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Report

Mating Type and the Genetic Basis of Self-Fertility in the Model Fungus *Aspergillus nidulans*

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Summary

Sexual reproduction occurs in two fundamentally different ways: by outcrossing, in which two distinct partners contribute nuclei, or by self-fertilization (selfing), in which both nuclei are derived from the same individual. Selfing is common in flowering plants, fungi, and some animal taxa [1–5]. We investigated the genetic basis of selfing in the homothallic fungus *Aspergillus nidulans*. We demonstrate that alpha and high-mobility group domain mating-type (*MAT*) genes, found in outcrossing species, are both present in the genome of *A. nidulans* and that their expression is required for normal sexual development and ascospore production. Balanced overexpression of *MAT* genes suppressed vegetative growth and stimulated sexual differentiation under conditions unfavorable for sex. Sexual reproduction was correlated with significantly increased expression of *MAT* genes and key genes of a pheromone-response MAP-kinase signaling pathway involved in heterothallic outcrossing. Mutation of a component MAP-kinase *mpkB* gene resulted in sterility. These results indicate that selfing in *A. nidulans* involves activation of the same mating pathways characteristic of sex in outcrossing species, i.e., self-fertilization does not bypass requirements for outcrossing sex but instead requires activation of these pathways within a single individual. However, unlike heterothallic species, aspects of pheromone signaling appeared to be independent of *MAT* control.

Results and Discussion

Self-fertilization is a common phenomenon in flowering plants, fungi, and certain animal taxa and is thought to confer ecological benefits, such as reproductive assurance, in areas where compatible mating partners are scarce, and maintenance of well-adapted genotypes [1–4]. It is generally believed that self-fertilization has evolved from ancestral outcrossing systems [1, 2, 6–8]. Although much is understood about the genetic controls and signaling pathways involved with outcrossing in plants and fungi, surprisingly little is known about the genetics and cellular basis of self-fertilization [9–11]. Main insights have come from studies in plant species in which mutations in self-incompatibility systems give rise to self-fertility [1, 2], from yeasts in which silent gene cassettes enable self-fertility through mating-type conversion [9, 12, 13], from filamentous fungi in which linkage or fusion of *MAT* genes is associated with self-compatibility [6, 7], and from the pathogen *Cryptococcus neoformans* in which sexual reproduction can occur between partners of the same mating type [14].

In the present study, we investigated the genetic basis of self-fertility in the ascomycete fungus *Aspergillus nidulans* (teleomorph *Emericella nidulans*). Here, individual isolates, composed of hyphae originating from a uninucleate haploid spore, are able to complete the sexual cycle under suitable environmental conditions [15–17]. Nuclear fusion occurs within reproductive structures termed "cleistothecia," which are surrounded by specialized "Hülle" cells, with the diploid zygote undergoing meiotic division to yield haploid ascospores [17–19]. Ascomycete fungi provide a good model system because much is known about the genetic basis of mating pathways in heterothallic (obligately outcrossing) species [6, 11, 13, 20]. In filamentous species, "mating-type" genes act as master regulators of sexual reproduction conferring sexual identity for mating (processes leading to fusion of isolates of opposite mating type) and are also thought to be involved with later stages of sexual development and nuclear fusion [6, 11, 20–23].

Identification of Mating-Type Genes in *A. nidulans*

Mating-type (*MAT*) genes have been cloned from hemiascomycete yeast and euascomycete filamentous fungal species. They encode proteins with DNA-binding motifs, consistent with a role as transcriptional activators [10]. Highly dissimilar regions of DNA termed "idiomorphs," containing one to three *MAT* genes, have been identified in isolates of opposite mating type in heterothallic species [6]. By convention, *MAT1-1* (abbreviated *MAT-1*) isolates contain a *MAT* gene encoding a conserved alpha (α)-domain protein, whereas *MAT1-2* (abbreviated *MAT-2*) isolates contain a *MAT* gene encoding a conserved class of high mobility group (HMG)-domain protein [24]. Gene-disruption studies have shown that *MAT* genes are required for normal sexual development in heterothallic species [6].

