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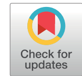
Regular Articles

Evolution of the mating types and mating strategies in prominent genera in the *Botryosphaeriaceae*



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ABSTRACT

Little is known regarding mating strategies in the *Botryosphaeriaceae*. To understand sexual reproduction in this fungal family, the mating type genes of *Botryosphaeria dothidea* and *Macrophomina phaseolina*, as well as several species of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were characterized from whole genome assemblies. Comparisons between the mating type loci of these fungi showed that the mating type genes are highly variable, but in most cases the organization of these genes is conserved. Of the species considered, nine were homothallic and seven were heterothallic. Mating type gene fragments were discovered flanking the mating type regions, which indicates both ongoing and ancestral recombination occurring within the mating type region. Ancestral reconstruction analysis further indicated that heterothallism is the ancestral state in the *Botryosphaeriaceae* and this is supported by the presence of mating type gene fragments in homothallic species. The results also show that at least five transitions from heterothallism to homothallism have taken place in the *Botryosphaeriaceae*. The study provides a foundation for comparison of mating type evolution between *Botryosphaeriaceae* and other fungi and also provides valuable markers for population biology studies in this family.

1. Introduction

Two modes of sexual reproduction exist in fungi, heterothallism and homothallism. In heterothallic fungi, two individuals of opposite mating compatibility types must be present in order for sexual reproduction to take place (Ni et al., 2011). Homothallism is characterized by indiscrimate mating, i.e. one fungal individual can mate with any other individual of its species, including itself. Both thallism states have advantages. Homothallism allows unrestricted sexual compatibility that is beneficial when the probability of encountering a mating partner is low and the benefits of occasional outcrossing outweigh the cost of more frequent haploid selfing. Conversely, heterothallism is better suited to situations where mating partners are encountered frequently, but the fitness cost for selfing is high (Billiard et al., 2012).

Ascomycetes have a bipolar mating system that is usually determined by a single locus (Butler, 2010). The ascomycete mating type genes occur at the *MAT1* locus. In heterothallic fungi the genes are idiomorphic between the two mating types; that is the genes at the *MAT1* locus are not homologous between the two mating types (Idnurm, 2011). The *MAT1-1* idiomorph is defined as containing the *MAT1-1-1* gene that possesses an alpha box protein domain (Turgeon and Yoder, 2000). Likewise the *MAT1-2* idiomorph contains the *MAT1-2-1* gene that produces a protein with the high mobility group (HMG) domain (Turgeon and Yoder, 2000).

The gene organization at the *MAT1* locus, as well as the position of the locus in the genome is moderately conserved in the Ascomycota. The *MAT1* locus may contain either the *MAT1-1-1* or *MAT1-2-1*, alone or with one or more ancillary mating type genes. The naming of mating type genes follows the system described by Turgeon and Yoder (2000) and has recently been revised in order to correct inconsistent/incorrect application of the mating type gene nomenclature (Wilken et al., 2017). In many ascomycetes, the *MAT1* locus is flanked by the *SLA2* and *APN2*/DNA lyase genes (Debuchy and Turgeon, 2006).

Homothallism can occur in various forms, i.e. primary homothallism, pseudohomothallism and mating type switching (Wilson et al., 2015). Primary homothalism occurs when both mating type idiomorphs are present in one genome, either at the same locus (Pöggeler et al., 1997; Yun et al., 1999, 2000) or at unlinked loci (Galagan et al., 2005; Lopes et al., 2017). Additionally the *MAT1-1-1* and *MAT1-2-1* genes can be fused in a single functional gene that contains both the alpha-box and HMG domains (Yun et al., 1999).

The *Botryosphaeriaceae* is a family of fungi that commonly occur in woody plants as endophytes and many species are also important opportunisitic tree pathogens (Slippers and Wingfield, 2007). Sexual structures in these fungi are rarely observed in nature or under laboratory conditions (Phillips et al., 2013; Slippers et al., 2017). This,

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however, does not necessarily imply that species of *Botryosphaeriaceae* fail to undergo sexual reproduction. For example, physical evidence of sexual reproduction has never been observed for *Diplodia sapinea*, but most likely takes place when considering population genetics data (Bihon et al., 2012a, 2012b). Consistent with this view, the mating type genes in *D. sapinea* were subsequently characterized and these showed that the fungus is heterothallic (Bihon et al., 2014). The *MAT1-1* idiomorph of *D. sapinea* contains the *MAT1-1-1* gene, as well as the *MAT1-1-8* gene, whereas the *MAT1-2* idiomorph contains the *MAT1-2-1* genes, as well as a novel *MAT1-2-5* gene. Bihon et al. (2014) further showed that all but one of the populations considered did not deviate significantly deviate from the 1:1 ratio between the two mating type idiomorphs, further supporting sexual reproduction in this species.

The mating type genes in several other *Botryosphaeriales* have recently been characterized (Amorim et al., 2017; Lopes et al., 2017; Marsberg et al., 2016; Wang et al., 2016). A genome assembly for *Neofusicoccum parvum* was used to identify the mating type genes of this species and revealed both *MAT1-1* and *MAT1-2* idiomorphs in the genome, indicating primary homothallism (Lopes et al., 2017). Primer sets that allowed amplification of the *MAT1-1-1* and *MAT1-2-1* genes from other species in this genus were also developed (Lopes et al., 2017). Similarly, a genome assembly was used to characterize the mating type genes of *Botryosphaeria dothidea*, which was shown to be homothallic (Marsberg et al., 2016). The mating type locus in *Phyllosticta citricarpa* (Phyllostictaceae) (Amorim et al., 2017; Wang et al., 2016) and *P. capitalensis* (Wang et al., 2016) has also been characterized using a similar genomics based approach, showing that *P. citricarpa* is heterothallic and *P. capitalensis* is homothallic.

Recent studies on the mating type genes in species of *Botryosphaeriales* have highlighted various knowledge gaps regarding sexual reproduction in these fungi. For example, questions arise regarding the conservation of mating types, i.e. the conservation of nucleotide and amino acid sequence of the mating type genes, the conservation of the genes occurring at the mating type locus and the conservation of the genomic location of the mating type locus. It is also not known how thallism has evolved in this family; whether the most recent common ancestor of the *Botryosphaeriaceae* was homothallic or heterothallic or how frequently changes to the ancestral mating strategy have evolved. Furthermore, a scarcity of mating type markers capable of amplifying a broad range of species, other than those for *Neofusicoccum* spp. developed by Lopes et al. (2017), limits our capacity to investigate thallism and mating type ratios in *Botryosphaeriaceae* species, for which genomic data are not available.

Knowledge regarding the mating types and mode of reproduction is lacking for some of the most commonly occurring genera in the *Botryosphaeriaceae*. Consequently, the aim of this study was to characterize the mating type loci of several species of the *Botryosphaeriaceae* using both publicly available genomic data and genomic assemblies determined as part of this study. The architecture of these mating type loci and their flanking genes were compared and their phylogenetic relationships analysed to reconstruct the evolutionary history of mating strategies in this family. A set of primers was also designed to facilitate future studies of mating systems in the *Botryosphaeriaceae*.

2. Materials and methods

2.1. Acquisition of genome sequences

Genomic sequence data used in analyses were acquired either from incomplete *de novo* genome assemblies or from public genomic databases (Table 1). The incomplete *de novo* genome sequencing was performed using paired-end reads on a Illumina Hiseq 2500 platform. Sequencing reads were trimmed using Trimmomatic 0.36 (Bolger et al., 2014) and assembled with Velvet (Zerbino and Birney, 2008) and Velvetoptimiser (Gladman and Seemann, 2012).

2.2. Characterization of mating type and surrounding genes

The presence of mating type genes from the genome sequences was determined by performing a local tBLASTx (Camacho et al., 2009) search using the MAT and adjacent genes from *D. sapinea* (KF551229 and KF551228) (Bihon et al., 2014). The relevant contigs were extracted from genome sequences and annotated using WebAugustus (http://bioinf.uni-greifswald.de/augustus/). The identities of the annotated genes were determined by BLASTp against the NCBI nr-database. The annotations were further manually refined by comparison with existing homologs on NCBI. The resultant annotated loci of sequence data generated in this study were deposited in GenBank (Table 1). The mating type gene annotations from publically available genome data are provided in Supplementary File 1. Mating type genes were assessed for functional domains by performing a search against NCBI's conserved domain database (CDD BLAST) (Marchler-Bauer et al., 2014).

2.3. Comparison of mating type locus organization

In order to compare the organization of the mating type genes and flanking genes between species, BLASTn was used to assess the similarity between sequences. EasyFig version 2.2.2 (Sullivan et al., 2011) was used to perform synteny analysis based on pairwise BLASTn similarity between taxa using a maximum E-value cut-off of 0.0001. This approach was also used to assess the presence of partial MAT gene fragments at the mating type locus. Intergenic regions on either side of the mating type genes were investigated for the presence of sequences with homology to the *MAT1-1-1, MAT1-1-8, MAT1-2-1* or *MAT1-2-5* genes. Where only a single mating type was available for a species (i.e. *D. corticola*, *D. scrobiculata* and *L. pseudotheobromea*), the genes of the opposite mating type from the closest available species was used to determine the presence of mating type gene fragments in the intergenic regions flanking the mating type genes.

