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MINI-REVIEW

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Mating-type genes for classical strain improvements of ascomycetes

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Abstract The ability to mate fungi in the laboratory is a valuable tool for genetic analysis and for classical strain improvement. In ascomycetous fungi, mating typically occurs between morphologically identical partners that are distinguished by their mating type. In most cases, the single mating-type locus conferring mating behavior consists of dissimilar DNA sequences (idiomorphs) in the mating partners. All ascomycete mating-type idiomorphs encode proteins with confirmed or putative DNA-binding motifs. These proteins control, as master regulatory transcription factors, pathways of cell speciation and sexual morphogenesis. Mating-type organization of four of the six classes of ascomycetes has been studied at the molecular level over the past 20 years. This review gives a short overview of the structural organization of the mating-type loci of yeasts and filamentous ascomycetes. In addition, this review describes how the availability of mating-type sequences allows the investigation of key issues concerning genetic and phylogenetic analyses of fungal species.

Introduction

Sexuality in fungi has long been recognized as an interesting aspect of biology. In the largest class of fungi, the ascomycetes, sexual reproduction has been studied for more than 80 years. Ascomycetes are characterized by the formation of ascospores in a sac-like structure, the ascus, and have been grouped into six classes, according to the development of fruiting bodies, the structure of the ascus wall, and the method of discharge of the ascospores. These include: *Hemiascomycetes*, *Plectomycetes*, *Pyrenomycetes*, *Discomycetes*, *Laboulbeniomycetes*, and *Loculoascomycetes* (Hawksworth et al. 1995).

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Furthermore, two morphologically distinct groups can be distinguished among the ascomycetes, which are the unicellular hemiascomycetous yeasts and the mycelial fungi. The best-known yeast, the baker's yeast *Saccharomyces cerevisiae*, is the economically most useful of all fungi, being used for bread making, brewing, and wine making.

However, other ascomycetes are equally as important as the baker's yeast. Ascomycetes are the primary agents of decay in cycling of carbon, nitrogen, and other nutrients. They can cause serious diseases in plants and animals by their direct attack, and as producers of mycotoxins contaminate foodstuffs. However, many also carry economic advantages, being important to the drug, food, and chemical industries, for example as producers of antibiotics (Brakhage and Turner 1995).

Moreover, some members of the discomyceteous class establish a symbiotic association, ectomycorrhizae, with roots of gymnosperm and angiosperm trees and shrubs. This mutual symbiosis has beneficial effects on growth rates of forest trees (Rygiewicz and Andersen 1994). Ectomycorrhizal fungi belonging to the genera *Tuber* (truffles) and *Morchella* (morel) are edible. Although the mycelium of some of these fungi can grow on defined media, fruiting bodies are only produced when grown in tight association with trees. Truffles are of particular interest because of their unique flavor and texture, which makes them highly prized and referred to as a 'vegetable gold'.

Strain improvement of ascomycetes relies on random mutagenesis or on classical breeding and genetic crossing of two strains, followed by screening for mutants and progeny exhibiting enhanced properties of interest. The successful mating of fungi in the laboratory is the prerequisite for classical genetics. In the sexually reproducing ascomycetes, the four products of each meiosis stay together as a tetrad of ascospores. Consequently, one major advantage of many ascomycetes as an experimental system is that they can be easily subjected to powerful genetic analyses. Tetrad analysis supplies comprehensive information about segregation patterns, gene linkage,

and recombination events, which is needed as a basis for molecular genetics in both fundamental research and biotechnology (Esser 1996). Comprehensive knowledge of the molecular nature of mating types will thus reduce the amount of effort required for the breeding process. This review aims to give an overview of the mating system of ascomycetes and to describe recent molecular analyses of mating-type genes in ascomycetes.

The mating system of ascomycetes

In 1920, Dodge discovered the bipolar mating system in the ascomycetous fungus *Ascobolus magnificus*. The term 'bipolar' describes a mating system with one locus and denotes the number of mating types among the progeny of a single cross. Thus, in *A. magnificus* the mating group to which a strain belongs is genetically determined by two mating types at a single locus. Since compatible haploid strains are morphologically indistinguishable and not differentiated into male and female sexes, the mating partners are distinguished only by their mating types (Bistis 1998). The genetic breeding mechanism of fungi in which sexual reproduction occurs only between strains of the opposite mating type is called heterothallism. Most heterothallic species of ascomycetes contain a single mating-type locus with two alternate alleles. The DNA sequences at the mating-type locus are completely different in strains carrying different mating types and, therefore, have been termed idiomorphs instead of alleles to emphasize that the sequences at the same locus in different strains are dissimilar (Glass et al. 1988; Hiscock and Kües 1999; Metzenberg and Glass 1990).

In contrast to the mating system in the ascomycetes, basidiomycetes have evolved multiple mating types (Kothe 2001). A mating system with multiple mating types has only been reported in the filamentous ascomycete *Glomerella cingulata* (Cisar and TeBeest 1999). The mating types of heterothallic ascomycetes were designated α and a in the budding yeast *Saccharomyces cerevisiae*, or *h*– (*matM*) and *h*+ (*matP*) in the yeast *Schizosaccharomyces pombe*. In euascomycetes, the mating types were called *mat A* and *mat a*, or *mat* – and *mat* + in members of the pyrenomycetes, *MAT1–1* and *MAT1–2* in members of the loculoascomycetes and discomycetes (Coppin et al. 1997; Glass and Staben 1997; Perkins 1999; Turgeon and Yoder 2000). The life cycles of the best-known heterothallic yeast *Saccharomyces cerevisiae* and the well-known heterothallic filamentous ascomycete *Neurospora crassa* are illustrated in Fig. 1.