Intriguingly, mating-type genes have also been found in homothallic (self-fertile) euascomycetes. Some

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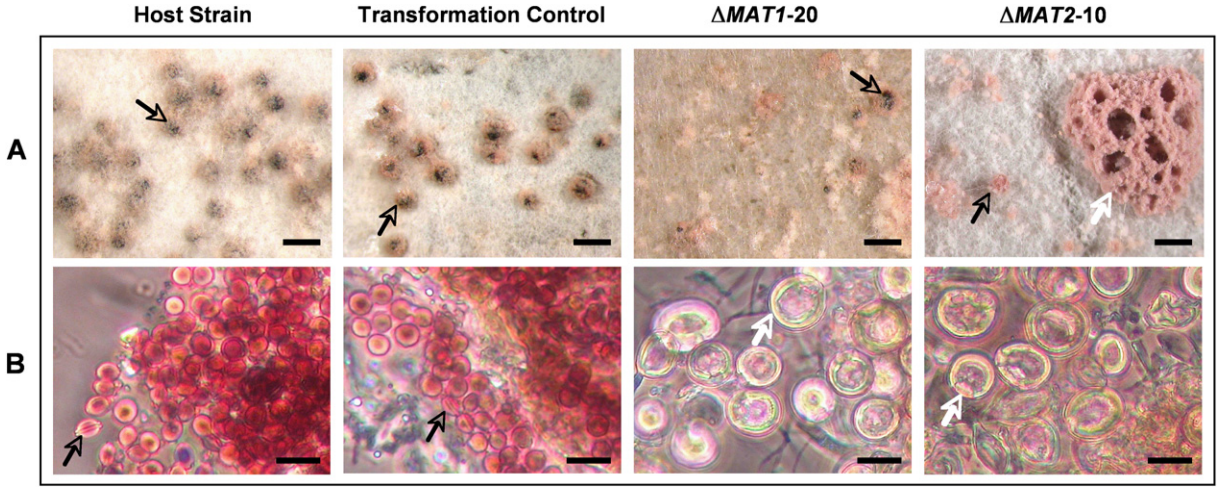

Figure 1. Developmental Features of Control and Δ*MAT* Gene Deletion Strains

Panels show growth after 14 days on ACM (supplemented with 10 mM uracil and uridine) in the dark at 28°C of host (2–155) and transformation (PG Con2) control strains and Δ*MAT1*-20 and Δ*MAT2*-10 gene deletion/replacement strains.

(A) Colony appearance showing dark-shelled cleistothecia (black arrow) covered by salmon-gold colored Hülle cells. Also note macroscopic aggregation of Hülle cells (white arrow) in the Δ*MAT2*-10 strain. Scale bars indicate 200 μm.

(B) Squashes of cleistothecia and adherent Hülle cells showing prolific red-colored ascospore production (black arrow) in control strains or thick-walled Hülle cells alone (white arrow) and showing no evidence of ascospore formation in gene deletion strains. Scale bars indicate 10 μm.

species have both α- and HMG *MAT* genes present, either fused together, linked in the same region, or at separate loci [6–8, 25]. Other species may have only an α-domain gene [6]. *MAT* genes from homothallic species appear functional in heterothallic species [6, 7]. It has been proposed that *MAT* genes may be involved in sexual development of homothallic species [6, 20], although a requirement has only been demonstrated experimentally for *Gibberella zeae* and *Sordaria macrospora* [26, 27].

By using conventional molecular approaches, we identified a 6.1 kb region from *A. nidulans* isolate 2-1 containing a putative *MAT* gene encoding a 318 amino acid HMG protein (Figures S1 and S2 in the Supplemental Data available online). During the course of this work, genome sequence data were released for *A. nidulans* isolate FGSC A4 revealing an identical HMG *MAT* sequence [28]. As noted in a preliminary report [29], we also detected a putative α-domain *MAT* gene, encoding a 361 amino acid polypeptide. A full description of cloning, RACE-PCR, sequence analysis of *MAT* genes, and corresponding proteins is presented in the Supplemental Data. The α- and HMG *MAT* genes mapped to *A. nidulans* chromosome 6 and 3, respectively (Figure S2). In accordance with standard nomenclature ([24]; B.G. Turgeon, personal communication), the α-domain gene was designated *MAT1-1* (abbreviated *MAT1*), and the HMG gene was designated *MAT2-1* (abbreviated *MAT2*). Unlinked *MAT* loci have also recently been detected in the related homothallic *Neosartorya fischeri* [25].