2.4. Phylogenetic comparisons and ancestral state reconstruction

Phylogenetic analyses of the *MAT1-1-1* and *MAT1-2-1* genes were performed using the nucleotide sequence data of the protein coding regions. Sequences were aligned using MAFFT version 7 (Katoh and Standley, 2013). RAxML (Stamatakis, 2014) was used to generate Maximum Likelihood phylogenetic trees using a GTRCATI model and a thousand bootstrap replicates to assess branch support. Additionally, Bayesian statistical inference was also applied to generate phylogenetic trees and posterior probability node support for the mating type genes. Each gene alignment was subjected to hierarchical Likelihood ratio tests using MrModeltest2.2 (Nylander, 2004). Bayesian analysis was performed for one million generations on MrBayes version 3.1 (Ronquist and Huelsenbeck, 2003). The burn-in was determined using Tracer (Rambaut and Drummond, 2003), prior to tree and parameter summarization.

Reconstruction of the ancestral thallic state was performed using Mesquite 3.10 (Maddison and Maddison, 2016). The topology of the multi-gene phylogenetic tree of Slippers et al. (2013) was used as a backbone to map characters. The character states mapped onto the phylogenetic backbone were heterothallism, homothallism or unknown. Species were designated as heterothallic when a single mating type (either *MAT1-1* or *MAT1-2*) was present in the genome. Conversely, homothallic species were identified by the presence of both mating types in a single genome. Character state data used in this analysis included those generated in the current study, as well as previous studies of the *Botryosphaeriales* (Lopes et al., 2017; Wang et al., 2016). An unordered parsimony model was used to map character states onto the tree and to reconstruct ancestral states.

Table 1
Data on genome sequences used in this study including details of mating type and thallism.

Species	Reference collection number[a]	Other collection numbers or strain	Thallism	Mating type	Accession numbers		
					MAT locus	Genome	Genome reference
Botryosphaeria dothidea		LW030101	Homothallic			MDSR00000000	(Liu et al., 2016)
Diplodia corticola	CBS112549		Heterothallic	*MAT1-1*		MNUE01000001	
D. sapinea	CBS117911	CMW190	Heterothallic	*MAT1-1*	KF551229	AXCF00000000	(van der Nest et al., 2014)
	CBS138184	CMW39103		*MAT1-2*	KF551228	JHUM00000000	
D. seriata		F98.1	Heterothallic	*MAT1-1*		MSZU00000000	(Robert-Siegwald et al., 2017)
		UCDDS831		*MAT1-2*		LAQI00000000	(Morales-Cruz et al., 2015)
	CBS112555	**CMW 31769**		*MAT1-2*	**KX787890**		
D. scrobiculata	CBS139796	CMW30223	Heterothallic	*MAT1-1*		LAEG00000000	(Wingfield et al., 2015)
Lasiodiplodia gonubiensis	**CBS115812**	**CMW 14077**	Homothallic		**KX787887**		
L. pseudotheobromae	**CBS116459**	**CMW 31774**	Heterothallic	*MAT1-1*	**KX787888**		
L. theobromae	**CBS164.96**	**CMW40942**	Heterothallic	*MAT1-1*	**KX787889**		
		CSS001		*MAT1-2*		MDYX00000000	(Yan et al., 2017)
Macrophomina phaseolina		MS6	Heterothallic	*MAT1-1*		AHHD00000000	(Islam et al., 2012)
		MP00003		*MAT1-2*		LHTM00000000	
Neofusicoccum australe		**CMW 6837**	Homothallic		**KY775140**		
					KY775142		
N. cordaticola	**CBS123634**	**CMW13992**	Homothallic		**KY612503**		
					KY612506		
	CBS123638	**CMW14124**			**KX766040**		
					KX766043		
N. kwambonambiense	**CBS123639**	**CMW14023**	Homothallic		**KY612505**		
					KY612507		
	CBS123642	**CMW14155**			**KX766039**		
					KX766045		
N. luteum		**CMW 9076**	Homothallic		**KY775141**		
					KY775143		
N. parvum		UCRNP2	Homothallic		KB915846	AORE00000000	(Blanco-Ulate et al., 2013)
					KB916244		
		UCD646So				PRJNA321421	(Massonnet et al., 2016)
		CMW9080			**KY612501**		
					KY612508		
	CBS123649	**CMW14085**			**KX766038**		
					KX766044		
N. ribis	**CBS115475**	**CMW7772**	Homothallic		**KY612504**		
					KY612509		
	CBS121.26	**CMW7054**			**KX766036**		
					KX766041		
N. umdonicola	**CBS123645**	**CMW14058**	Homothallic		**KY612502**		
					KY612510		
	CBS123644	**CMW14106**			**KX766037**		
					KX766042		

[a] Entries in boldface represents isolates where mating type sequences were obtained from incomplete genomes in the present study.

2.5. Primer design and PCR amplification

Primers for amplification of the primary mating type genes have previously been designed for *D. sapinea* (Bihon et al., 2014) and *Neofusicoccum* spp. (Lopes et al., 2017). The *MAT1-1-1* and *MAT1-2-1* gene sequences of the *Lasiodiplodia* spp. and *B. dothidea* were used to design primers for each genus, respectively (Table 2). Additionally, the *MAT1-1-1* gene was used to design primers for amplification in the other *Diplodia* species, but not for the *MAT1-2-1* gene. This was because the existing primers (DipHMG) were able to amplify in *D.* seriata, which is the only other *MAT1-2* isolate with sequence data available for that genus. Primer3 (Rozen and Skaletsky, 1999) was used to determine optimal primer location. For the *MAT1-1-1* primers, the three *Lasiodiplodia* spp. were aligned and primers were designed at conserved regions so as to potentially amplify across many species in the genus. For the *MAT1-2-1* primers, sequences of *L. gonubiensis* and *L. theobromae* were aligned and primers were designed at conserved regions. The same approach was used for the *Diplodia MAT1-1-1* primers using sequences of *D. sapinea, D. scrobiculata* and *D. corticola*. Separate primer pairs were designed to amplify both the *MAT1-1-1* and *MAT1-2-1* genes from *B. dothidea*.

Template DNA for polymerase chain reactions (PCRs) was extracted using the protocol described by Möller et al. (1992). PCRs were set up as follows: 1 × PCR reaction buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM of each primer, 1 U of FastStart Taq Polymerase (Roche) and PCR grade water to the final reaction volume of 25 µl. Between 2 and 20 ng of gDNA was used as template for PCR. Thermocycling was performed on a Veriti Thermal Cycler (Applied Biosystems) using an initial step of 95 °C for five minutes, followed by 35 cycles of 95 °C for 30 s, 58 °C for 45 s, 72 °C for 60 s, and a final extention step of 72 °C for 4 min. Resultant amplicons were sequenced using the same primers as those used in PCR.

3. Results

3.1. Mating type genes of Botryosphaeriaceae

Genes showing strong homology to the mating type genes of *D. sapinea* were identified from 28 *Botryosphaeriaceae* genomes, 16 of which were from incomplete genome sequences generated in this study. The genes of each mating type idiomorph (*MAT1-1* and *MAT1-2*) always co-occurred in the genomes, i.e. *MAT1-1-1* and *MAT1-1-8* always co-occured, *MAT1-2-1* and *MAT1-2-5* always co-occurred. The *MAT1-1-1* and *MAT1-2-1* genes had strong homology to conserved protein

Table 2
Primer pairs for amplification of mating type gene fragments.

Primer name	Sequence	Tm (C)	Fragment size	Species with successful amplification
Botryosphaeria dothidea MAT1-1-1			820 bp	*Botryosphaeria dothidea* (CMW8000)
BdM1f	GACTCGTCCACCTCACTTCA	63.5		
BdM1r	CAGAGTAGTGGTGCCAGAGG	63.0		
Botryosphaeria dothidea MAT1-2-1			444 bp	*Botryosphaeria dothidea* (CMW8000)
BdM2f	GACCGCAGAGATCAAGAAGC	64.5		
BdM2r	TCAGCGAGAAGGCCATAGTT	64.2		
Diplodia MAT1-1-1			750–870 bp	*Diplodia sapinea* (CBS117911)
DipM1f3	AGCTCATCCGTCGCTTCATTC	68		*D. scrobiculata* (CBS139796)
DipMr3	CGGTGCATGTCGAATGC	65.8		*D. corticola* (CMW38070)
Lasiodiplodia MAT1-1-1			820–850 bp	*Lasiodiplodia theobromae* (CBS164.96)
LasM1f	AACTGCTTCGTTGCCTTCC	64.7		*L. pseudotheobromae* (CBS116459)
LasM1r2	AGACAGGTCAGGGTCAATGG	64.5		*L. gonubiensis* (CBS115812)
				L. citricola (CMW37047)
				L. iraniensis (CMW37051)
Lasiodiplodia MAT1-2-1			711 bp	*L. theobromae* (CMW32498)
LasM2f1	ACCGCAGGGACAACCAC	65.4		*L. gonubiensis* (CBS115812)
LasM2r1	GTTGCGCTGGGAAGCAG	66.4		*L. gilanensis* (CMW37048)
				L. mediterranea (CMW43392)
				L. lignicola (CMW40932)
				D. scrobiculata (CBS118110)
				D. seriata (CBS112555)

Table 3
Conserved protein domains present in *Botryosphaeriaceae* mating type genes.

