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Genetics

Heterologous expression of mating-type genes in filamentous fungi

(sexual reproduction/vegetative incompatibility)

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ABSTRACT ***Podospora anserina* and *Neurospora crassa*, two filamentous heterothallic ascomycetes, have a single mating-type locus with two alternate forms called *mat*+ and *mat*− and *A* and *a*, respectively. Mating type controls entry into the sexual cycle, events subsequent to fertilization, and, in *N. crassa*, prevents the formation of mixed mating-type heterokaryons. The mating types of these two organisms display similarity in their DNA structure and in the encoded polypeptides involved in fertilization. Here we show that this molecular similarity reflects a functional homology with respect to mating identity. Transformation experiments show that the *N. crassa* mating-type genes can provide the fertilization functions in *P. anserina* strains devoid of mating specificity as well as in *mat*+ and *mat*− strains. Reciprocally, the introduction of *P. anserina* mating-type genes confers mating activity in *N. crassa*. Functional identity between the mating types is not observed for vegetative incompatibility or for post-fertilization events such as meiosis and ascosporogenesis.**

In heterothallic ascomycetes, sexual reproduction is under the control of mating types that are specified by a single locus with two alternate forms. The mating type regions have been cloned from a number of heterothallic ascomycetes [*Saccharomyces cerevisiae*, ref. 1 for review; *Schizosaccharomyces pombe*, ref. 2 for review; *Neurospora crassa* (3); *Podospora anserina* (4); and *Cochliobolus heterostrophus* (5)], and all share a number of common features. Each uses a region of DNA, variable in length, that is essentially nonhomologous between the two mating types and that controls various aspects of mating and meiosis. The term "idiomorph" has been introduced to denote evolutionarily dissimilar sequences (like mating type) found at identical loci in different strains (6). In addition, common transcriptional factor motifs can be found in the putative polypeptides encoded by one or the other mating type regions of the various fungi (5, 7–13).

Our aim in this study was to determine if the structural similarities in the mating regions of *P. anserina* and *N. crassa* also reflect conservation of mating type function. *P. anserina* and *N. crassa* have two mating types, designated *mat*− and *mat*+ (14) and *A* and *a* (15), respectively. Although *P. anserina* and *N. crassa* are both members of the Sordariaceae and cytological events associated with sexual development and meiosis are similar in the two organisms (16, 17), mating reactions between the two species have never been observed. In *N. crassa*, mating type also functions to control heterokaryon formation between *A* and *a* strains; if hyphae of opposite mating types fuse during vegetative growth, the resulting heterokaryotic cells are inhibited in their growth (18). Unlike *N. crassa*, *P. anserina* does not exhibit mating-type-associated heterokaryon incompatibility. In fact, opposite mating-type nuclei are compartmentalized within single ascospores, resulting in the characteristic "pseudohomothallic" phenotype of wild-type strains (14).

The *N. crassa A* and *a* idiomorphs are 5.3 and 3.2 kbp in length (3); regions conferring both mating and heterokaryon incompatibility have been localized to two open reading frames (ORFs), one in each idiomorph (*mt A-1* and *mt a-1*, respectively) (11, 12). The *P. anserina mat*− and *mat*+ idiomorphs are 4.7 and 3.5 kbp in length (13); regions conferring mating activity have been localized to two ORFs (*FMR1* and *FPR1*, respectively). A comparison of the *FMR1* and *mt A-1* ORFs revealed that in the amino-terminal portion of the putative polypeptides, 106 amino acids out of 196 are identical (13). Although the *FPR1* and *mt a-1* ORFs are divergent, a region encompassing the putative DNA binding domain is highly conserved between the two ORFs. Regions within the *mat*− and *A* idiomorphs in addition to *FMR1* and *mt A-1*, respectively, are required for productive ascospore formation (13, 19).

In this study, we report that the molecular similarity between the *FPR1/mt a-1* and *FMR1/mt A-1* ORFs reflects a functional homology with respect to mating identity. Transformation experiments of *P. anserina* strains with *N. crassa* mating-type genes argue that, in a *P. anserina* context, *A* acts like *mat*− and *a* acts like *mat*+, with respect to mating. Similarly, the introduction of *mat*− and *mat*+ into *N. crassa* confers *A* and *a* mating specificity, respectively. Functional identity between the idiomorphs is not observed for the incompatibility process nor for post-fertilization events necessary for the development of the perithecium.