Functional Characterization of Mating-Type Genes

In heterothallic species, isolates of complementary *MAT-1* and *MAT-2* genotype are required for completion of the sexual cycle. MAT-1 and MAT-2 proteins have been shown to interact [30], possibly explaining the need for complementary *MAT* genes. The fact that *A. nidulans* contained both α and HMG *MAT* genes within the same genome provided a possible explanation for self-fertility. Three approaches were used to assess the functionality of *MAT* genes in *A. nidulans*.

First, *MAT1* and *MAT2* were individually deleted by gene replacement (Supplemental Data) [31]. A total of 40 putative *MAT1* and 37 putative *MAT2* deletion mutants were obtained, all of which showed defects in sexual development. Two *MAT1* (Δ*MAT1*-9 and Δ*MAT1*-20) and two *MAT2* (Δ*MAT2*-10 and Δ*MAT2*-11) transformants were chosen for further study. There was no discernable effect on vegetative growth or asexual sporulation. Regarding sexual development, both Δ*MAT1* mutants formed Hülle cells and cleistothecia under conditions inducing the sexual cycle (Figure 1), but cleistothecia were lower in number and smaller than those of control strains (Table 1). Critically, cleistothecia were sterile—being devoid of ascospores, with only a granular amorphous mass visible within. Similarly, the Δ*MAT2* mutants formed Hülle cells and cleistothecia under sexual conditions, although numbers of cleistothecia were again significantly lower than controls (~11%; Table 1), and both exhibited occasional proliferation of Hülle cells that aggregated to form ornate macroscopic structures (Figure 1). Cleistothecia were again sterile. Complementation of the *MAT*-gene deletants was achieved by crossing Δ*MAT1*-20 and Δ*MAT2*-10, resulting in the occasional formation of cleistothecia containing ascospores, as observed in outcrossing of *G. zeae MAT* mutants [26].

Second, an antisense RNA approach was used to disrupt *MAT2* gene function [32]. Transformants exhibited significantly delayed and less abundant sexual development compared to control strains, although there was no clear reduction in *MAT2* mRNA levels (Figure S3).

Table 1. Numbers and Size of Cleistothecia formed by Host 2–155 and Transformation Control PG Con2 Strains and Δ*MAT1* and Δ*MAT2* Gene Deletion Strains

Strain	Cleistothecia (per cm^2)[a,b,c]	Cleistothecia diameter (μm)[a,c,d]
2-155	332.4 ± 22.1 a	171.0 ± 6.4 a
PG Con2	314.2 ± 17.4 a	162.0 ± 4.5 a
Δ*MAT1*-9	265.3 ± 20.7 b	145.0 ± 9.8 b
Δ*MAT1*-20	256.9 ± 22.5 b	120.0 ± 7.5 b
Δ*MAT2*-10	34.0 ± 3.4 c	146.0 ± 12.2 b
Δ*MAT2*-11	37.2 ± 2.8 c	134.5 ± 7.0 b

[a] Cleistothecia, defined as dark-walled spheres >70 μm diameter for scoring purposes, produced after incubation on ACM (sealed plates supplemented with 10 mM uracil and uridine) in the dark at 28°C for 14 days.
[b] Mean of 12 replicates ± SEM. Strains show a significant ($p < 0.01$) variation in number of cleistothecia (one-way ANOVA on log-transformed data: $F = 116.3$; D.F. 5, 66).
[c] Values in the same column followed by the same letter do not differ significantly ($p < 0.05$) according to SPSS contrast test.
[d] Mean of 20 replicates ± SEM. Strains show a significant ($p < 0.01$) variation in diameter of cleistothecia (one-way ANOVA on Welch-corrected variance: $F = 7.5$; D.F. 5, 52).

