallic fungi have a *MAT1-1/2* genotype (Turgeon and Yoder, 2000); both *MAT1* idiomorphs occur in one genome, making them self-fertile. In heterothallic species, the absence of either idiomorph results in self-sterility that necessitates outcrossing

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between two individuals of opposite mating type for sexual reproduction (Kronstad and Staben, 1997; Nelson, 1996). Although it is widely accepted that the presence of both idiomorphs is necessary for sexual reproduction, fungal mating strategies are diverse and many exceptions to this rule have been observed (Heitman, 2015). For example, some species are self-fertile despite only possessing one *MAT1* idiomorph (unisexual reproduction; Alby and Bennett, 2011; Glass and Smith, 1994; Lin et al., 2005; Wilson et al., 2015).

The two idiomorphs of the ascomycete *MAT1* locus each contain at least one open reading frame (ORF) with a characteristic motif (Turgeon and Yoder, 2000). The *MAT1-1* idiomorph is defined by an ORF with an alpha domain (*MAT1-1-1*), although up to two additional “accessory” ORFs can occur in this idiomorph. The *MAT1-2* idiomorph generally has a single ORF (*MAT1-2-1*) with an HMG-box motif. The functions of each of these *MAT1* genes is not fully understood, but it is believed that each idiomorph encodes transcription factors (Herskowitz, 1989) that ultimately perform a dual function. Firstly, the transcription factors mediate a hormonal recognition mechanism between individuals by producing a pheromone and receptors for the pheromone of the opposite mating type (Coppin et al., 1997; Glass et al., 1990; Ni et al., 2011). Secondly, these genes are involved in the formation of sexual structures (Coppin et al., 1997). Molecular studies have shown that the *MAT1-1-1* gene alone is able to induce fertilization, but in *Podospira anserina*, the accessory genes analogous to *MAT1-1-2* and *MAT1-1-3* are needed for the sexual structures to develop fully (Debuchy et al., 1993), supporting the dual function. As the only consistently occurring ORF on the *MAT1-2* idiomorph, *MAT1-2-1* appears to be involved in ascum development and is the sole determinant of the necessary functions in this mating type (Coppin et al., 1997; Staben and Yanofsky, 1990).

Of the five Microascales (Sordariomycetes) families (Maharachchikumbura et al., 2015; Réblová et al., 2011) only three species in the predominantly plant-associated, agriculturally important Ceratocystidaceae (De Beer et al., 2014) have been studied extensively in terms of mating type genetics. The sweet potato pathogen, *Ceratocystis fimbriata* s.s., is homothallic and undergoes unidirectional mating type switching whereby it loses its *MAT1-2-1* gene and becomes self-sterile (Harrington and McNew, 1997; Wilken et al., 2014). The other two Ceratocystidaceae species studied are members of the genus *Huntia* that typically show a saprophytic association with tree wounds (Van Wyk et al., 2006). Both of the studied species in this genus are heterothallic (Wilson et al., 2015), although *H. moniliformis* is also capable of unisexual reproduction; since it contains a single *MAT1* idiomorph, yet produces ascumata (Wilson et al., 2015). One trait that unites the diverse mating strategies in Ceratocystidaceae is their deviation from the consensus gene order of the Sordariomycetes. The cytoskeleton assembly control (*SLA2*) and DNA lyase (*APN2*) genes that flank the *MAT* locus in almost all Sordariomycetes (Debuchy and Turgeon, 2006) have an altered order and orientation in the Ceratocystidaceae. The genes that typically flank the downstream region of *MAT1* loci have shifted to an upstream position in *C. fimbriata*. A similar shift is evident in the two studied *Huntia* species, although the *APN2* gene has shifted to a genomic position far from the *MAT1* locus (Wilson et al., 2015).