MATERIALS AND METHODS

***P. anserina* Strains.** The characteristics of *P. anserina* were first described by Rizet and Engelmann (14). The strains used in this study were derived from homokaryotic spores containing only one or the other mating type. The transformation recipients carry the *leu1-1* mutation. The *mat0* strain was isolated from the progeny of a *AS4.44 mat*+ *mat*−* homokaryotic strain crossed by a *mat*+ tester [the asterisk (*) indicates the transgenic mating type].

***N. crassa* Strains.** The following *N. crassa* strains were used as transformation recipients: *aro-9; qa-2 a* and *nic-3; aro-9; qa-2 A*.

Plasmid Constructs. The plasmid KSR1RV (13) is a 4.2-kbp *Eco*RI–*Eco*RV fragment that includes all the informative *mat*+ specific fragment; plasmid pHMTPP (13) contains the 5.7-kbp *Pst* I–*Pst* I *mat*− specific fragment (see Fig. 1). The plasmid pMTAG-2 contains the 1.2-kbp *Pst* I–*Bam*HI *mt A-1* specific fragment and pmt6.818 is a 6.8-kbp *Eco*RV fragment that includes *A* (3) (Fig. 1). The plasmid pCSN4 contains the 1.9-kbp *Bam*HI–*Eco*RV *mt a-1* specific fragment and pCSN27 is a 6.5-kbp *Hin*dIII–*Sac* I fragment that includes *a* (3) (Fig. 1). The plasmid pM33-18M3 contains the *leu1*+ gene

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Abbreviation: ORF, open reading frame.

of *P. anserina* (20). For cotransformations into *N. crassa* strains, either pSV50 (21) or pQa2 was used (3).

Transformation Methods. The various *A* and *a* mating-type constructs were introduced into *P. anserina* by cotransformation with pM33-18M3 (20). Transformation assays were performed as described (4). DNA-mediated transformation of *N. crassa* strains with the *mat*+ and *mat*− constructs was according to Glass *et al.* (3).

Crossing Procedures. Mating type of *P. anserina* transformants was assayed by confronting transformed strains with *mat*+ or *mat*− testers on each side of a plate and also by spraying microconidia of the opposite mating type onto transformant strains. Mating-type assays of *N. crassa* transformants and dual-mating strains were performed according to Glass *et al.* (3). Dual-mating strains were also plated singly onto mating medium (22) and onto mating-type tester plates seeded with either *fl; inl A* and *fl; inl a* strains.

Genomic DNA Preparation and Analysis. For *P. anserina*, genomic DNA was prepared from lyophilized mycelium according to a miniprep method (23). For *N. crassa*, genomic DNA was isolated by a modification of the method of Stevens and Metzenberg (24). DNA blot analysis was as described (4, 11).

Light Microscopy Preparation. Asci were fixed in fresh Lu's fixative (butanol/propionic acid/10% aqueous chromic acid, 9:6:2 by volume). After a 10-min hydrolysis at 70°C, asci were stained in two drops of 2% hematoxylin mixed on the slide with one drop of a ferric acetate solution.

RESULTS

Transfer of *Neurospora* Mating-Type Idiomorphs into *Podospora*

The *mt A-1* and *mt a-1* Genes of *N. crassa* Can Complement a Sterile *mat0* Strain of *P. anserina* with Respect to the Fertilization Process. The *mat0* strain contains a deletion of 5–8 kbp that includes the entire *mat*+ idiomorph (Fig. 1). This strain differentiates female and male reproductive structures but cannot mate with either *mat*+ or *mat*− reference strains. We define mating as the induction of perithecial development, irrespective of ascospore formation. The *mat0* strain can be complemented by the introduction of *FPR1* and *FMR1* (Fig. 1) to give rise to strains that mate as *mat*+ or *mat*− strains, respectively. The introduction of the *mat*+ (pKSR1RV) and *mat*− (pHMTPP) (Fig. 1) into *mat0* gives rise to fertile *mat*+ or *mat*− strains, respectively (E. Coppin, S.A., and V. Contamine, unpublished results).