The budding yeast *Saccharomyces cerevisiae* exists in two haploid cell types, α and a, and a diploid cell type, α/a. The difference between the three cell types is to be found at the mating-type locus (*MAT*). A haploid cell with a MATα allele is an α cell, a haploid cell with a *MATa* locus is an *a* cell, and a diploid cell is heterozygous and contains both the *MATα* and the *MATa* allele. During the mating process, haploid α and *a* cells respond to peptide pheromones secreted by the opposite cell type

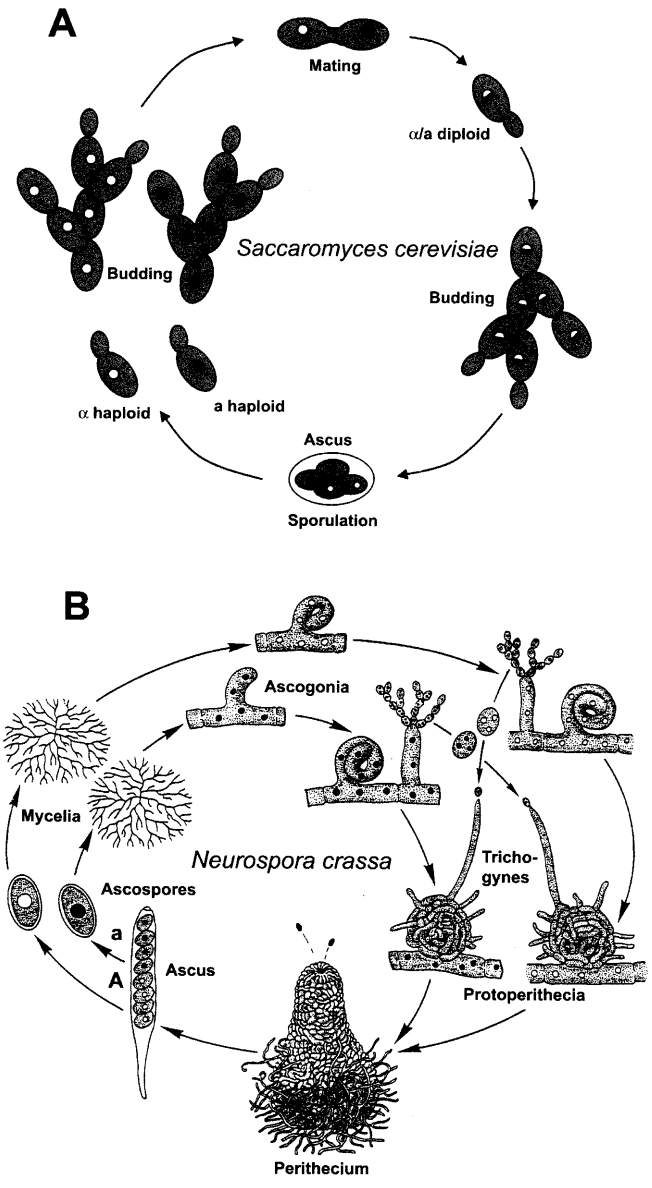

Fig. 1A, B Life cycle of the unicellular yeast *Saccharomyces cerevisiae* (**A**) and the heterothallic pyrenomycete *Neurospora crassa* (**B**)

to undergo sexual differentiation and cell fusion to form diploid α/a cells. When starved of both carbon and fixed nitrogen, α/a cells undergo meiosis and sporulation to yield four haploid ascospores. Vegetative reproduction occurs via budding in the haploid as well as in the diploid phase (Herskowitz 1989).

In *N. crassa*, mature haploid ascospores bearing the mating type *mat A* or *mat a* germinate upon heat activation and produce a multinucleate mycelium. Haploid strains of *N. crassa* are hermaphroditic. Both strains, *mat A* and *mat a*, differentiate female sex organs, the ascogonia, which are surrounded by vegetative hyphae to form protoperithecia. Via receptive hyphae (trichogynes) protoperithecia are fertilized by male conidia (vegetative spores) of the opposite mating type. The fertilized proto-

perithecia develop into perithecia. Within the perithecia, the asci are formed after a dikaryotic phase. Nuclear fusion occurs in the ascus mother cell and only between nuclei of opposite mating types. Meiosis and a postmeiotic division result in eight ascospores. Four of the spores in each ascus carry the *mat A* and four spores the *mat a* mating type. In contrast to *S. cerevisiae* where cell fusion is immediately followed by karyogamy, in *N. crassa* nuclear fusion is temporally and spatially separated from mating (Raju 1992).

In addition to heterothallism, a second mating system can be observed in ascomycetes, which is designated homothallism. Homothallic species possess no genetically definable mating type and are self-fertile (Glass et al. 1990b). The mycelium, which is derived from a uninucleate ascospore or from a vegetative conidium of a homothallic fungus, is able to complete the sexual cycle without the interaction of a mating partner. A third reproductive strategy occurring in filamentous fungi is called pseudohomothallism (Dodge 1957) or sometimes secondary homothallism. Pseudohomothallic members of the ascomycetes (e.g., the pyrenomycetes *Neurospora tetrasperma* and *Podospora anserina*) develop four-spored asci in which most ascospores contain two nuclei, one of each mating type (Raju and Perkins 1994). A typical binucleate ascospore gives rise to a self-fertile mycelium, but only because it is a heterokaryon containing both *mat A* and *mat a* nuclei. A few asci contain five ascospores, three binucleate and two small uninucleate ascospores, which produce homokaryons.

The yeast *S. cerevisiae* can switch its mating type through a recombination event in which α or a sequences at the mating-type locus are replaced by opposite mating-type sequences copied from one of two unexpressed, silent donor loci, *HMLα* and *HMRa*. The result of this recombination is a conversion of the mating-type locus and involves the replacement of the portion of the mating-type locus that determines the sexual identity of the cell (Haber 1998). Mating-type switching occurs in approximately 86% of all cell divisions of haploid cells, thus leading to a culture of α and a cells, which are able to undergo plasmogamy and form diploid cells without the need of a mating partner. Yeasts, which undergo mating-type switching and, hence, do not require a mating partner are referred to as homothallic (Mortimer and Hawthorne 1969). In filamentous ascomycetes, mating-type switching has been observed only in a few cases and, unlike in yeasts, is always unidirectional. A specific mating type is converted into another, but never back into the original mating type (Harrington and McNew 1997; Perkins 1987). In *Ceratocystis*, it is reported that the *MAT-2* mating-type gene is deleted during unidirectional mating-type switching (Witthun et al. 2000).