Species	Gene	Amino acid interval		E value
		MATalpha	MAT_HMG-box	
Botryosphaeria dothidea	*MAT1-1-1*	31–160		4.19E−38
	MAT1-2-1		174–248	2.55E−29
Diplodia scrobiculata	*MAT1-1-1*	63–205		5.09E−41
D. corticola	*MAT1-1-1*	87–223		1.84E−36
D. sapinea	*MAT1-1-1*	40–210		5.50E−41
	MAT1-2-1		130–206	4.09E−38
D. seriata	*MAT1-1-1*	48–210		8.19E−43
	MAT1-2-1		129–205	1.89E−33
Lasiodiplodia gonubiensis	*MAT1-1-1*	80–198		1.17E−37
	MAT1-2-1		180–253	5.92E−32
L. theobromae	*MAT1-1-1*	71–201		5.26E−39
	MAT1-2-1		191–260	2.18E−25
L. pseudotheobromae	*MAT1-1-1*	72–196		4.70E−39
Macrophomina phaseolina	*MAT1-1-1*	49–199		7.14E−38
	MAT1-2-1		190–264	3.23E−26
Neofusicoccum australe	*MAT1-1-1*	32–207		5.01E−40
	MAT1-2-1		181–255	6.11E−35
N. cordaticola	*MAT1-1-1*	31–207		8.84E−42
	MAT1-2-1		221–295	5.76E−36
N. kwambonambiense	*MAT1-1-1*	31–210		6.99E−41
	MAT1-2-1		221–295	7.01E−36
N. luteum	*MAT1-1-1*	32–207		9.14E−40
	MAT1-2-1		181–255	6.17E−35
N. parvum	*MAT1-1-1*	31–207		6.85E−42
	MAT1-2-1		221–295	1.55E−35
N. ribis	*MAT1-1-1*	31–207		5.63E−41
	MAT1-2-1		221–295	7.53E−36
N. umdonicola	*MAT1-1-1*	31–217		1.35E−41
	MAT1-2-1		221–295	7.01E−36

domains based on CDD BLAST (Table 3). The *MAT1-1-1* genes all contained the MATalpha (pfam04769) domain and the *MAT1-2-1* genes all contained the MAT_HMG-box (cd01389) domain.

Based on the presence of the mating type genes identified in each genome, species could be designated as either heterothallic or homothallic. *Botryosphaeria dothidea, L. gonubiense*, as well as all species of *Neofusicoccum* examined were homothallic with the genomes containing both the *MAT1-1* and *MAT1-2* idiomorphs. *Diplodia corticola*, *D. scrobiculata, D. seriata, L. theobromea, L. pseudotheobromea* and *M. phaseolina* were identified as heterothallic. Of the heterothallic species with only a single representative genome, all were of isolates possessing the *MAT1-1* idiomorph.

Table 4
Nucleotide and amino acid conservation of mating type genes of species of *Botryosphaeriaceae*.

Gene	Nucleotide conservation[a]	%	Amino acid conservation[a]	%
MAT1-1-1	313/1167	26.82	93/388	23.97
MAT1-1-8	209/933	22.40	47/232	20.26
MAT1-2-1	217/942	23.04	51/313	16.29
MAT1-2-5	166/783	21.20	42/248	16.94
APN2	936/1881	49.76	363/629	57.71
CIA30	398/642	61.99	160/214	74.77
CoxVIa	286/384	74.48	90/127	70.87
APC5	798/1398	57.08	308/478	64.44
MCP	541/834	64.87	236/277	85.20
SAICARsyn	577/1957	29.48	222/318	69.81

[a] Identity at sites not containing any gaps in the alignment.

The nucleotide and amino acid sequence conservation of the mating type genes was considerably lower than that of the adjacent genes (Table 4). The size of the coding sequences for the mating type genes, as well as the size and location of the introns was generally conserved (Supplementary File 3). There were, however, some exceptions. The *MAT1-1-1* gene of *M. phaseolina* contained an intron more than double the length of those in other species; the *MAT1-1-8* gene of *B. dothidea* was significantly longer than that of other *Botryosphaeriaceae*; the *MAT1-2-1* gene of both *D. sapinea* and *D. seriata* contained a single intron, compared to the two in the other *Botryosphaeriaceae*.

3.2. Organization of mating type loci

Three types of gene arrangements were observed at the mating type locus. In most species (Fig. 1), the mating type genes occurred between a syntenic cluster of six genes and a gene with a putative integral membrane (*PIM*) protein (also referred to as Pleckstrin homology domain containing protein and DUF2404), similar to that described by Bihon et al. (2014). The syntenic cluster consisted of six genes with a conserved order and orientation. These six genes were all protein coding and are as follows: DNA lyase (*APN2*), Cytochrome *c* oxidase subunit VIa (*CoxVIa*), Anaphase-promoting complex subunit 5 (*APC5*), Complex I intermediate-associated protein 30 (*CIA30*), Mitochondrial carrier protein (*MCP*), Phosphoribosylaminoimidazolesuccinocarboxamide Synthetase (*SAICARsyn*).