We tested whether the *mt a-1* and *mt A-1* mating-type genes of *N. crassa* can confer mating capacity in *P. anserina* by DNA-mediated transformation of a *mat0 leu1-1* strain. The vector pM33-18M3 was cotransformed with plasmids containing either *mt A-1* (pMTAG-2) or *mt a-1* (pCSN4) (Fig. 1). When transformation was assessed with the *mt A-1* fragment, 7 of 100 (leu^+) transformants acquired the ability to mate with a *P. anserina mat*+ strain; we call these transformants *mat0 mt A-1** (Table 1). When transformation was assessed with the *mt a-1* fragment, 9 of 100 (leu^+) transformants mated with a *P. anserina mat*− strain; we call these transformants *mat0 mt a-1** (Table 1).

In all of the *mat0 mt A-1** transformants examined, sequences corresponding to *mt A-1* were detected; hybridization to *mt A-1* could not be detected in *mat0*, *mat*+, or *mat*− strains (Fig. 2*C*). Similarly, all *mat0 mt a-1** transformants contained the *mt a-1* specific fragment, whereas no hybridization to *mt a-1* to DNA from *mat0*, *mat*+, or *mat*− strains was observed (Fig. 2*D*). Hybridization to *mat*+ or *mat*− sequences was not detected in either class of transformants (data not shown).

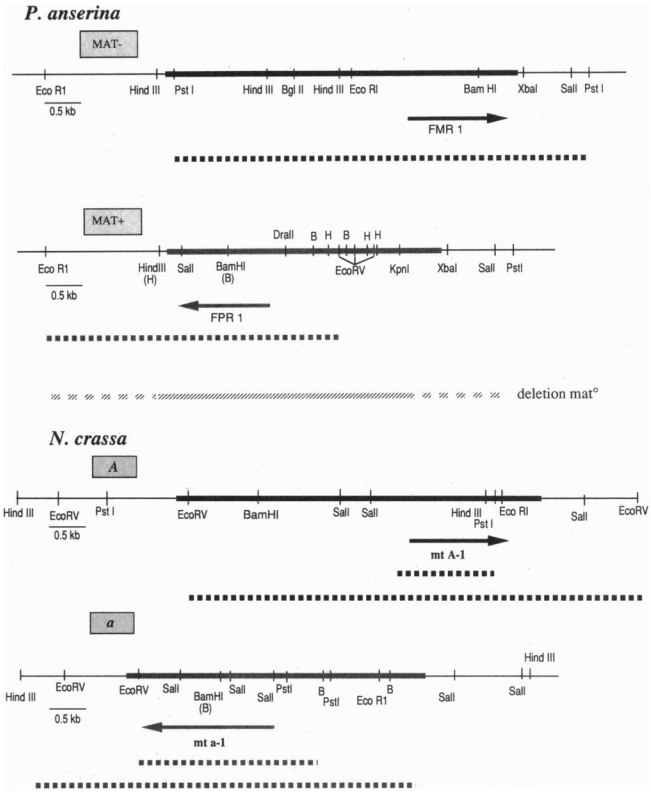

FIG. 1. Restriction maps of *MAT* loci in *P. anserina* (*Upper*) and in *N. crassa* (*Lower*). ■ and ▩, specific sequences of each idiomorph; transcription direction of ORFs involved in fertilization process are indicated by an arrow; ■■■ and ■■■, fragments tested in transformation experiments, subcloned in plasmids (see text). DNA fragment deleted in the *mat0* strain is indicated; the precise limits are not yet determined.

Transformation of *P. anserina mat*− or *mat*+ Strains by the *mt a-1* or *mt A-1* Genes, Respectively, Gives Rise to Self-Mating, Dual-Mating Strains. The plasmids pM33-18M3 and pMTAG-2 (*mt A-1*) were introduced by DNA-mediated transformation into *mat*+ *leu1-1* spheroplasts; similarly, *mat*− *leu1-1* spheroplasts were cotransformed by pM33-18M3 and pCSN4 (*mt a-1*). Twenty-three *mat*+ *leu*+* transformants and 34 *mat*− *leu*+* transformants behaved like self-mating and dual-mating strains (Table 1). Molecular analysis confirmed that the self/dual-mating *mat*+ transformants contained *mt A-1* and not *mat*−, and the self/dual-mating *mat*− transformants contained *mt a-1* and not *mat*+ (data not shown). Although the *mat*− *mt a-1** and *mat*+ *mt A-1** transformants mated with both *mat*+ and *mat*−, ascospores were obtained only in crosses involving a tester strain of the opposite mating type than the recipient (Table 1).