Structure and function of yeast mating-type genes

Saccharomyces cerevisiae

The genes in the mating-type locus of the sexually reproducing budding yeast have been well characterized. In 1981, the complete DNA sequence of the *MAT*α and *MATa* alleles was determined (Astell et al. 1981). The *MAT* alleles encode gene regulatory proteins, which in combination with other regulatory proteins are responsible for a distinct pattern of expression in the three yeast cell types (Herskowitz 1989; Hiscock and Kües 1999; Johnson 1995). Each mating type locus carries two genes, the *MATα* locus α1 and α2, and the *MATa* locus *a1* and *a2* (Fig. 2, Table 1). The α1 gene encodes a specific class of fungal transcription factors, namely an α1-domain transcription factor, and is a positive regulator of transcription of α-specific genes. Members of the α-specific genes are genes required for cell-cell recognition and fusion, and include genes for the α-factor pheromone (*MFα1* and *MFα2*) and a-factor receptor (*STE3*). The gene product of α2 is a homeodomain protein, which acts as a negative regulator of transcription of a-specific genes, e.g., genes coding for the a-factor pheromone (*MFA1* and *MFA2*) and the α-factor receptor (*STE2*). Thus, the reason why an α cell displays an α phenotype is that the α-specific genes are expressed, whereas a-specific genes are not. In the *MATa* locus of a cells, only the *a1* gene encodes a functional protein, a homeodomain transcription factor. However, unlike the α2 homeodomain protein of α cells, the a1 protein does not play a role in determining the a cell type. In the absence of the α2 protein, a-specific genes are expressed constitutively in a cells and α-specific genes are not expressed because of the absence of the positive regulator α1. The second gene *a2* of the *MATa* idiomorph encodes a nonfunctional version of α2 (Dolan and Fields 1991). In diploid α/a cells the homeodomain proteins α2 and a1 interact to form a novel negative regulatory protein. The heterodimer α2/a1 in combination with other regulatory proteins represses the expression of the α1 gene, which eliminates the transcription of α-specific genes and turns off the expression of haploid specific genes. In addition to its function in mating and meiosis, it was reported that the mating-type genes of *S. cerevisiae* play a role in maintaining cell wall integrity and heat shock response (Verna and Ballester 1999). Recently, Steinberg-Neifach and Eshel (2000) demonstrated that the simultaneous expression of both mating-type idiomorphs in haploid cells suppresses mutations in the yeast microtubule motor genes. In addition, they suggest that in diploid cells properties of microtubules are modified in a pathway controlled by the mating-type genes.

Schizosaccharomyces pombe

In the fission yeast *Schizosaccharomyces pombe*, the *mat1-M* idiomorph of *h+* cells carrys two genes, namely

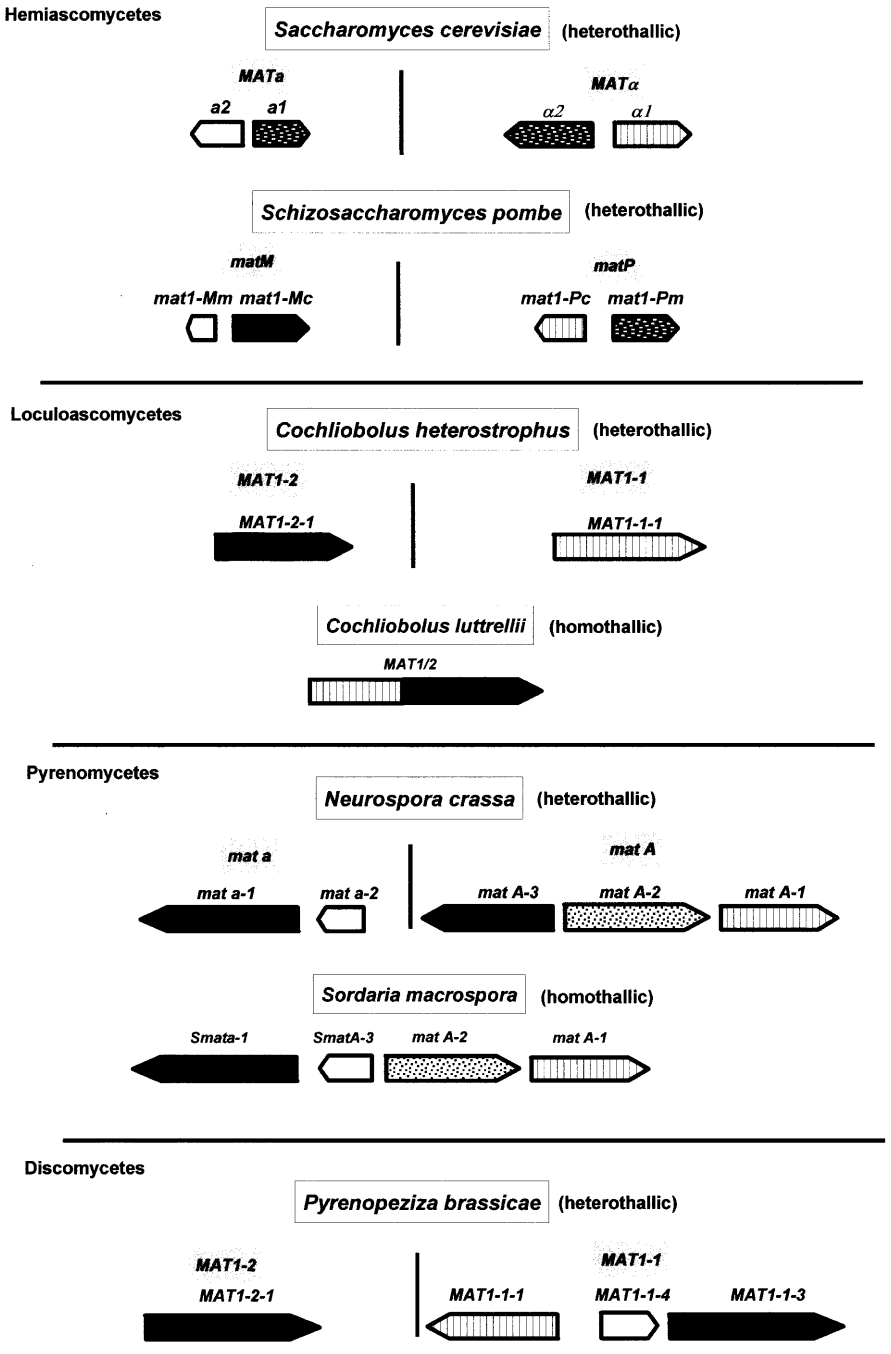

Fig. 2 Comparison of mating-type loci from heterothallic and homothallic ascomycetes. The *arrowed* boxes represent the orientation and size of the open reading frames in the mating-type loci; *black boxes* genes encoding HMG domain proteins; *striped boxes* genes encoding α-domain proteins; *white dotted boxes* genes encoding homeodomain proteins; *black dotted boxes* genes encoding MAT A-2 domain proteins; *white boxes* genes encoding proteins of unknown function

mat1-Mc and *mat1-Mm* (Kelly et al. 1988; Table 1). The translation product of *mat1-Mc* shows a region of similarity to known DNA binding sequences carrying a HMG (high mobility group) domain (Grosschedl et al. 1994), whereas *mat1-Mm* encodes an unknown class of proteins (Fig. 2). The proteins encoded by the two genes of the *mat1-P* idiomorph, *mat1-Pc* and *mat1-Pm*, display similarities to the *S. cerevisiae* α-domain protein α1 and to the homeodomain proteins, respectively (Kelly et al. 1988; Table 1 and Fig. 2). The transcription of all four genes is strongly induced upon nitrogen starvation and all of the *S. pombe* mating-type proteins are required for meiotic competence in a *h–/h+* diploid cell (Willer et al. 1995).