Finally, *MAT1* and *MAT2* were overexpressed under the control of an inducible *alcA* promoter, either individually (OvEMAT1 and OvEMAT2 strains) or together (OvEMAT1+2 strain) (Supplemental Data). On ACM, all transformants grew as the control strain after point or confluent inoculations, completing the sexual cycle by producing fertile cleistothecia. However, differing results were obtained when transformants were grown on *alcA*-inducing media, which does not normally support sexual development. After point inoculations, OvEMAT1 and OvEMAT2 replicates grew at the same rate as the control, but they failed to produce cleistothecia or Hülle cells. In contrast, point-inoculated OvEMAT1+2 transformants showed no growth. When transformants were initially grown on cellophane sheets on ACM before transfer to the inducing medium, OvEMAT1, OvEMAT2, and the control continued vegetative growth after transfer but failed to produce cleistothecia or Hülle cells. In contrast, OvEMAT1+2 strains exhibited no further vegetative growth and instead produced cleistothecia containing ascospores from the mycelium already formed. RT-PCR confirmed overexpression of *MAT* genes (Figure S4).

These results are highly significant because they indicate that *MAT* genes, by definition involved in mating in outcrossing species, are also involved with sexual development in the self-fertile species *A. nidulans*. The gene-deletion studies demonstrated that both *MAT1* and *MAT2* are required for the production of sexual ascospores, coincident with a preliminary report of *MAT* deletion in *A. nidulans* [33]. Deletion of *MAT* genes in *G. zeae* and *S. macrospora* also rendered these homothallics self-sterile [26, 27], and *MAT*α locus gene(s) are linked to self fertility in *C. neoformans* [34]. At least 40 additional genes are required for, or correlated with, sex in *A. nidulans* (see recent review [17]). The observation that Hülle cells and sterile cleistothecia were formed even in the absence of *MAT* genes indicates that they act downstream of key initiators of sexual development such as *VeA*, *NsdD*, and *StuA* [17]. Given the requirement of *MAT* genes for ascospore production in *A. nidulans*, it may be speculated that *MAT* expression contributes to nuclear identity within homothallic species, as suggested for heterothallic species [20, 35]. This might be one factor involved in "relative heterothallism," whereby some self-fertile isolates exhibit preferential outcrossing [18, 36]. *MAT2* might also have a role in regulating Hülle-cell development, given the formation of abnormal Hülle aggregates in the Δ*MAT2* strains.

The overexpression studies showed that *MAT* gene expression at abnormally high levels resulted in suppression of vegetative growth and stimulation of sexual differentiation under conditions unfavorable for sex. Similar vegetative-growth arrest at the onset of sex occurs in yeast [22, 37], and *MAT* gene overexpression has been shown to repress growth or be lethal in heterothallic *P. anserina* [35]. Growth suppression and sexual stimulation occurred only in OvEMAT1+2, suggesting that balanced expression of *MAT1* and *MAT2* genes is required for normal sexual reproduction.

Pheromone-Response MAP-Kinase Signaling Pathway

A key role of *MAT* genes in heterothallic species is to regulate expression of a pheromone-signaling system involved with detection of a mating partner [11, 23, 37]. This pathway has been best characterized in *Saccharomyces cerevisiae*, in which mating-type-dependant expression of pheromone-precursor and receptor genes is observed [37]. Binding of diffusible peptide pheromones to cognate receptors triggers G protein-mediated signal transmission through a mitogen-activated protein (MAP) kinase cascade ultimately targeting a homeodomain transcription factor [22, 37]. The result is two-fold: cell-cycle arrest and activation of mating-specific genes including those encoding proteins required for nuclear-membrane fusion [11, 37, 38]. Although less is known about signaling pathways in heterothallic euascomycetes, components similar to those in yeasts are highly conserved across fungal taxa [10, 11, 39]. Genes encoding α- and a-factor-like pheromone precursors and receptors have been characterized from filamentous species, with evidence of mating-type-dependent expression [23] and a role in mate recognition [40]. Meanwhile, various components of G protein-linked MAP-kinase cascades are essential for sexual development in *Magnaporthe*, *Cryphonectria*, and *Neurospora* species [11, 41]. Elements of the pheromone-signaling pathway have also been detected in homothallic species, most notably in *A. nidulans* (see review article [17]).