As *mt A-1** and *mt a-1** segregate independently of the *P. anserina MAT* locus, spores containing *mat*+ and *mt a-1* information, or *mat*− and *mt A-1* information, were recovered. They did not display any peculiar phenotype compared to standard *mat*+ or *mat*− strains (Table 1).

Sexual Development Is Initiated But Ascospore Formation Is Rarely Achieved in Either *mt A-1*, *mt a-1* or in *A*, *a P. anserina* Transformants. Large numbers of perithecia and ascospores are produced in intraspecific crosses between sexually compatible strains of *P. anserina* or *N. crassa*. In contrast, perithecia were less abundant and smaller in size and produced very few ascospores in crosses between *mat0 mt A-1** or *mat0 mt a-1** transformants and *mat*+ or *mat*−, respectively. In wild-type *P. anserina* perithecia, the nuclei of biparental origin become isolated into dikaryotic cells. In the top of the hook cell (crozier), the conjugate mitoses are

Table 1. Expression of the *N. crassa mt A-1* and *mt a-1* genes in *P. anserina mat0*, *mat+*, and *mat−* strains and *P. anserina mat+* and *mat−* in *N. crassa A* and *a* strains

Genotype†	Perithecium induction			Ascospore formation		
	On *mat+*	On *mat−*	On self	With *mat+*	With *mat−*	With self
P. anserina						
mat0	−	−	−			
*mat0 mt A-1** or *mat0 A**	+	−	−	§		
*mat0 mt a-1** or *mat0 a**	−	+	−		¶	
mat0 mt A-1/mt a-1**‡	+	+	+	−	−	−
mat+	−	+	−		+	
*mat+ mt A-1** or *mat+ A**	+	+	+	−	+	−
*mat+ mt a-1** or *mat+ a**‡	−	+	−		+	
mat−	+	−	−	+		
*mat− mt a+1** or *mat− a**	+	+	+	+	−	−
*mat− mt A-1** or *mat− A**‡	+	−	−	+		
	On *A*	On *a*	On self	With *A*	With *a*	With self
N. crassa						
A	−	+	−		+	
*A mat−**	−	+	−		+	
*A mat+**	+	+	+	−	+	−
a	+	−	−	+		
*a mat−**	+	+	+	+	−	−
*a mat+**	+	−	−	+		

†All strains are *P. anserina* or *N. crassa* transformed strains with one mating type indicated by the asterisk (*) except three strains (‡) obtained in two steps: transformation of *mat−* or *mat+* strain by *mta-1* or *mtA-1* respectively, followed by crossing (see detail in text).

§Perithecia filled with croziers, but only few asci with rare and abnormal ascospores.

¶Perithecia barren at early prophase, few of them forming some asci with four ascospores.

followed by formation of septae that divide the crozier into three cells; the central cell contains two nuclei of opposite mating type. Karyogamy is initiated and the diploid nucleus proceeds directly into meiosis and ascospore formation. Crozier development in two *mat0 mt A-1** × *mat+* crosses was normal, but the two nuclei in the penultimate crozier-cell did not undergo karyogamy. Instead, they began a new round of synchronized mitoses and the perithecia became filled with several "crozier-trees" from which only rare asci (1–10) were formed (Fig. 3*a*). The asci showed variability in the stage of arrest and only abnormal ascospores were observed. In a *mat0 mt a-1** × *mat−* cross, the majority of asci were blocked at prophase I; a few perithecia contained asci with four ascospores (Fig. 3*b*).

In a second experiment, the entire *A* (pmt6.818) or *a* (pCSN27) idiomorphs (Fig. 1) were introduced into a *mat0 leu1-1* strain by cotransformation with pM33-18M3. Among 90 leu+ (*A*) transformants, 34 had acquired the ability to mate with a *P. anserina mat+* strain; 64 transformants of 182 leu+ (*a*) mated with a *mat−* strain. The perithecia of two *mat0 a** × *mat−* and two *mat0 A** × *mat+* crosses were examined cytologically; sexual development was blocked at precisely the same stage as the *mat0 mt a-1** × *mat−* or *mat0 mt A-1** × *mat+* crosses, respectively (Table 1).