Yarrowia lipolytica

In the dimorphic alkane-utilizing yeast *Yarrowia lipolytica*, only the mating-type idiomorph *MATA* has been analyzed at the molecular level. *Y. lipolytica* is a heterothallic fungus and has a sexual life cycle with a haplophase and a diplophase. Two cells of opposite mating types, *A* and *B*, can conjugate and form a diploid zygote. Mating-type switching has not been demonstrated in *Y. lipolytica*. The *MATA* idiomorph was cloned on the basis of its ability to induce sporulation in a diploid *B*/*B* strain, which was constructed by protoplast fusion (Kurischko et al. 1992). This strain normally behaves like a haploid

Table 1 Mating-type genes from ascomycetes (*aa* amino acids, *ND* not determined)

Species	Mating system	Mating-type idiomorph	Genes	Type/length of encoded protein	Reference
Hemiascomycetes					
Saccaromyces cerevisiae	Heterothallic	*MATα*	*α1*	α-box/175 aa	Astell et al. 1981
			α2	Homeodomain/210 aa	
		MATa	*a1*	Homeodomain/126 aa	
			a2	?/120 aa	
Schizosaccharomyces pombe	Heterothallic	*matP*	*mat1-Pm*	Homeodomain/159 aa	Kelly et al. 1988
			mat1-Pc	α-box/118 aa	
		matM	*mat1-Mm*	?/42 aa	
			mat1-Mc	HMG-domain/181 aa	
Yarrowia lipolytica	Heterothallic	*MATA*	*MATA1*	?/119 aa	Kurischko et al. 1999
			MATA2	HMG-domain/291 aa	
		MATB	ND		
Candida albicans	Asexual	*MTLα*	*MTLα1*	α-box/193 aa	Hull and Johnson 1999
			MTLα2	Homeodomain/186 aa	
			OBPα	Oxysterol-binding protein 429 aa	
			PAPα	Poly (A) polymerase/558 aa	
			PIKα	Phosphatidyl inositol 4 kinase/977 aa	
		MTLa	*MTLa1*	Homeodomain/210 aa	
			OBPa	Oxysterol binding protein 433 aa	
			PAPa	Poly (A) polymerase/555 aa	
			PIKa	Phosphatidyl inositol 4 kinase/956 aa	
Loculoascomycetes					
Cochliobolus heterostrophus	Heterothallic	*MAT1-1*	*MAT1-1-1*	α-box/383 aa	Turgeon et al. 1993
		MAT1-2	*MAT1-2-1*	HMG-domain/343 aa	
Cochliobolus lutrellii	Homothallic	*MAT-1/2*	*MAT1/2-1*	α-box/HMG-domain (f)/562 aa	Yun et al. 1999
Cochliobolus homomorphus	Homothallic	*MAT2/1*	*MAT2/1-1*	HMG-domain/α-box (f)/705 aa	
Cochliobolus kusanoi	Homothallic	*MA-1/2*	*MAT1/2-1*	HMG–domain (f)/393 aa	
			MAT1/2-2	α-box/379 aa	
			MAT1/2-3	?/40 aa	
Cochliobolus cymbopogonis	Homothallic	*MAT1/2*	*MAT1/2-1*	HMG-domain (f)/326 aa	
			MAT1/2-2	α-box/354 aa	
Alternaria alternata	Asexual	*MAT1-1*	*MAT1-1-1*	α-box/389a	Arie et al. 2000
		MAT1-2	*MAT2-1-1*	HMG-domain/342 aa	
Pyrenomycetes					
Neurospora crassa	Heterothallic	*mat A*	*mat A-1*	α-box 293 aa	Glass et al. 1990a
			mat A-2	MAT A-2 domain/373 aa	Ferreira et al. 1996
			mat A-3	HMG-domain 342 aa	
		mat a	*mat a-1*	HMG 382 aa	Staben and Yanofsky 1990
			mat a-2	?/79 aa	Pöggeler and Kück 2000
Sordaria macrospora	Homothallic	*mat*	*SmtA-1*	α-box 306 aa	Pöggeler et al. 1997
			SmtA-2	MAT A-2 domain/359 aa	
			SmtA-3	?/124 aa	Pöggeler and Kück 2000
			Smta-1	HMG 287 aa	Pöggeler et al. 1997
Podospora anserina	Heterothallic	*mat –*	*FMR1*	α-box/305 aa	Debuchy and Coppin 1992
			SMR1	MAT A-2 domain/356 aa	Debuchy et al. 1993
			SMR2	HMG-domain/288 aa	
		mat +	*FPR1*	HMG-domain 402 aa	Debuchy and Coppin 1992
Giberella fujikuroi	Heterothallic	*MAT1-1*	*MAT1-1-1*	α-box/382 aa	Yun et al. 2000
			MAT1-1-2	MAT A-2 domain/433 aa	
			MAT1-1-3	HMG-domain/174 aa	
		MAT1-2	*MAT1-2-1*	HMG-domain/223 aa	

Table 1 continued

Species	Mating system	Mating-type idiomorph	Genes	Type/length of encoded protein	Reference
Fusarium oxysporum	Asexual	*MAT1-1*	*MAT1-1-1*	α-box/382 aa	Yun et al. 2000
			MAT1-1-2	MAT A-2 domain/431 aa	
			MAT1-1-3	HMG-domain/174 aa	
		MAT1-2	*MAT1-2-1*	HMG-domain/223 aa	
Giberella zeae	Homothallic	*MAT*	*MAT1-1-1*	α-box/345 aa	
			MAT1-1-2	MAT A-2 domain/463 aa	
			MAT1-1-3	HMG-domain/181 aa	
			MAT1-2-1	HMG-domain/253 aa	
Discomycetes					
Pyrenopeziza brassicae	Heterothallic	*MAT1-1*	*MAT1-1-1*	α-box/344 aa	Singh and Ashby 1998, 1999
			MAT1-1-3	Methallothionein/86 aa	
			MAT1-1-4	HMG-domain/417 aa	
		MAT1-2	*MAT1-2-1*	HMG-domain/424 aa	

B strain, that means it conjugates with *A* strains, but is unable to sporulate. The introduction of the *MATA* idiomorph into the diploid *B*/*B* strain represses its mating capacity, but induces sporulation. Sequence analysis of the *MATA* idiomorph revealed two genes, *MATA1* and *MATA2* (Table 1). The *MATA1* gene is required for the induction of sporulation and encodes a protein, which shows no homology to any other known protein. The *MATA2* gene is necessary for repression of mating and encodes a HMG-domain protein (Kurischko et al. 1999). The identification of flanking sequences of the *MATA* idiomorph will enable the cloning and characterization of the *MATB* idiomorph in the near future.