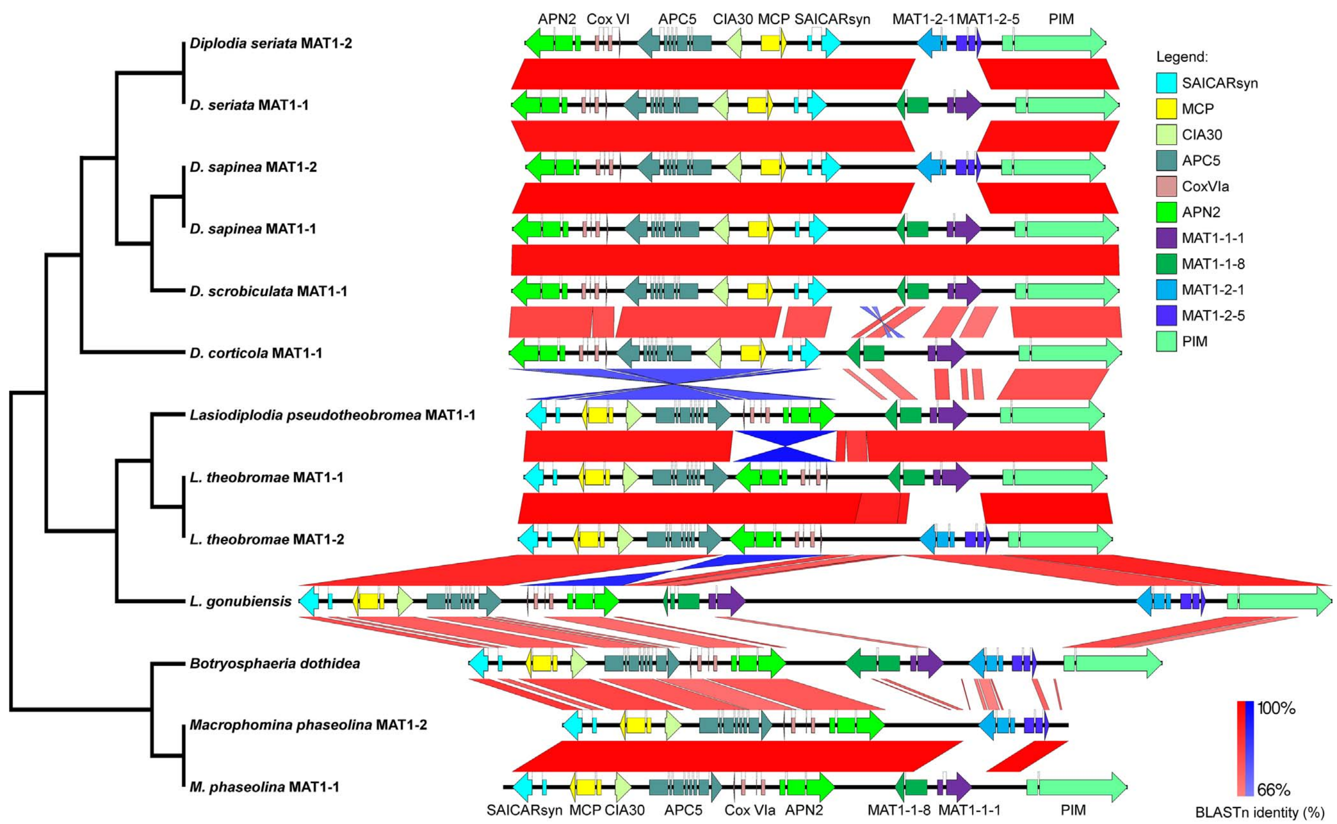

Fig. 1. Pairwise mating type locus comparison between species of *Botryosphaeriaceae* (excluding *Neofusicoccum* spp.). Black horizontal lines represent genomic sequences, annotated with colour coded arrows representing genes. Red and blue boxes between genomic sequences indicates pair wise similarity based on BLASTn, red boxes indicate that both regions are in the same orientation and blue boxes indicate that regions are orientated in opposite directions. Genes are abbreviated as follows: DNA lyase (*APN2*), Cytochrome *c* oxidase subunit Via (*Cox-Via*), Anaphase-promoting complex subunit 5 (*APC5*), Complex I intermediate-associated protein 30 (*CIA30*), Mitochondrial carrier protein (*MCP*), Phosphoribosylaminoimidazolesuccinocarboxamide Synthetase (*SAICARsyn*), Putative integral membrane protein (*PIM*). The relationships between taxa are indicated by the cladogram on the left. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A second organization (Supplementary File 2), seen only in the *Neofusicoccum parvum/N. ribis* complex (*N. cordaticola, N. kwambomambiense, N. parvum, N. ribis* and *N. umdonicola*), was similar to that described by Lopes et al. (2017) where the *MAT1-1* and *MAT1-2* genes occur at separate genomic loci and neither occurring in close proximity to the syntenic cluster that flanks the mating type genes in the other genera of *Botryosphaeriaceae*. However, investigation of these genes in the *N. parvum* UCD646So genome (Massonnet et al., 2016) indicated that the *MAT1-1* mating type is located on the same chromosome (contig 3 from Massonnet et al. (2016)) as the *APN2-SAICARsyn* cluster, but 242 kb distant from it. Furthermore, the *MAT1-2* mating type occurred on a separate chromosome (contig 36 from Massonnet et al. (2016)) very close (12 kb) to the telomere.

The third type of gene arrangement (Supplementary File 2) was observed in *N. australe* and *N. luteum* where the *MAT1-2* idiomorph occurred directly downstream of the *APN2-SAICARsyn* cluster. The *MAT1-1* idiomorph occurred on a separate contig flanked on one side by phosphoglucomutase and beta-glycosidase genes. The proximity of the two mating types could not be determined in *N. australe* and *N. luteum*.

The orientation of the mating type genes was well conserved in all species of *Botryosphaeriaceae*. We orientated the mating type locus by the direction of the *MAT1-1-1* gene. The two genes of each idiomorph (*MAT1-1-1* and *MAT1-1-8*, and *MAT1-2-1* and *MAT1-2-5*) were always arranged in opposite directions. Furthermore, the *MAT1-2-1* gene was always orientated in the opposite direction as the *MAT1-1-1* gene.

Although the orientation of the mating type idiomorphs was conserved in the species of *Botryosphaeriaceae* considered, several rearrangements were observed (Fig. 1). In all the *Diplodia* species, the syntenic cluster preceding the mating type locus was in the *APN2* to *SAICARsyn* orientation, as described by Bihon et al. (2014). In most other species of *Botryosphaeriaceae,* these six genes were in the opposite orientation with *APN2* being the most proximal to the MAT genes. In *N. parvum,* the *APN2-SAICARsyn* cluster also occurred in the same orientation to the *MAT1-1* idiomorph, similar to the situation in *Diplodia,* although it was not directly adjacent to it. In *L. theobromae,* the position and orientation of *APN2* and *CoxVIa* was inverted.

Both *L. gonubiensis* and *B. dothidea* had both the *MAT1-1* and *MAT1-2* idiomorphs at the same locus (Fig. 1) and were, therefore, considered homothallic. In both these species, the *MAT1-1* and *MAT1-2* idiomorphs occurred adjacent to one another, with the *MAT1-1* idiomorph preceding the *MAT1-2* idiomorph. *Lasiodiplodia gonubiensis* had a very large (~15 kb) intergenic region between the genes of the two idiomorphs. This intergenic region consisted of low complexity, A-T rich sequence.

With the exception of the genes flanking the *MAT1-2* idiomorph, the mating type idiomorph organization of the four additional species in the *Neofusicoccum parvum/N. ribis* complex was identical to that previously found in *Neofusicoccum parvum* (Lopes et al., 2017). The *MAT1-1* idiomorphs occurred between the AMP-binding protein and major facilitator superfamily (MFS) allantoate encoding genes. The *MAT1-2* idiomorphs were flanked on one side either by a putative mutator element gene (*N. parvum*), a DNA helicase gene (*N. ribis* and *N. umdonicola*) or a retrotransposon *gag* protein (*N. cordaticola)*. The *MAT1-2* idiomorphs were flanked on the other side by the *PIM* gene followed by a putative cutinase gene. No genes resembling the mating type genes were present adjacent to the *APN2-SAICARsyn* syntenic cluster in the species of the *Neofusicoccum parvum/N. ribis* complex, but were rather followed by an F-box protein.

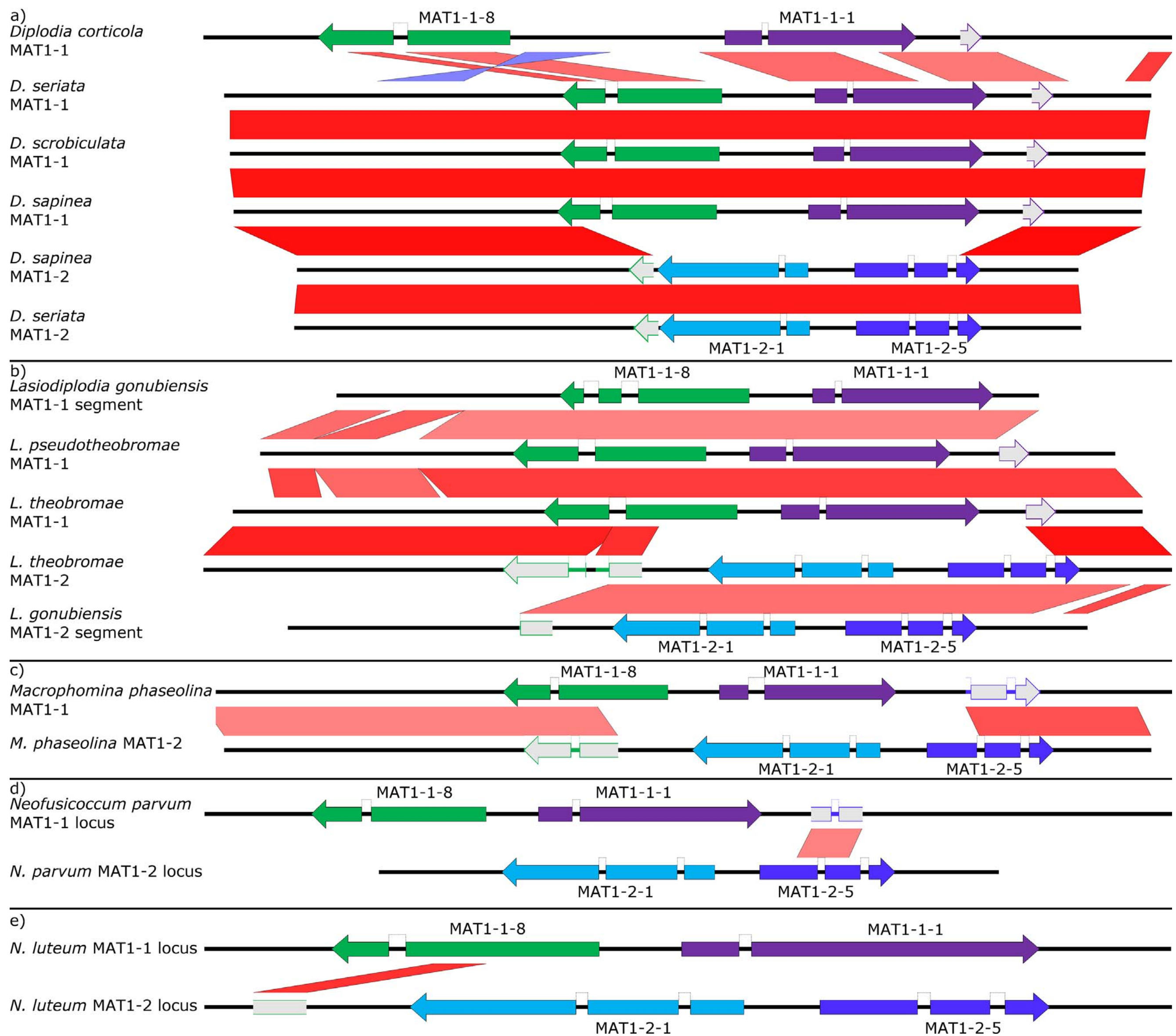

Fig. 2. Comparison of mating type loci to indicate intergenic regions with homology to the mating type genes of the opposite idiomorph. Comparison of mating type sequences of (a) *Diplodia* spp., (b) *Lasiodiplodia* spp., (c) *Macrophomina phaseolina*, (d) *Neofusicoccum parvum* and (e) *N. luteum*. Black horizontal lines represent genomic sequences, annotated with colour coded arrows representing genes. Red and blue boxes between genomic sequences indicates pair wise similarity based on BLASTn, red boxes indicate that both regions are in the same orientation and blue boxes indicate that regions are orientated in opposite directions. Light grey arrows indicate truncated gene sequences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Mating type gene fragments