The *P. anserina mat0 A** and *mat0 a** transformants did not give a visible mating reaction when seeded with conidia from a *N. crassa A* or *a* strain.

Unlike in *N. crassa*, the *mt A-1* and the *mt a-1* Genes Do Not Confer Vegetative Incompatibility in *P. anserina*. In *N. crassa*, partial diploids heterozygous for mating type [constructed by DNA-mediated transformation (3) or by crosses involving translocation strains (25)] display growth inhibition, referred to as mating-type-associated vegetative incompatibility. In contrast, the introduction of *mt A-1* and *mt a-1* into *P. anserina* does not induce an incompatibility response. Some *mat− mt A-1*/mt a-1** and *mat+ mt A-1*/mt a-1** strains were recovered among progeny from crosses of *mat+ mt A-1** × *mat− mt a-1**. These strains were self-mating, behaved macroscopically like *mat− mt a-1** or *mat+ mt A-1**, and grew at wild-type rates. A *mat0 mt A-1*/mt a-1** strain was recovered from a *mat+ mt A-1*/mt a-1** × *mat0 mat−** cross (Table 1) that also grew normally and was self-mating. Finally, an inhibition in vegetative growth was not observed when fusion of *mat0 mt A-1** and *mat0 mt a-1** hypha occurred.

Transfer of *Podospora* Mating-Type Idiomorphs into *Neurospora*

The Introduction of the *P. anserina mat+* and *mat−* Idiomorphs into *N. crassa* Confers Mating Function But Ascospores Are Not Observed. The *mat+* plasmid and *mat−* plasmid (Fig. 1) were introduced into *N. crassa A* and *a* spheroplasts by cotransformation with pQa2. Both dual and self-mating strains could be recovered from *A* and *a* transformants. We call these self-mating *A* transformants *A mat+** and the self-mating *a* transformants *a mat−**.

Ascospores were isolated from crosses between the *A mat+** × *fl; inl a* and *a mat−** × *fl; inl A*. Self-mating and dual-mating single ascospore progeny were recovered from both crosses (Table 1). DNA fragments hybridizing to *mat+* in the *A mat+** strains could be detected in the self/dual-mating progeny (Fig. 2*B*). One of the ascospore progeny (Fig. 2*B*, lane 5) was originally a self/dual mater, but upon subsequent subculture, both self/dual mating and *mat+* sequences were lost. In the *a mat−** strains, DNA fragments hybridizing to *mat−* could be detected in all of the self/dual-mating progeny (Fig. 2*A*). Hybridization of *mat+* and *mat−* to DNA from *A* or *a* strains could not be detected under conditions of high stringency (Fig. 2 *A* and *B*).

The *A mat+** strains are self- and dual-maters, but ascospore formation only occurs when the strains are mated to an *a* mating type (Table 1). Similarly, the *a mat−** strains mate with both *A* and *a* strains, but ascospore formation only occurs in crosses with *A* strains. These results are similar to what has been observed when the *A* and *a* idiomorphs are introduced into *N. crassa* sterile mutants: the ability to mate is restored but ascospores are seldom produced (3). The *a mat−** strains mated nearly as well with both *A* and *a* strains to induce perithecial development; however, in the *A mat+** × *A* crosses, fewer perithecia were produced as compared to matings with *a* strains. Most of the self/dual-mating *A mat+** strains lose *a* (*mat+*) mating activity upon repeated subculture. The basis of the loss of *mat+* activity in subculture of *A mat+** strains is unknown.

Cytological analyses of the *A mat+** or *a mat−** strains showed that sexual development in these strains was normal until 3–4 days postfertilization. Further development of the perithecia was arrested; perithecia were smaller than in fertile crosses and beak formation was rudimentary. Within the developing perithecium, the block in sexual cycle occurred prior to, or at the initiation of, crozier formation.