Candida albicans

Recently, Hull and Johnson (1999) made an exciting observation in the pathogenic yeast *Candida albicans*. This yeast was thought to be constitutively diploid and to reproduce only asexually. Through available genomic sequences and chromosome walking, they identified genomic regions with the potential to encode proteins with more than 50% derived amino acid homology to the master sexual cycle regulators α1, α2, and a1 of the *S. cerevisiae* mating-type idiomorphs *MATα* and *MATa*. These sequences were termed 'mating type-like locus' *MTLα* and *MTLa*. The *MTL* locus is heterozygous in the diploid *C. albicans* with the *MTLa* idiomorph contained in one chromosome and the *MTLα* idiomorph in the other. In addition to the putative transcriptional regulators, the *C. albicans MTL* idiomorphs bear several genes not seen in other fungal mating-type loci. The products of these genes show similarities to poly (A) polymerases, oxysterol-binding proteins, and to phosphatidylinositol kinases (Table 1). However, these extra three genes found in variable order in the mating-type loci might have been by chance acquired at the locus without a direct link to mating function. In analogy to *S. cerevisiae*, it was postulated that the diploid *C. albicans* would be an α/a strain and as such was not expected to mate. However, recently two research groups demonstrated that *C. albicans* can be forced to mate under certain conditions (Hull et al. 2000; Magee and Magee 2000). Hull et al. (2000) deleted specific *MTL* sequences to create compatible *MTLa/mtlα* and *mtla/MTLα* mating partners and showed that these strains were capable of mating after inoculation into a mammalian host. The obtained mating products contained genetic information from both parents, were tetrasomic in at least two chromosomes, and exhibited a higher than 2n DNA content.

Magee and Magee (2000) also constructed strains containing only *MTLα* or *MTLa*. They took advantage of the location of *MTL* on chromosome 5 that can be reduced to monosomy by growing cells on sorbose. In *C. albicans* the level of expression of the *SOU1* gene required for sorbose assimilation is determined by the copy number of chromosome 5, such that monosomic strains assimilate sorbose, whereas disomic strains do not (Janbon et al. 1998). It was demonstrated that *MTLα* and *MTLa* strains containing different auxotrophic markers mated, but strains carrying like mating-types did not. Progeny occurred on selective agar plates, which contained both *MTL* idiomorphs and molecular markers from both parents, were tetraploid and mononucleate.

The findings of both research groups suggest that mating may occur naturally. However, it remains to be clarified whether *C. albicans* undergoes meiosis and whether mating generates tetraploid or haploid intermediates. Compatible mating partners could be generated either by loss or repression at the *MTL* locus. Following that, the tetraploid progeny might undergo meiosis or experience chromosome loss to reduce the progeny to the diploid state. Alternatively, a compatible haploid mating partner could be generated by meiosis and could produce diploid progeny (Gow et al. 2000). The discovery of sexuality in *C. albicans* will have a major impact on the future of molecular genetics of this fungal pathogen, since formation of recombinants with genetic characters from both parents can be simply produced by mixing homozygous strains of opposite mating types.

Structure and organization of mating-type loci from heterothallic filamentous ascomycetes

Contemporary knowledge of mating-type systems in filamentous ascomycetes is summarized in the reviews by Coppin et al. (1997), Kronstad and Staben (1997), Hiscock and Kües (1999), and Shiu and Glass (2000). Therefore, this review intends to give a short overview of the mating-type loci of filamentous ascomycetes and primarily to highlight recent findings. Although some features are shared with hemiascomycetous yeasts, the organization of mating-type loci of heterothallic ascomycetes is clearly different. Mating-type idiomorphs have been cloned and sequenced from a number of heterothallic ascomycetes, including the pyrenomycetes *N. crassa* (Glass et al. 1990a; Ferreira et al. 1996; Staben and Yanofsky 1990), *Podospora anserina* (Debuchy and Coppin 1992; Debuchy et al. 1993) and *Gibberella fujikuroi* (Yun et al. 2000), the loculoascomycete *Cochliobolus heterostrophus* (Turgeon et al. 1993), and the discomycete *Pyrenopeziza brassicae* (Singh and Ashby 1998, 1999).

Pyrenomycetes

The mating-type idiomorphs of *N. crassa*, *P. anserina*, and *G. fujikuroi* contain the same basic set of genes (Fig. 2, Table 1). Molecular characterization and genetic analysis of mutants identified the *mat a-1* gene of the *N. crassa mat a* idiomorph as the main regulator of sexual development in *a* strains. The translation product of *mat a-1* is characterized by a conserved region found in HMG transcription factors (Staben and Yanofsky 1990). DNA-binding activity of the MAT a-1 protein has been demonstrated (Philley and Staben 1994). Recently, a second open reading frame (ORF), *mat a-2*, encoding a protein of 79 amino acids was identified in the *N. crassa mat a* idiomorph. RT-PCR analysis revealed that this ORF is co-transcribed with the *mat a-1* gene. Database searches discovered significant similarities between MAT a-2 and the mating-type protein SMT A-3 from the homothallic pyrenomycete *Sordaria macrospora*, but failed to show similarities to any other known protein (Pöggeler and Kück 2000). In the *P. anserina mat+* idiomorph, sequences similar to *mat a-2* have not yet been detected.

In *N. crassa A* strains, the main regulator for sexual development is encoded by the *mat A-1* gene (Glass et al. 1990a). The MAT A-1 polypeptide contains a motif that shows similarities to the α1-domain of the *S. cerevisiae* α1 mating-type protein. Apart from *mat A-1*, the *N. crassa A* idiomorph contains two additional genes, *mat A-2* and *mat A-3*. Both of these genes are constitutively expressed during the vegetative as well as the sexual phase (Ferreira et al. 1996). Mutational analysis of the *N. crassa mat A-2* and *mat A-3* genes, and of their counterparts *SMR1* and *SMR2* in *P. anserina*, revealed that both genes are involved in ascosporogenesis (Ferreira et al. 1998; Zickler et al. 1995).