Sequences with homology to either the *MAT1-1-8* or *MAT1-2-5* genes were observed in the intergenic regions flanking the mating types genes of most species considered in this study (Fig. 2). All heterothallic species of *Botryosphaeriaceae* had sequences with homology to the 3′ end of the *MAT1-2-5* gene in the 3′ intergenic region of the *MAT1-1* idiomorph. Furthermore, all heterothallic species had sequences with homology to the 3′ end of the *MAT1-1-8* gene in the 5′ intergenic region of the *MAT1-2* idiomorph (Fig. 2). Despite the strong homology to the *MAT1-1-8* and *MAT1-2-5* genes, these sequences did not code for any proteins or contain open reading frames as no start codon was present.

Sequences with homology to mating type genes also occured in homothallic species of *Botryosphaeriaceae.* In *L. gonubiensis*, the sequence upstream of the *MAT1-2-1* gene had homology to the middle region of its *MAT1-1-8* gene. In contrast, *B. dothidea*, had no such homologous sequences in the intergenic region surrounding its mating type genes. In the five species residing in the *Neofusicoccum parvum/N. ribis* complex, the 3′ intergenic sequences of the *MAT1-1* idiomorph had homology to the middle of the *MAT1-2-5* gene (Fig. 2d, only *N. parvum* shown). In *N. australe* and *N. luteum* the 5′ intergenic region of the *MAT1-2* idiomorph contained sequence homologous to the middle of the *MAT1-1-8* gene (Fig. 2e, *N. luteum* illustrated).

3.4. Phylogeny of mating type genes and ancestral reconstruction of thallism

Both maximum likelihood and Bayesian inference yielded phylogenetic trees with identical topologies for both the *MAT1-1-1* and *MAT1-2-1* genes. Consequently, the bootstrap and posterior probability support values were combined on a single tree for each gene (Fig. 3). The phylograms for these genes had similar topologies with strong support for the major clades, corresponding to the different genera used in this study.

The ancestral reconstruction analysis (Fig. 4) traced the character

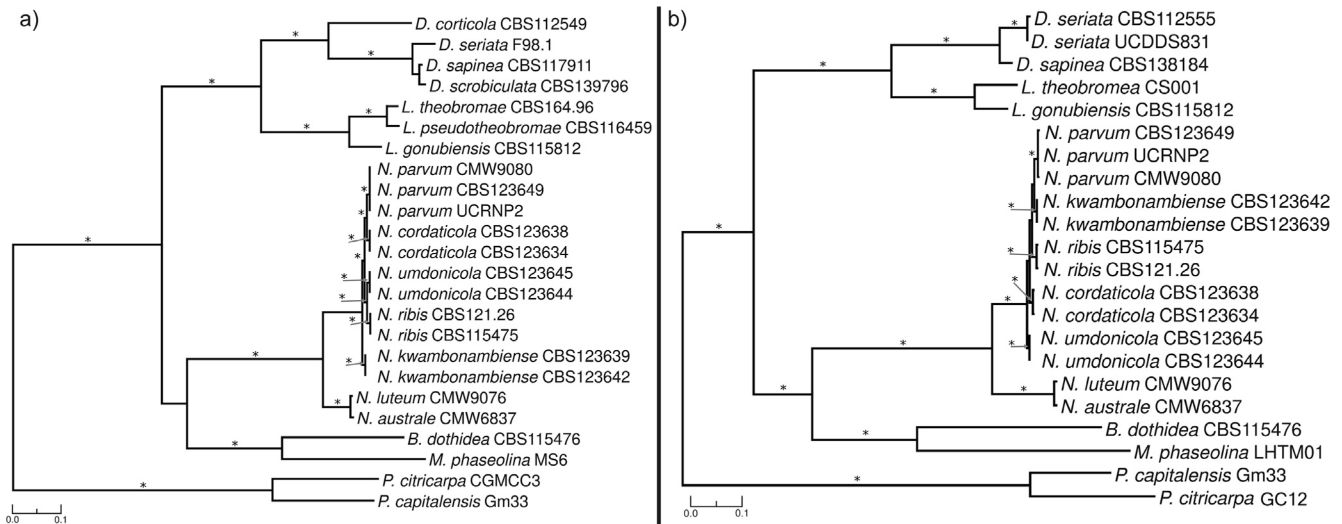

Fig. 3. Maximum likelihood phylogenetic tree of the (a) *MAT1-1-1* and (b) *MAT1-2-1* genes from species of *Botryosphaeriaceae*. Branches with bootstrap support higher than 85% and Bayesian inference posterior probabilities higher than 0.95 are indicated using an asterisk (*) symbol. Both phylogenetic trees are rooted using *Phyllosticta citricarpa* and *P. capitalensis.*

history onto the phylogenetic tree and suggested that heterothallism is the ancestral state in this family. Six character state changes occurred across this phylogeny, including one state change in the outgroup (*Phyllostictaceae)* lineage. Five independent transitions from heterothallism to homothallism were observed in the *Botryosphaeriaceae*. No transition from homothallism to heterothallism was observed.

3.5. Mating type primers and amplification

Primers could successfully amplify MAT1-1-1/MAT1-2-1 gene fragments in the species for which they were designed, as well as for some additional species indicated in Table 2. Primers were not designed for *Neofusicoccum* spp. because these were already available from the study of Lopes et al. (2017).

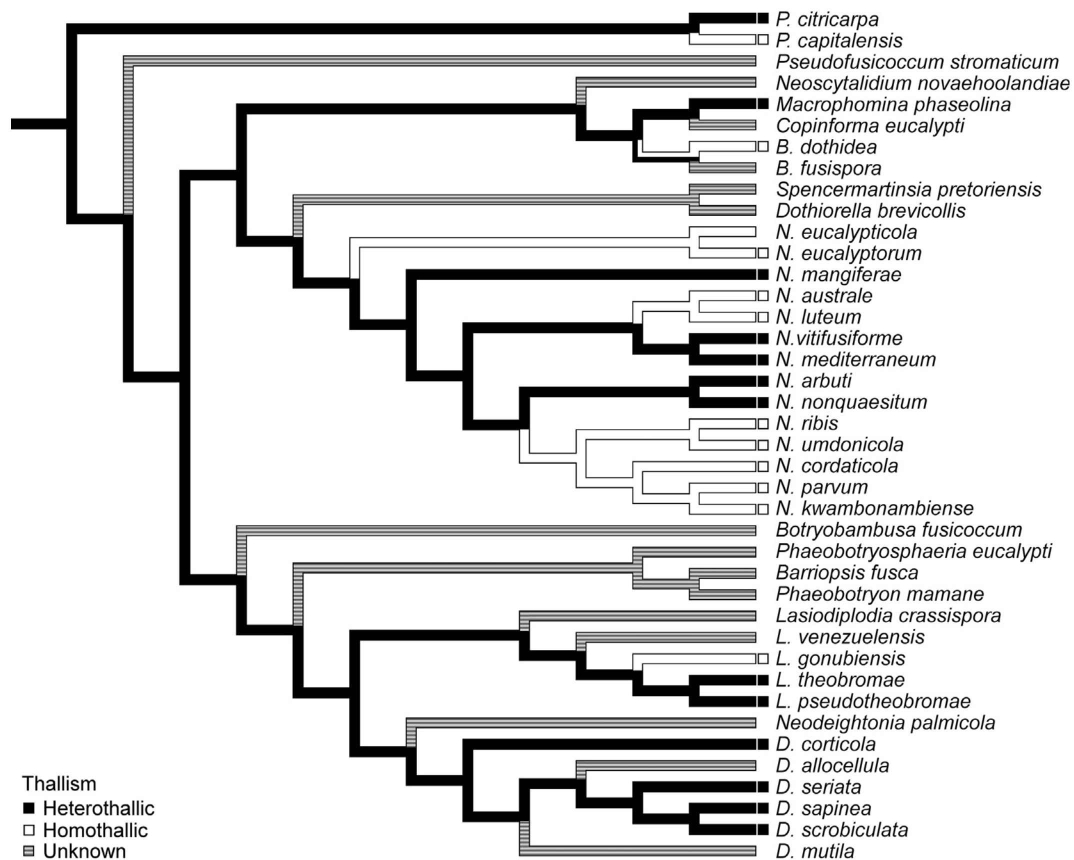

Fig. 4. Ancestral state reconstruction of sexual thallism of species of *Botryosphaeriaceae*. Two character states were mapped onto the phylogenetic tree: Heterothallism (solid) and homothallism (open). Grey lines denote undetermined states.