The *mat+* and *mat−* Idiomorphs Do Not Confer Vegetative Incompatibility in *N. crassa*. The introduction of *A* or *a* into *N. crassa a* or *A* spheroplasts, respectively, results in a reduction of transformation efficiencies; surviving transformants either contain copies of the introduced mating-type sequence that were disrupted by the transformation event or display a phenotype characteristic of the incompatibility response (3). When *mat+* and *mat−* sequences were cotransformed with the pQa2 vector into *N. crassa A* and *a* sphero-

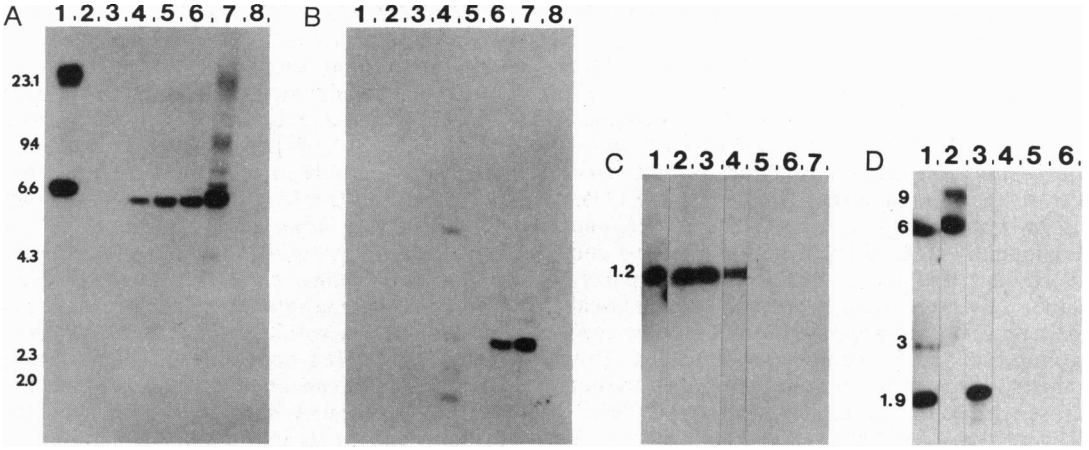

FIG. 2. (*A*) Southern blots of genomic DNA from *N. crassa a mat−** and untransformed *A* and *a* strains hybridized to ^{32}P-labeled pHMTPP. DNAs were digested with *Bam*HI and *Pst* I. Lane 1, size markers (in kbp); lane 2, no DNA; lane 3, untransformed *A* strain; lane 4, initial *a mat−** transformant; lanes 5–7, self/dual-mating progeny from *a mat−** × *A* cross; lane 8, untransformed *a* strain. (*B*) Southern blots of genomic DNA from *N. crassa A mat+** and untransformed *A* and *a* strains hybridized to ^{32}P-labeled pKSR1RV. DNAs were digested with *Hin*dIII. Lane 1, size markers (in kbp); lane 2, no DNA; lane 3, untransformed *A* strain; lane 4, initial *A mat+** transformant; lanes 5–7, self/dual-mating progeny from an *A mat+** × *a* cross; lane 8, untransformed *a* strain. Progeny in lane 5 was originally a self/dual-mater but has lost the *mat+** sequences upon subculture. (*C*) Southern blots of genomic DNA from *mat0 mt A-1** and from untransformed *mat0, mat−*, and *mat+* strains hybridized with the ^{32}P-labeled *Eco*RI–*Bam*HI insert from pMTAG-2. DNAs were digested with *Bam*HI and *Eco*RI. Lane 1, pMTAG-2; lanes 2–4, *mat0 mt A-1** transformants; lanes 5–7, untransformed *mat0, mat−*, and *mat+* strains, respectively. (*D*) Southern blots of genomic DNA from *mat0 mt a-1** and *mat+ mt a-1** transformants and from untransformed *mat0, mat−*, and *mat+* strains hybridized with the ^{32}P-labeled *Eco*RV insert from pCSN4. DNAs were digested with *Eco*RV. Lane 1, pCSN4; lanes 2 and 3, *mat0 mt a-1** transformants; lanes 4–6, untransformed *mat0, mat−*, and *mat+* strains, respectively. One transformant presented the expected *Eco*RV fragment of 1.9 kbp (lane 3), but the other (lane 2) presented two major bands of 6 and 9 kbp (*Eco*RV site may be disrupted). All hybridizations were performed under conditions of high stringency.

plasts, respectively, approximately one-third of the *A* and *a* transformants were self-fertile and mated with both *A* and *a* strains. The presence of *mat+* and *mat−* could be detected in all of the self-mating/dual-mating transformants (data not shown). In contrast, self/dual-mating strains can only be constructed in *N. crassa* by introducing opposite mating-type sequences into a strain containing a *tol* mutation; the unlinked suppressor *tol* allows the coexistence of both mating-type sequences within a single nucleus (26). Thus, in contrast to *mt A-1* and *mt a-1*, the introduction of *mat+* into *A* spheroplasts and *mat−* into *a* spheroplasts does not induce an incompatibility response in a *N. crassa tol*$^+$ background.