Fig. 3 Highly conserved MAT-A2 motif found in MAT A-2 homologues of pyrenomycetes. NcMAT A-2 (*N. crassa*), SmSMT A-2 (*S. macrospora*), PaSMR1 *(P. anserina*), GfMAT1-1-2 (*G. fujikuroi*), FoMAT1-1-2 (*F. oxysporum*), GzMAT1-1-2 (*G. zeae*). Identical amino acid residues are *shaded in black* and functional similar residues are *boxed in gray*

The gene product of *mat A-2* and *SMR1*, respectively, contains a highly conserved motif, hereafter referred to as MAT A-2 domain that has no significant similarity to characterized DNA-binding domains (Fig. 3). It was proposed that this motif might correspond to a new DNA binding domain (Coppin et al. 1997). Proteins carrying this domain appear to be uniquely encoded in the mating-type loci of pyrenomycetes (Table 1, Fig. 3). A HMG domain was found in the translation products of *mat A-3* and *SMR2* (Table 1).

In the closely related pyrenomycetes *N. crassa* and *P. anserina*, mating types display not only a high degree of sequence and organizational similarity but also a functional identity (Arnaise et al. 1993).

In *N. crassa*, mating type also controls vegetative incompatibility. This means that if hyphae of opposite mating types fuse during vegetative growth, the resulting heterokaryotic cells will be growth inhibited and die (Glass and Kuldau 1992; Leslie 1993). Functional identity between *N. crassa* and *P. anserina* mating-type idiomorphs has not been observed with regard to the incompatibility process (Arnaise et al. 1993).

Loculoascomycetes

A slightly different organization of mating-type idiomorphs has been found in heterothallic members of the loculoascomycetes. Each idiomorph contains only one single gene, one of which encodes a HMG-domain protein and is a homologue of *N. crassa mat a-1*, while the other encodes an α-domain protein and is a homologue of *N. crassa mat A-1* (Turgeon et al. 1993; Fig. 2, Table 1). The mating-type specific homologues of *C. heterostrophus* are also functionally conserved and can induce perithecial development when expressed in *N. crassa* and *P. anserina* (Christiansen et al. 1993).

Discomycetes

In the *MAT1-1* idiomorph of the heterothallic discomycete *P. brassicae*, a metallothionein protein is encoded (*MAT1-1-4* gene) in addition to a α-domain (*MAT1-1-1* gene) and a HMG protein (*MAT1-1-3* gene) (Singh and Ashby 1998, 1999; Fig. 2, Table 1). A gene encoding a MAT A-2 domain protein (Fig. 3) was not found in the

P. brassicae MAT1-1 idiomorph. The *MAT1-2* idiomorph carries one gene (*MAT1-2-1*) encoding an HMG-domain protein (Table 1). Within 30 field isolates from different geographical locations, molecular analyses by means of PCR using primers specific for the four *P. brassicae* mating-type genes and by Southern blot analyses of the genomic DNA of the closely related discomycete *Tapesia yallundae* revealed that the mating-type gene structure and organization is conserved within field populations of *P. brassicae* and in *T. yallundae*. The results obtained in these experiments are consistent with the mating-type designations established by crossing experiments (Singh et al. 1999). However, mating-type-specific hybridization could not be obtained in heterologous gel-blot hybridization analysis with genomic DNA of the more distantly related discomycete *Ascobolus stercorarius*, and may thus reflect a divergence of mating-type sequences within the discomycetous class (Singh et al. 1999).

Mating-type genes in homothallic and asexual filamentous ascomycetes

Homothallic filamentous ascomycetes

Homologues of mating type genes have been found not only in homothallic but also in asexual filamentous fungi (Coppin et al. 1997; Shiu and Glass 2000). In pyrenomycetes, hybridization analysis revealed that sequences from the *N. crassa* idiomorphs are conserved in homothallic species (Beatty et al. 1994; Glass et al. 1990b). Two groups of homothallic species were distinguished in this study. One group contains sequences similar to the *mat A* idiomorph (*A*-type) and the second group accommodates sequences similar to both *mat A* and *mat a* idiomorphs (*A/a*-type) (Glass et al. 1988, 1990b). The mating type locus from *Neurospora africana*, an *A*-type homothallic species, contains a *mat A-1* homologue shown to function as a mating activator in *N. crassa* (Glass and Smith 1994). In *Sordaria macrospora*, an *A/a*-type homothallic species, the mating-type locus contains sequences homologous to both the *mat A* and the *mat a* idiomorph of *N. crassa* (Pöggeler et al. 1997). Within the mating-type locus of *S. macrospora* four ORFs, *Smta-1*, *SmtA-3*, *SmtA-2*, and *SmtA-1*, were identified. Whilst three of the *S. macrospora* genes show strong sequence similarity to the corresponding *N. crassa* mating-type genes, *SmtA-3* displays a chimeric character (Table 1, Fig. 2). The 5' sequences of *SmtA-3* correspond to the *N. crassa mat A-3* gene and the 3' end of the gene shows significant homology to the *N. crassa mat a-2* gene (Pöggeler and Kück 2000). All of the *S. macrospora* mating-type genes are transcriptionally expressed. In addition, the introduction of the homothallic *S. macrospora* mating-type genes into haploid strains of both mating types of *P. anserina* demonstrated that homothallic genes induce fruiting body formation. These results indicate that both *mat A*- and *mat a*-specific genes are not pseudogenes in homothallic species (Pöggeler et al. 1997). As in *S. macrospora*, the mating-type locus of the homothallic plant pathogen *Gibberella zeae* carries homologues of all four genes found in both idiomorphs of the heterothallic *G. fujikuroi*. However, a fused mating-type gene was not identified in the *G. zeae* mating-type locus (Yun et al. 2000; Table 1).

In the loculoascomycetous genus *Cochliobolus*, all homothallic species analyzed to date carry sequences homologous to both mating-type idiomorphs within one genome. In contrast to heterothallic species of the genus *Cochliobolus*, which display a highly conserved structural organization of the mating type loci, the structural organization of each locus is unique in the homothallic species. The genes are either, as in *C. luttrellii* and *C. homomorphus*, fused to a single ORF or are not fused, for example in *C. kusanoi* and *C. cymbopogonis*. Whilst in the latter, PCR and gel blot analysis revealed no evidence for linkage within 30 kb, both mating-type genes are closely linked in *C. kusanoi* (Yun et al. 1999; Table 1). Expression of a fused mating-type gene of a homothallic species confers self-fertility on a mating-type deletion strain of the heterothallic *C. heterostropus*, suggesting that mating-type locus of a homothallic species is sufficient to change the reproductive life style. Comparison of mating type sequences from the heterothallic *C. heterostrophus* and the closely related homothallic *C. luttrellii* provided evidence that heterothallism is ancestral to homothallism. The genetic mechanism by which the fusion of mating-type genes in a homothallic species has evolved might be a recombination event between islands of identity within dissimilar mating-type sequences of the heterothallic ancestor. Phylogenetic analyses support a convergent origin for homothallism in the genus *Cochliobolus* (Turgeon 1998; Yun et al. 1999).