4. Discussion

This study characterized mating type genes in sixteen species residing in five genera of the *Botryosphaeriaceae.* The gene content and order within the *MAT1-1* and *MAT1-2* idiomorphs was strongly conserved. The genomic position of the *MAT1* locus was less conserved, with one or both of the *Neofusicoccum* MAT idiomorphs not in close proximity to the *APN2-SAICARsyn* gene cluster. Mating type gene fragments were present in the intergenic regions flanking the mating type genes in all heterothallic species and to a lesser extent in homothallic species. Phylogenetic analyses of the *MAT1-1-1* and *MAT1-2-1* genes produced phylogenetic trees congruent with the accepted phylogenetic topology for the *Botryosphaeriaceae*. Ancestral reconstruction suggested that heterothallism is the ancestral state for the *Botryosphaeriaceae* and that at least five transitions to homothallism have occurred.

4.1. Mating type genes of Botryosphaeriaceae

The mating type genes characterised in this study had low overall levels of nucleotide and amino acid sequence conservation. Despite the low sequence conservation among taxa in the *Botryosphaeriaceae,* the MATalpha_HMGbox domain of the *MAT1-1-1* gene and the MATA_HMG-box domain of the *MAT1-2-1* gene were conserved. This is consistent with the fact that mating type genes in the fungi have few differences within a species, but are highly divergent between species (Turgeon, 1998).

The *MAT1-2-1* gene in *D. sapinea* and *D. seriata* has lost the intron that interrupts the MATA_HMG-box domain in other species. The amino acid sequences flanking the lost intron site remained intact in the *MAT1-2-1* gene. This was consistent with the Poly-A primed mRNA derived intron loss model proposed for *Cryptococcus* (Sharpton et al., 2008). It is a strongly conserved trend that the MATalpha and the MAT_HMG-box domains are interrupted by introns in many ascomycetes (Arie et al., 2000; De Miccolis Angelini et al., 2016; Duong et al., 2013; Paoletti et al., 2005). No other instances of intron loss occuring within the fungal mating type genes have been reported.

The *MAT1-1-8* and *MAT1-2-5* genes were consistently present in the *Botryosphaeriaceae* from the *MAT1-1* and *MAT1-2* idiomorphs, respectively. This is consistent with the situation previously reported in *D. sapinea* (Bihon et al., 2014) and *N. parvum* (Lopes et al., 2017). Both the *MAT1-1-8* and *MAT1-2-5* genes have also been reported in the *Phyllostictaceae* (Amorim et al., 2017; Wang et al., 2016). The *MAT1-1-8* and *MAT1-2-5* genes have not been encountered in the mating type idiomorphs in other orders of Dothideomycetes (Arie et al., 2000; Barve et al., 2003; Bennett et al., 2003; Cozijnsen and Howlett, 2003; Turgeon et al., 1993; Waalwijk et al., 2002).

4.2. Organization of mating type loci

Other than in the case of *Neofusicoccum* spp., the position of the mating type locus was conserved in species of *Botryosphaeriaceae*. The *MAT1* locus was consistently flanked by the *APN2-SAICARsyn* gene cluster and the PIM gene in species from *Botryosphaeria*, *Diplodia*, *Lasiodiplodia* and *Macrophomina*. This finding was consistent with the position of the *MAT1* locus in species of *Phyllostictaceae*, with the exception that the PIM gene has been incorporated into the idiomorphic region in *P. citricarpa* (Amorim et al., 2017; Wang et al., 2016).

The mating type idiomorphs did not occur in close proximity to one another in two *Neofusicoccum* species. Species in the *Neofusicoccum parvum/N. ribis* complex had neither mating type idiomorphs in close proximity to one another or to the *APN2-SAICARsyn* cluster. This was in agreement with the results of the previous study on *N. parvum* (Lopes et al., 2017). *Neofusicoccum australe* and *N. luteum* had the *MAT1-2* idiomorph associated with the *APN2-SAICARsyn* cluster, but the *MAT1-1* idiomorph did not occur in close proximity. The occurrence of the two mating types at unlinked regions is not without precedent in fungi and has been observed in *Aspergillus nidulans* (Galagan et al., 2005), *Curvularia cymbopogonis* (Yun et al., 1999) and *Neosartorya fischeri* (Rydholm et al., 2007).

There was no evidence of mating type switching in the mating type loci in the species of *Botryosphaeriaceae*. Direct repeat-mediated DNA deletion of one or more mating type gene has been implicated in unidirectional mating type switching, i.e. self-fertile to self-sterile conversion (Wilken et al., 2014; Xu et al., 2016; Yun et al., 2017). However, no such direct repeats were observed in the present study. Mating type switching has also never been reported for any species of *Botryosphaeriaceae*. It is thus unlikely that unidirectional mating type switching takes place in the *Botryosphaeriaceae.*

4.3. Mating type gene fragments

All heterothallic species of *Botryosphaeriaceae* in this study had gene fragments corresponding to either the *MAT1-1-8* or *MAT1-2-5* gene in the intergenic region flanking the mating type idiomorph. This has previously also been observed in *D. sapinea* (Bihon et al., 2014). Such gene fragments have often been observed at the MAT locus in other fungi (De Miccolis Angelini et al., 2016; Duong et al., 2013; King et al., 2015; Paoletti et al., 2005; Terhem et al., 2015; Tsui et al., 2013; Zaffarano et al., 2010). Invariably in all known cases, gene fragments correspond to a mating type gene of the opposite idiomorph.

The pattern of mating type gene fragments observed in the *Botryosphaeriaceae* can be explained by two hypothesis (Fig. 5). One is that the mating type gene fragments are remnants of a deletion event (Fig. 5a) involved in an ancestral shift from homothallism to heterothallism (Paoletti et al., 2005; Terhem et al., 2015). This hypothesis requires a homothallic ancestor and two independent deletion events. The other hypothesis interprets the mating type gene fragments as the result of unequal recombination (Fig. 5b) occurring between idiomorphs in a heterothallic species (De Miccolis Angelini et al., 2016; Tsui et al., 2013; Zaffarano et al., 2010). This hypothesis requires a heterothallic ancestor and at least two unequal recombination events. Both these hypotheses would result in an idiomorphic locus with part of the mating type genes existing outside the idiomorphic region (Fig. 5c). The sequence similarity of the gene fragments to the original genes would make continued recombination possible and prevent the two sequences from diverging in heterothallic species.

Mating type gene fragments occurred in some homothallic species of *Botryosphaeriaceae*, but with less sequence conservation to the complete gene than seen in heterothallic species. This can be interpreted as evidence of a heterothallic to homothallic transition. Such gene fragments would have had to exist prior to the transition to homothallism. Once both idiomorphs had become established at different positions in a genome, recombination between the fragments and complete genes would become much less likely. A reduction in recombination between mating type gene fragments and mating genes would allow mutations to accumulate and the sequences to diverge (Fig. 5e). Mating type gene fragments have not previously been reported from homothallic fungi (Debuchy and Turgeon, 2006; Galagan et al., 2005).

4.4. Phylogeny of mating type genes and ancestral reconstruction of thallism

Both the *MAT1-1-1* and *MAT1-2-1* phylogenies were congruent with one another and with the accepted multigene phylogeny of the *Botryosphaeriaceae* (Slippers et al., 2013). This was consistent with the finding from other studies suggesting that phylogenies of mating type genes are generally consistent with those of other genes (Duong et al., 2013; Turgeon, 1998; Waalwijk et al., 2002; Yokoyama et al., 2006).