DISCUSSION

This study shows that the mating-type regulators of *N. crassa* and *P. anserina* are interchangeable with respect to mating function, even though mating reactions between the two species have never been observed. However, the mating-type regulators of *N. crassa* and *P. anserina* are not interchangeable with regard to postfertilization functions such as meiosis and ascospore formation or as vegetative incompatibility genes.

Fertilization Functions of Mating-Type Genes Are Conserved Between *P. anserina* and *N. crassa*. The products of the mating-type genes in *N. crassa* and *P. anserina*, which are thought to encode regulatory polypeptides, present some common motifs: *FMR1, mt A-1*, and *S. cerevisiae MATα1* polypeptides have a common motif called an α box (5, 11, 13). In *S. cerevisiae*, it has been shown that *MATα1* encodes a polypeptide that transcriptionally activates genes responsible for conferring α cell type, including the gene for α pheromone (MFα1) and the receptor for the **a** pheromone (*STE3*) (7). A high-mobility group (HMG) domain is present in *FPR1, mt a-1*, and *S. pombe Mc* polypeptides (5, 12, 13). Polypeptides that bear a HMG domain, but that are not involved in mating specificity, have been shown to bind DNA of putative target genes (27). Genetic and sequence analyses, plus the behavior of transformants, suggest that the products of *mt A-1, mt a-1, FMR1*, and *FPR1* function as transcriptional activators in *N. crassa* and *P. anserina* to confer mating cell type. Potential target genes include those for the mating-specific pheromones and their receptors and genes involved in the fusion of cells with opposite mating type. In support of this hypothesis, what appears to be the *A*-specific pheromone gene has been cloned from *N. crassa*; the transcription of this gene requires

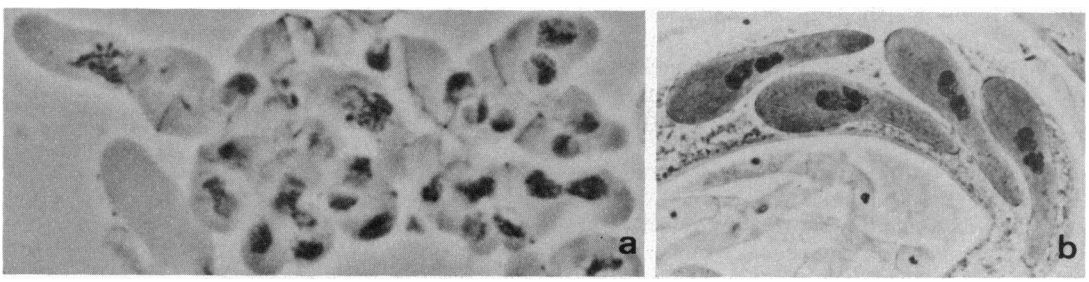

FIG. 3. Cytological analysis of perithecia in *P. anserina mat0 mt A-1** × *mat+* and *mat0 mt a-1** × *mat−* crosses. All crosses were performed by confronting mycelia of the transformant with the tester strain and by spermatizing. (*a*) *mat0 mt A-1** × *mat+* cross showing abnormal grouping of croziers blocked at caryogamy. (*b*) *mat0 mt a-1** × *mat−* cross showing wild-type ascus with four ascospores. (×720.)

a functional *mt A-1* product (T. Randall and R. L. Metzenberg, unpublished results).