As described above, conservation of mating-type function was demonstrated in homothallic filamentous ascomycetes, suggesting that functional mating-type sequences are required for sexual reproduction in homothallic species. Beside their function in the fertilization process, another role of heterothallic mating-type genes is the establishment of nuclear identity (Arnaise et al. 1997; Coppin and Debuchy 2000; Debuchy 1999; Zickler et al. 1995).

Asexual filamentous ascomycetes

Although sexual reproduction is absent in a large number of filamentous ascomycetes, mating-type sequences have been isolated from several asexual fungi. The asexual fungus *Bipolaris sacchari*, a pathogen of sugarcane, carries a homologue of the *MAT1-2-1* gene of its heterothallic relative *C. heterostrophus*. Sequence comparisons revealed that the *B. sacchari MAT1-2-1* gene, encoding an HMG-domain protein, is 98% identical to the *C. heterostrophus MAT1-2-1* gene. Moreover, the *B. sacchari MAT1-2-1* is functional in *C. heterostrophus*, since a *C. heterostrophus MAT-1* strain transformed with the *B. sacchari MAT1-2-1* gene is able to produce perithecia.

In contrast, when either of the *C. heterostrophus* mating-type genes was transformed into *B. sacchari*, the recipients were unable to self or to cross with other *B. sacchari* strains. Thus, the asexual nature of *B. sacchari* is not due to absence or mutations of the mating-type gene. Transcripts of the native *B. sacchari MAT1-2-1* gene have not been detected in *B. sacchari* wild-type strains, however, the *B. sacchari MAT1-2-1* gene is expressed in transgenic *C. heterostrophus MAT-1* strains (Sharon et al. 1996; Turgeon et al. 1995). Recently, the asexual loculoascomycete *Alternaria alternata* and the asexual pyrenomycetes *Fusarium oxysporum* were also proved to have mating-type loci, which are structurally similar to those of heterothallic relatives (Table 1). Heterologous expression of *A. alternata* mating-type genes in *C. heterostrophus* showed that *MAT1-1-1* as well as *MAT 1-2-1* are functional and, consequently, asexuality must be attributed to a different entity other than mating-type genes. In contrast to *B. sacchari*, mating-type genes were expressed in *A. alternata* and *F. oxysporum* wild type strains (Arie et al. 2000; Yun et al. 2000).

Mating-type genes and biotechnology

During the last 20 years much progress has been made in elucidating mating-type loci of ascomycetes at the molecular level. Knowledge of the molecular basis of mating types has given insights into fundamental processes such as evolution of homothallism, heterothallism, and asexuality, but also has applied aspects and can facilitate research on ascomycetous species of industrial interest in the future. Mating-type sequences can be used as phylogenetic and diagnostic markers and may also be used to convert homothallic to heterothallic or asexual to sexual strains.

Molecular mating-type assays

On the basis of the knowledge of the molecular structure of mating-type loci in ascomycetes, Arie et al. (1997) developed a PCR-based approach to obtain mating-type genes from different fungal species. Degenerated primers were designed to amplify the conserved HMG-domain, encoding sequences of loculoascomycetes and pyrenomycetes homologues to the *C. heterostrophus MAT1-2-1* gene and the *N. crassa mat a-1* gene, respectively. This approach has also been successfully applied to many different members of loculoascomycetes, pyrenomycetes, and discomycetes (Arie et al. 1999; Covert et al. 1999; Dyer et al. 2001; Kerényi et al. 1999; Witthun et al. 2000). As the approach of Arie et al. (1997) identified only one of the idiomorphs, several groups created rapid and reliable molecular assays by applying primers for both mating-type idiomorphs to genomic DNA in a single PCR (Dyer et al. 2001; Steenkamp et al. 2000; Wallace and Covert 2000). This technique facilitates the identification of sexually compatible pairs of opposite mating types in many heterothallic species. The molecular mating-type scoring reduces the amount of effort required to screen field populations for sexual fertility and increases the efficiency of the process through which new mating populations are identified (Covert et al. 1999; Steenkamp et al. 2000). Based on the results obtained by the amplification of mating-type genes, the existing arbitrary mating-type terminology of many fungal strains was replaced (Arie et al. 1997; Kerényi et al. 1999).

The availability of molecular diagnostics for mating types facilitates a more-thorough analysis of the allegedly asexual fungi. The finding that asexual ascomycetes carry mating-type genes (Table 1) introduces the possibility of sexual reproduction of several members of purportedly asexual ascomycetes being induced in the laboratory, as was shown for classic 'asexual' yeast *C. albicans* (Hull et al. 2000; Magee and Magee 2000). Thus, mating types may be found or else engineered in other asexual fungi, which have so far not been accessible to classical genetic methods.

Alternatively, sexual development might be imposed on asexual fungi by molecular genetic manipulation. This was demonstrated by Sharon et al. (1996) in transgenic *B. sacchari* strains carrying multiple copies of *C. heterostrophus* mating-type genes. The transgenic strains are sometimes capable of initiating sexual development with a *C. heterostrophus* strain. Sharon et al. (1996) suggested by that the heterothallic mating partner contributes factors that allow the initiation of interspecific sexual development. A similar approach may be chosen to initiate sexual development in other asexual ascomycetes.

Mating-type genes as a source for phylogenetic analyses

Molecular analysis of mating-type genes is a powerful tool for the evolutionary investigation of reproductive life styles and species relationships. This was impressively demonstrated in the plant pathogens *Cochliobolus victoriae* and *Cochliobolus carbonum*. Gel blot analysis of DNAs from *C. carbonum* detected that field isolates from diverse geographical locations contained either the *MAT1-1* or the *MAT1-2* idiomorph, but never both together. In contrast, *C. victoriae* isolates contained only the *MAT1-2* idiomorph and were found to be either female sterile or completely infertile. Since crosses between *C. victoriae* and *C. carbonum* could be readily obtained, it was suggested that these two are members of the same species. All members of the documented *C. victoriae* field population are thought to be derived from a *MAT1-2 C. carbonum* strain. *C. victoriae* results from horizontal transfer of genes for pathogenicity to oats into a strain of *C. carbonum*, which was *MAT1-2* and female sterile (Christiansen et al. 1998).