Ancestral state reconstruction suggested that heterothallism is the ancestral state in the *Botryosphaeriales* and that multiple, independent shifts to homothallism have occurred. Similarly, studies on numerous taxa of ascomycetes have also indicated that shifts from heterothallism

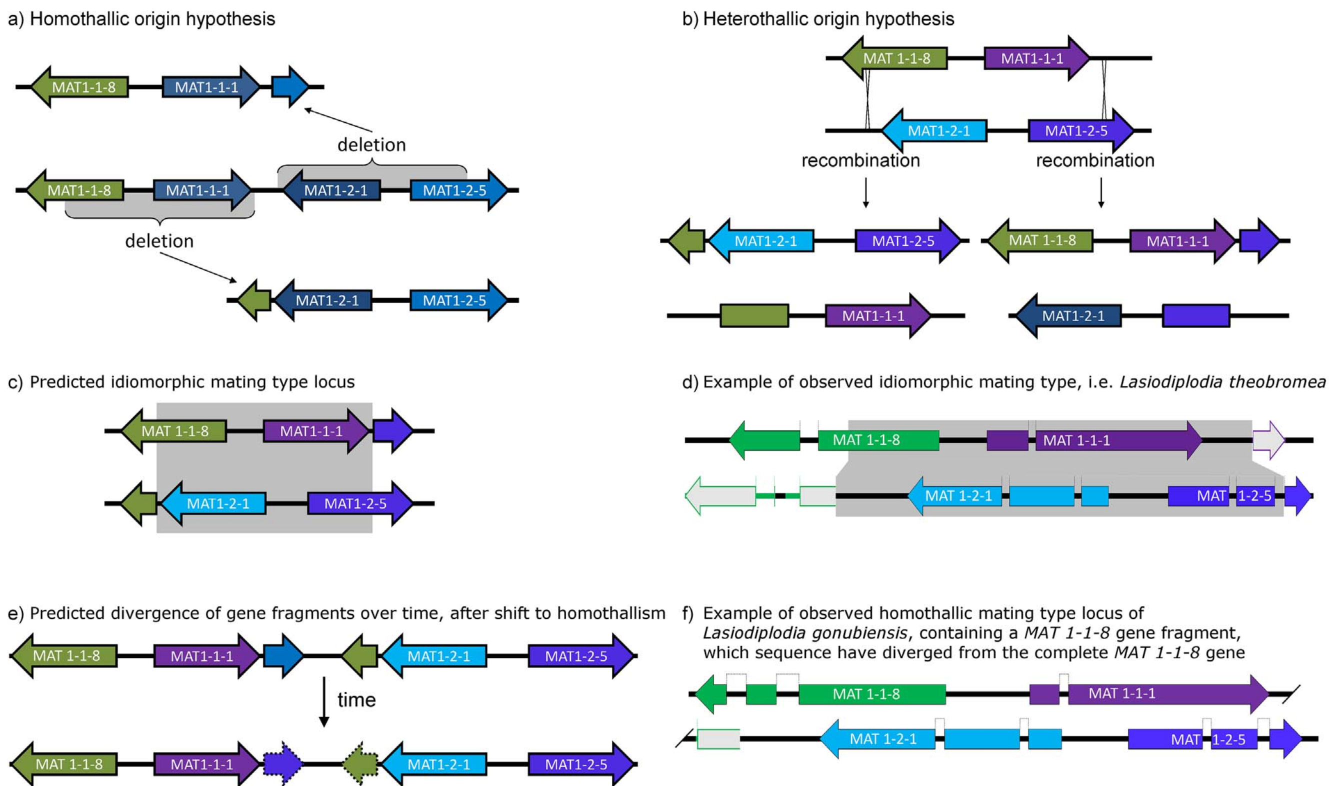

Fig. 5. Proposed models for the evolution of mating type in the *Botryosphaeriaceae*. Two mechanisms are hypothesized for the formation of heterothallic mating type idiomorphs containing partial gene fragments of either the *MAT1-1-8* or *MAT1-2-5* genes: (a) a primary homothallic, ancestral mating type locus that undergoes two independent deletions and (b) a heterothallic, ancestral mating type locus that undergoes two unequal crossing over/recombination events. (c) Both these mechanisms (in a and b) will result in a heterothallic mating type locus containing 3′ gene fragments of the *MAT1-1-8* or *MAT1-2-5* genes at the *MAT1-2* and *MAT1-1* idiomorph. Sequence similarity between the *MAT1-1-8/MAT1-2-5* genes and their fragments on the opposite idiomorph will permit homologous recombination within mating type genes and so reduce the effective size of the idiomorphic region (indicated by a grey block). Mating type gene fragments will maintain their sequence similarity to the complete *MAT1-1-8* or *MAT1-2-5* as long as recombination occurs between them. (d) An example of a heterothallic mating type locus with idiomorphic region not containing the 3′ ends of the *MAT1-1-8* and *MAT1-2-5* genes is shown, i.e. *Lasiodiplodia theobromae*. (e) If a subsequent heterothallic to homothallic conversion occurs both or one or none of the mating type gene fragments may be present. In this case the mating type gene fragments will no longer be maintained by recombination and will accumulate mutations that will reduce their homology to the complete genes over time, for example (f) the *MAT1-1-8* fragment in *Lasiodiplodia gonubiensis*.

to homothallism are common (Gioti et al., 2012; Inderbitzin et al., 2005; Nygren et al., 2011; Yokoyama et al., 2006; Yun et al., 1999). In contrast, there are no studies unambiguously showing a transition from homothallism to heterothallism. This is supported by population genetic models predicting that it is unlikely for heterothallism to become established in a homothallic population (Nauta and Hoekstra, 1992).

4.5. Mating type markers

Mating type markers that can be applied in many species and other populations of *Botryosphaeriaceae* were developed. Primers capable of amplifying the mating type genes of spesific species in *Botryosphaeria*, *Diplodia* and *Lasiodiplodia* were designed. Additionally the *Lasiodiplodia* mating type primers could also amplify the mating type genes of other *Lasiodiplodia* spp. and even some *Diplodia* spp. These primers will supplement the primers previously created for *D. sapinea* (Bihon et al., 2014) and *Neofusicoccum* spp. (Lopes et al., 2017). Mating type gene markers are immensely valuable population genetic tools for studying the presence or absence of sexual reproduction in plant pathogenic fungi (Bihon et al., 2014; Groenewald et al., 2006; Paoletti et al., 2005). Primer sets that allow amplification in a wide array of species are thus very valuable.

5. Conclusions

This study is the first to characterize and compare the *MAT1* loci of species across many genera of the *Botryosphaeriaceae*. It illustrates the inherent variability in the DNA sequence of mating type genes, but also the organizational conformity within and outside the mating type regions in most species of *Botryosphaeriaceae*. The ubiquitous presence of mating type gene fragments outside the idiomorphic regions in heterothallic species emphasises the role of unequal crossing over. Their presence inside idiomorphic regions in homothallic species adds evidence to support multiple independent shifts from heterothallism. The application of the mating type markers developed in this and other studies will add valuable tools to characterize the population biology in the species of *Botryosphaeriaceae.* Together these data provide a foundation for future studies on the biology and evolution of species in this family.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.fgb.2018.03.003.

References

Amorim, R., et al., 2017. MAT gene idiomorphs suggest a heterothallic sexual cycle in the citrus pathogen *Phyllosticta citricarpa*. Eur. J. Plant Pathol. 147, 325–337.

Arie, T., et al., 2000. Mating-type genes from asexual phytopathogenic ascomycetes *Fusarium oxysporum* and *Alternaria alternata*. Mol. Plant Microbe Interact. 13, 1330–1339.

Barve, M.P., et al., 2003. Cloning and characterization of the mating type (MAT) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) and a MAT phylogeny of legume-associated *Ascochyta* spp. Fungal Genet. Biol. 39, 151–167.

Bennett, R.S., et al., 2003. Identity and conservation of mating type genes in geographically diverse isolates of *Phaeosphaeria nodorum*. Fungal Genet. Biol. 40, 25–37.

Bihon, W., et al., 2012a. High levels of genetic diversity and cryptic recombination is widespread in introduced *Diplodia pinea* populations. Australas. Plant Pathol. 41, 41–46.

Bihon, W., et al., 2012b. Diverse sources of infection and cryptic recombination revealed in South African *Diplodia pinea* populations. Fungal Biol. 116, 112–120.

Bihon, W., et al., 2014. MAT gene idiomorphs suggest a heterothallic sexual cycle in a predominantly asexual and important pine pathogen. Fungal Genet. Biol. 62, 55–61.

Billiard, S., et al., 2012. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J. Evol. Biol. 25, 1020–1038.

Blanco-Ulate, B., et al., 2013. Draft genome sequence of *Neofusicoccum parvum* isolate UCR-NP2, a fungal vascular pathogen associated with grapevine cankers. Genome Announ. 1, e00339–13.

Bolger, A.M. et al., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. btu170.

Butler, G., 2010. Fungal sex and pathogenesis. Clin. Microbiol. Rev. 23, 140–159.

Camacho, C., et al., 2009. BLAST+: architecture and applications. BMC Bioinf. 10, 421.

Cozijnsen, A.J., Howlett, B.J., 2003. Characterisation of the mating-type locus of the plant pathogenic ascomycete *Leptosphaeria maculans*. Curr. Genet. 43, 351–357.

De Miccolis Angelini, R.M., et al., 2016. Molecular analysis of the mating type (MAT1) locus in strains of the heterothallic ascomycete *Botrytis cinerea*. Plant. Pathol. 65, 1321–1332.