The filamentous ascomycete *C. heterostrophus* is a distant relative of *N. crassa* and *P. anserina*. Preliminary experiments show that the introduction of the *C. heterostrophus* mating-type sequences *MAT1-1* and *MAT1-2* into a *P. anserina mat0* strain confers fertilization functions of *mat*− and *mat*+, respectively (S.A., unpublished results). The *MAT1-1* ORF contains an α box similar to *mt A-1* and *FMR1*, and *MAT1-2* ORF contains a HMG domain similar to *mt a-1* and *FPR1*. These results, and those included in this paper, suggest that the conserved motifs represent a functional polypeptide domain that is appropriate for mating-type control in the evolution of the filamentous ascomycetes. This suggests that the mating-type idiomorphs themselves are not a barrier to cross-species mating specificity, but that differences in target genes may result in reproductive isolation. A comparative analysis of the various mating-type regulators will provide valuable information about the conservation of fertilization functions and their regulation of the events associated with mating.

***P. anserina FPR1* and *FMR1* Genes Are Not Functional Counterparts of *N. crassa mt A-1* and *mt a-1* Genes with Respect to Vegetative Incompatibility.** When *mt A-1* and *mt a-1* were introduced into a *P. anserina* background, mating-type-associated incompatibility was not induced. This suggests that *P. anserina* contains a suppressor similar to *tol* in *N. crassa*. In *Neurospora tetrasperma*, like *P. anserina*, opposite mating-type nuclei normally coexist in heterokaryotic vegetative hyphae. By genetic introgression experiments, it was determined that the tol^+ gene is absent or inactive in *N. tetrasperma* (28). In contrast to *mt A-1* and *mt a-1*, an incompatibility reaction was not elicited when *FMR1* and *FPR1* were introduced into *N. crassa* tol^+ strains. These observations indicate a functional difference between *FMR1/mt A-1* and *FPR1/mt a-1* sequences with respect to vegetative incompatibility, rather than the action of an unlinked suppressor, such as *tol*. A comparison of *FMR1* and *mt A-1* shows that 106 of the first 196 amino acids are identical between the two ORFs; the carboxyl-terminal portions of the putative polypeptides are dissimilar (13). A comparison of amino acid sequence between *mt a-1* and *FPR1* ORFs also shows that the carboxyl region is variable between the two polypeptides (13). This suggests that these regions of dissimilarity between *mt a-1/FPR1* and *mt A-1/FMR1* may function to regulate the incompatibility response.

Postfertilization Functions Encoded by the Idiomorphs Are Not Fully Conserved Between *N. crassa* and *P. anserina*. In *P. anserina* and *N. crassa*, the *mt A-1*, *mt a-1* and the *FPR1*, *FMR1* transformants do not (or very seldom) complete events associated with meiosis and ascosporogenesis. This was expected since *mt a-1*, *mt A-1*, *FPR1*, and *FMR1*, in their own context, are responsible for mating identity and perithecium induction but are not sufficient for ascus production. In *P. anserina*, two genes controlling postfertilization events are present in the upstream region of *FMR1* (R. Debuchy, S.A., and G. Lecellier, unpublished results), and a sequence in addition to the fragment that confers *mat*+ specificity improves fertility (13). By mutational analyses, an additional region has also been identified in the *A* idiomorph of *N. crassa* that is required for productive ascospore formation (19). An initial sequence comparison between *mat*− and *A* indicates that the region required for ascospore formation may be similar between the two idiomorphs (29).

When the *mat*+ or *mat*− idiomorphs are introduced into a *P. anserina mat0* strain, transformants behave as a *mat*+ or *mat*− reference strain, respectively, and produce fertile perithecia containing ascospores when mated with the opposite mating type. Thus, *mat*+ and *mat*− can be expressed fully even in an ectopic position in a *mat0* strain (E. Coppin, S.A., and V. Contamine, unpublished results). In contrast, when the *A* or *a* idiomorph was introduced in a *P. anserina mat0* strain, only the fertilization function was expressed; perithecial development was initiated, but ascospore formation was absent or aberrant. Two hypotheses can account for these data. (*i*) The postfertilization functions of the idiomorphs have not been conserved in evolution between these two fungi. (*ii*) The *N. crassa A* and *a* idiomorphs cannot be expressed fully in an ectopic position in *P. anserina*. The sporulation defect of *mat*− or *mat*+ *N. crassa* transformants could be explained either by the ectopic position of the transgenic mating type or by the presence of both mating-type sequences within a single nucleus. The resident idiomorph can exert a dominant effect on a transgenic one, as observed in *P. anserina* (4). The construction of genetic chimeras between *A/mat*− and *a/mat*+ and the introduction of these constructs into a *mat0* strain may help to resolve these issues concerning postfertilization functions.

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