The aim of this study was to describe the *MAT1* locus of two saprophytic, but host-specific species in the Gondwanamycetaceae, which is also a member of the Microascales (Réblová et al., 2011). These fungi (*Knoxdaviesia capensis* and *K. proteae*) occur in the seed cones of *Protea*, a keystone plant genus in the Cape Floristic Region of South Africa (Bergh et al., 2014; Cowling, 1992; Manning and Goldblatt, 2012). The arthropod, and possibly bird, vectors of these fungi disperse ascospores between *Protea*

flower heads (Roets et al., 2011b). After flowering, the *Protea* inflorescence matures into an enclosed seed cone in which *K. capensis* and *K. proteae* are visible on decaying floral structures as ascumata that present spore droplets on long ostiolar necks (Wingfield and Van Wyk, 1993; Wingfield et al., 1988). Although conidiophores may also be present, *K. capensis* and *K. proteae* sexual structures are abundant within infructescences (Wingfield and Van Wyk, 1993; Wingfield et al., 1988), indicating that sexual reproduction is prevalent and likely the dominant mode of reproduction at this stage of their life-cycle. As ascomycete fungi, *K. capensis* and *K. proteae* are haploid during the vegetative state and sexual reproduction would thus only add genetic diversity if it is not a result of self-fertilization (Fincham and Day, 1963; Milgroom, 1996; Moore and Novak Frazer, 2002). High gene and genotypic diversity and random allele association within two populations of *K. proteae* (Aylward et al., 2014, 2015b) and nine populations of *K. capensis* (Aylward, unpublished) strongly suggest that sexual reproduction in these species is non-selfing. *Protea*-associated *Knoxdaviesia* individuals, therefore, regularly recombine to produce genetically novel offspring, but whether outcrossing is a prerequisite for sexual reproduction in these species (*i.e.* heterothallism) or whether it is optional (*i.e.* homothallism) remains unknown.

The genomes of *K. capensis* and *K. proteae* have recently been sequenced (Aylward et al., 2016). In this study, we used these genomes to investigate the genetic basis of mating in *Knoxdaviesia*. In so doing, we tested the hypothesis that the genetic diversity observed in natural populations of these species is due to outcrossing driven by a heterothallic mating system. As a secondary aim, we compared the identified *Knoxdaviesia MAT1* loci to the three species in the Ceratocystidaceae with characterized *MAT1* loci.

2. Materials and methods

2.1. Fungal isolates and genome sequences used

The genomes of *K. capensis* CBS139037 (LNGK00000000.1) and *K. proteae* CBS140089 (LNGLO00000000.1) were sequenced in a previous study (Aylward et al., 2016) and are available in GenBank® (Benson et al., 2013). The *MAT1* locus of *C. fimbriata* CMW14799, previously characterized from its sequenced genome (Wilken et al., 2014), was also obtained from GenBank (KF033902.1; KF033903.1). Other than the *Knoxdaviesia* genome isolates, three additional strains of *K. capensis* (CMW40886, CMW40889, CMW40892) and *K. proteae* (CMW40879, CMW40882, CMW40883) were used in this study to perform crossing experiments. All isolates were routinely cultured on Potato Dextrose Agar (PDA; Merck, Wadeville, South Africa) for approximately seven days at 25 °C and maintained at 4 °C.

2.2. Identification of *MAT* loci from genome sequences

Ceratocystis fimbriata s.s. is currently the species most closely related to *Knoxdaviesia* that has a characterized *MAT1* locus with available gene models. The predicted proteins of the *MAT1* locus of *C. fimbriata* CMW14799 (AHV84683-84701) were used to search for the *MAT1* locus in the genomes of *K. capensis* and *K. proteae* by performing local BLASTx searches in CLC Genomics workbench 6 (CLC Bio, Denmark). Preliminary analysis identified a single *MAT1* idiomorph from each *Knoxdaviesia* genome and revealed that the two genomes contained opposite *MAT1* idiomorphs. Subsequently, we mapped the raw sequence reads from the *K. capensis* genome (GenBank Accession: SRX1453186, SRX1453795 and SRX1453796) to the *K. proteae MAT1* region in CLC Genomics workbench to identify the terminal ends of the *MAT1* locus in *K. proteae*.

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