Debuchy, R., Turgeon, B.G., 2006. Mating-type structure, evolution, and function in Euascomycetes. Growth, Differentiation and Sexuality. Springer, pp. 293–323.

Duong, T.A., et al., 2013. Characterization of the mating-type genes in *Leptographium procerum* and *Leptographium profanum*. Fungal Biol. 117, 411–421.

Galagan, J.E., et al., 2005. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature 438, 1105–1115.

Gioti, A., et al., 2012. Unidirectional evolutionary transitions in fungal mating systems and the role of transposable elements. Mol. Biol. Evol. 29, 3215–3226.

Gladman, S., Seemann, T., 2012. VelvetOptimiser. Victorian Bioinformatics Consortium, Clayton, Australia. <http://bioinformatics.net.au/software.velvetoptimiser.shtml>.

Groenewald, M., et al., 2006. Mating type gene analysis in apparently asexual *Cercospora* species is suggestive of cryptic sex. Fungal Genet. Biol. 43, 813–825.

Idnurm, A., 2011. Sex and speciation: the paradox that non-recombining DNA promotes recombination. Fung. Biol. Rev. 25, 121–127.

Inderbitzin, P., et al., 2005. Lateral transfer of mating system in *Stemphylium*. Proc. Natl. Acad. Sci. U.S.A. 102, 11390–11395.

Islam, M.S., et al., 2012. Tools to kill: genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genom. 13, 493.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

King, K.M., et al., 2015. Differences in MAT gene distribution and expression between *Rhynchosporium* species on grasses. Plant. Pathol. 64, 344–354.

Lopes, A., et al., 2017. Mating type genes in the genus *Neofusicoccum*: mating strategies and usefulness in species delimitation. Fungal Biol. 121, 394–404.

Liu, Z., et al., 2016. Draft genome sequence of *Botryosphaeria dothidea*, the pathogen of apple ring rot. Genome Announ. 4, e01142–16.

Maddison, W.P., Maddison, D.R., 2016. Mesquite: a modular system for evolutionary analysis V3.10. <http://mesquiteproject.org>.

Marchler-Bauer, A., et al., 2014. CDD: NCBI's conserved domain database. Nucleic Acids Res. gku1221.

Marsberg, A., et al., 2016. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. Mol. Plant Pathol. 18, 477–488.

Massonnet, M., et al., 2016. Condition-dependent co-regulation of genomic clusters of virulence factors in the grapevine trunk pathogen *Neofusicoccum parvum*. Mol. Plant Pathol. http://dx.doi.org/10.1111/mpp.12491.

Möller, E.M., et al., 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Res. 20, 6115.

Morales-Cruz, A., et al., 2015. Distinctive expansion of gene families associated with plant cell wall degradation, secondary metabolism, and nutrient uptake in the genomes of grapevine trunk pathogens. BMC Genom. 16, 469.

Nauta, M.J., Hoekstra, R.F., 1992. Evolution of reproductive systems in filamentous ascomycetes. I. Evolution of mating types. Heredity 68, 405–410.

Ni, M., et al., 2011. Sex in fungi. Annu. Rev. Genet. 45, 405–430.

Nygren, K., et al., 2011. A comprehensive phylogeny of *Neurospora* reveals a link between reproductive mode and molecular evolution in fungi. Mol. Phylogenet. Evol. 59, 649–663.

Nylander, J.A.A., 2004. MrModeltest v2: Evolutionary Biology Centre. Uppsala University.

Paoletti, M., et al., 2005. Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr. Biol. 15, 1242–1248.

Pöggeler, S., et al., 1997. Mating-type genes from the homothallic fungus *Sordaria macrospora* are functionally expressed in a heterothallic ascomycete. Genetics 147, 567–580.

Phillips, A.J.L., et al., 2013. The Botryosphaeriaceae: genera and species known from culture. Stud. Mycol. 76, 51–167.

Rambaut, A., Drummond, A., 2003. Tracer: A Program for Analysing Results from Bayesian MCMC Programs such as BEAST & MrBayes. University of Edinburgh, UK.

Robert-Siegwald, G., et al., 2017. Draft genome sequence of *Diplodia seriata* F98. 1, a fungal species involved in grapevine trunk diseases. Genome Announ. 5, e00061–17.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Rozen, S., Skaletsky, H., 1999. Primer3 on the WWW for general users and for biologist programmers. Bioinform. Methods Protoc. 365–386.

Rydholm, C., et al., 2007. DNA sequence characterization and molecular evolution of MAT1 and MAT2 mating-type loci of the self-compatible ascomycete mold *Neosartorya fischeri*. Eukaryot. Cell. 6, 868–874.

Sharpton, T.J., et al., 2008. Mechanisms of intron gain and loss in *Cryptococcus*. Genome Biol. 9, R24.

Slippers, B., et al., 2013. Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. Stud. Mycol. 76, 31–49.

Slippers, B., et al., 2017. Diversity in the Botryosphaeriales: looking back, looking forward. Fungal Biol.

Slippers, B., Wingfield, M.J., 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biol. Rev. 21, 90–106.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.

Sullivan, M.J., et al., 2011. Easyfig: a genome comparison visualizer. Bioinformatics 27, 1009–1010.

Terhem, R.B., et al., 2015. Mating type and sexual fruiting body of *Botrytis elliptica*, the causal agent of fire blight in lily. Eur. J. Plant Pathol. 142, 615–624.

Tsui, C.K.M., et al., 2013. Unequal recombination and evolution of the mating-type (MAT) loci in the pathogenic fungus *Grosmannia clavigera* and relatives. G3: Genes| Genomes| Genetics 3, 465–480.

Turgeon, B.G., 1998. Application of mating type gene technology to problems in fungal biology. Annu. Rev. Phytopathol. 36, 115–137.

Turgeon, B.G., et al., 1993. Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus*. Mol. Gen. Genet. MGG 238, 270–284.

Turgeon, B.G., Yoder, O.C., 2000. Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet. Biol. 31, 1–5.

van der Nest, M.A., et al., 2014. Draft genome sequences of *Diplodia sapinea, Ceratocystis manginecans*, and *Ceratocystis moniliformis*. IMA Fungus 5, 135–140.

Waalwijk, C., et al., 2002. Isolation and characterization of the mating-type idiomorphs from the wheat septoria leaf blotch fungus *Mycosphaerella graminicola*. Fungal Genet. Biol. 35, 277–286.

Wang, N.-Y., et al., 2016. Mating type and simple sequence repeat markers indicate a clonal population of *Phyllosticta citricarpa* in Florida. Phytopathology 106, 1300–1310.

Wilken, P.M., et al., 2014. DNA loss at the *Ceratocystis fimbriata* mating locus results in self-sterility. PLoS ONE 9, e92180.

Wilken, P.M., et al., 2017. Which MAT gene? Pezizomycotina (Ascomycota) mating-type gene nomenclature reconsidered. Fungal Biol. Rev. 31, 199–211.

Wilson, A.M., et al., 2015. Homothallism: an umbrella term for describing diverse sexual behaviours. IMA Fungus 6, 207–214.

Wingfield, B.D., et al., 2015. Draft genome sequences of *Chrysoporthe austroafricana, Diplodia scrobiculata, Fusarium nygamai, Leptographium lundbergii, Limonomyces culmigenus, Stagonosporopsis tanaceti,* and *Thielaviopsis punctulata*. IMA Fungus 6, 233–248.

Xu, L., et al., 2016. Direct repeat-mediated DNA deletion of the mating type *MAT1-2* genes results in unidirectional mating type switching in *Sclerotinia trifoliorum*. Sci. Rep. 6, 27083.

Yan, J.Y., et al., 2017. Comparative genome and transcriptome analyses reveal adaptations to opportunistic infections in woody plant degrading pathogens of Botryosphaeriaceae. DNA Res. dsx40.

Yokoyama, E., et al., 2006. Phylogenetic and structural analyses of the mating-type loci in Clavicipitaceae. FEMS Microbiol. Lett. 264, 182–191.

Yun, S.-H., et al., 2000. Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella/Fusarium* species. Fungal Genet. Biol. 31, 7–20.

Yun, S.-H., et al., 1999. Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proc. Natl. Acad. Sci. 96, 5592–5597.

Yun, S.-H., et al., 2017. Self-fertility in *Chromocrea spinulosa* is a consequence of direct repeat-mediated loss of *MAT1-2*, subsequent imbalance of nuclei differing in mating type, and recognition between unlike nuclei in a common cytoplasm. PLOS Genet. 13, e1006981.

Zaffarano, P.L., et al., 2010. Characterization of the mating type (MAT) locus in the *Phialocephala fortinii* sl–*Acephala applanata* species complex. Fungal Genet. Biol. 47, 761–772.

Zerbino, D.R., Birney, E., 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18, 821–829.