Several phylogenetic studies based on sequences of mating-type genes and other protein encoding genes or on ITS sequences suggest that mating-type genes evolve

very rapidly. In contrast to high variability between species, the mating-type genes appear to be conserved within species (Pöggeler 1999; Turgeon 1998). Rapid evolution of mating-type genes can be exploited as a molecular tool for phylogenetic analyses of closely related species, for species diagnosis, and for the classification of both sexual and asexual species.

Phylogenetic trees constructed with mating-type sequences were shown to resolve the relationship between plant pathogens. The phylogenetic analysis of species belonging to the genus *Cochliobolus* demonstrated that highly pathogenic species are all closely related. This relationship, however, remains unresolved in trees constructed with ITS or *gpd* sequences alone. In addition, the trees inferred from mating-type sequences identified closely related pairs of asexual/sexual or heterothallic/homothallic species of the genus *Cochliobolus* (Berbee 1999; Turgeon 1998).

Phylogenetic analysis using mating-type sequences is not only a powerful tool for the determination of relationships between pathogens, but also may prove to be an important method in identifying the relationship of economically valuable species belonging to the genus *Tuber*. Unlike other ectosymbiotic fungi, many species of the genus *Tuber* produce economically valuable, edible fruiting bodies. In addition, the beneficial effects of mutual association play a pivotal role in the growth and nutrition of forest trees (Rygiewicz and Andersen 1994), which encourage the large-scale production and commercialization of trees inoculated with *Tuber* species. An unambiguous identification of truffle mycorrhizae is essential in the nursery inoculation of host plants, because morphologically similar *Tuber* spp. may have rather different ecological growth requirements, as well as dissimilar organoleptic qualities and are, consequently, not of the same commercial value (Paolocci et al. 2000). Thus, mating-type sequences used as molecular markers could cater for a reliable species characterization.

Recognized and economically valuable ascomycetes are also those producing compounds for commerce, for example the β-lactam antibiotics penicillin and cephalosporin. The best known are *Pencillium chrysogenum*, *Aspergillus nidulans*, and *Acremonium chrysogenum*. In addition to these, the American Type Culture Collection (ATCC) contains 518, mainly asexual, fungi that have been reported to produce 207 different antibiotics (Jong and Donovick 1989). Mating-type sequences may be useful for the improvement of phylogenetic resolution among these species and presents an opportunity for this important group of fungi to be modified by the methods of classical genetics.

Moreover, ascomycetes are commercially interesting because of their ability to produce chemicals such as gibberellins, a large family of isoprenoid plant hormones, some of which are bioactive growth regulators, controlling seed germination, stem elongation, and flowering (Tudzynski 1999). Seven *G. fujikuroi* mating populations (MP A-G) have been described (Leslie 1991, 1995). Mating populations (MP) can be considered as biological species, as members of a given MP are able to reproduce sexually only with members of the same MP. Members of the MP C are able to produce large amounts of the gibberellins (Tudzynski 1999). The recently cloned and sequenced mating-type idiomorphs of the heterothallic rice pathogen *Giberrella fujikori*, MP A, will allow the distinction between compatible isolates of the opposite mating type of other MPs (Yun et al. 2000; Table 1). This is hoped to improve the success in crossing strains of the large collection of field isolates and might lead to classical genetic approaches for strain improvement of gibberellin-producing strains. Other members of the *Gibberella/Fusarium* complex impose economic consequences as plant pathogens and/or as producers of mycotoxins. Mating-type sequences should be exploited as phylogenetic markers in order to unravel species relationships within this complex.

Interspecific crossings

It appears that among *Saccharomyces* yeasts used in fabrication of wine, cider, and beer, stable interspecies hybrids are quite common. The specific properties of hybrid strains, for example the production of certain esters and higher alcohols, can offer an advantage in the brewing and wine making process (Masneuf et al. 1998). Thus, interspecific crossing should be one of the first methods to be considered for improvement of industrial yeasts, and a simple and reliable determination of mating types will improve the interspecific mating in the laboratory. The economically important genus *Saccharomyces* can be divided into two major groups: sensu stricto and sensu lato (Barnett 1992). The sensu stricto yeasts, which include *S. bayanus*, *S. cerecvisiae*, *S. paradoxus*, and *S. pastoranius*, represent a closely related biological complex (Kurtzman and Robnet 1991). In the laboratory, members of the sensu stricto group can be mated at low frequency and produce a viable offspring (Naumov 1996). For heterothallic strains, the hybrids can be selected by micro-manipulating the zygotes formed between meiotic segregants with opposite mating types. Although many of the yeasts proven to be of industrial importance are homothallic, this is not a bar to classical genetic manipulation (Ramírez et al. 1998). The knowledge of the structural organization of mating types in yeasts can be used to support the genetic tractability of homothallic species by making them heterothallic.

Homothallic filamentous ascomycetes produce self-fertile fruiting bodies. Thus, for genetic analyses, it is difficult to distinguish between self-fertile and hybrid perithecia in crosses. To circumvent this problem, color spore mutants or sterile mutant strains can be used for crossings of homothallic species (Esser and Straub 1958). In the first case, hybrid perithecia can be distinguished from self-fertile perithecia by the segregation pattern of the colored spores. In the latter case, complementation of the genetic defects results in the formation of fertile perithecia in the contact zone of two mutant

mycelia only, all of which are hybrid perithecia. However, color spore mutants and sterile mutant strains are not always available. Consequently, the replacement of homothallic mating-type loci by heterothallic mating-type idiomorphs or the deletion of single mating-type genes would expedite crossing procedures and genetic analysis. To date the feasibility of this manipulation has only been demonstrated in the reverse direction. Yun et al. (1999) replaced the mating-type idiomorph of a heterothallic *C. heterostrophus* strain with the mating-type locus from the homothallic *C. luttrellii* and showed that the transgenic strain was able to produce progeny. The introduction of the mating-type locus from the homothallic *S. macrospora* into a mating-type-deficient strain of *P. anserina* also led to the production of ascospores in *P. anserina* (S. Pöggeler, unpublished data).

Asexual fungi carry functional mating-type genes and most probably lack some attributes other than mating-type genes, which are required for mating (Arie et al. 2000; Sharon et al. 1996; Yun et al. 2000). If future analysis discloses the molecular basis of asexuality in fungi, then even fungi for which a perfect stage is not known could be modified by molecular genetic methods to become sexual and, subsequently, be subjected to modification through methods of classical genetics.

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