In the present study, we exploited newly available *A. nidulans* genome data to assess the concurrent expression of genes with possible involvement in a pheromone-response pathway during sexual development of a self-fertile species. RNA was extracted from 4-, 7-, or 10-day-old cultures, and semiquantitative RT-PCR was performed. Expression of genes was barely detectable from unsealed plates (Figure 2), indicating low-level expression under conditions favoring vegetative growth and asexual conidiation. In contrast, strong expression of *MAT1* and *MAT2* and all genes of the pheromone-response pathway was detected at all time points in

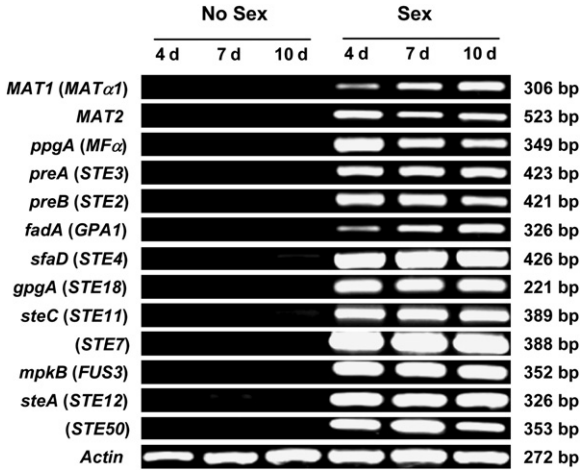

Figure 2. Expression of Genes of the Pheromone-Response Pathway

Semiquantitative RT-PCR showing expression of *MAT* genes and representative genes of the pheromone-response pathway of *A. nidulans*. The name of the *A. nidulans* gene (where identified) is shown to left-hand side with equivalent gene from *S. cerevisiae* in parentheses. Cultures were grown for 4, 7, or 10 days in the dark at 28°C on ACM unsealed ("No Sex") or sealed ("Sex") plates.

extracts from sealed plates, where sexual development was occurring because of restricted air exchange (Figure 2) [15, 17]. This included increased expression of a putative α-pheromone precursor (*ppgA*) [29], both a- and α-factor-like pheromone receptors (*preA* and *preB* respectively [29], later cotermed *gprB* and *gprA* by Seo et al. [42]), the subunits of a heterotrimeric G protein (*fadA*, *sfaD*, and *gpgA*) [43], three kinases of the MAP kinase cascade (*steC* [44], *STE7* equivalent, and *mpkB* [homolog of *FUS3* in *S. cerevisiae*]), a protein kinase regulator (*STE50* equivalent), as well as the target homeodomain transcription factor (*steA*) [45]. Expression of the actin control gene remained constant. Results were consistent with individual studies of *gprA* (*preB*) and *gprB* (*preA*) expression [42].

On the basis of the increase in *mpkB* transcription during sexual development, we further characterized the possible role of this MAP-kinase regulatory gene in cleistothecial formation in *A. nidulans* by generating an *mpkB* loss-of-function mutant. The Δ*mpkB* mutant showed no apparent change in asexual conidiation. However, a complete block in sexual reproduction was evident with no Hülle cells or cleistothecia observed in the Δ*mpkB* mutant after 15 days' growth, unlike control strains (Figure 3). Complementation with the wild-type allele restored sexual development in the mutant. The Δ*mpkB* phenotype bore some resemblance to the *A. nidulans steC* mutant regarding sexual development [44].

The data described are of significance because a definite correlation was evident between expression at a high level of genes known to be involved with sex in outcrossing heterothallic species and sexual development in the self-fertile *A. nidulans*. In parallel, fruiting between same-sex partners in *C. neoformans* also requires activation of the pheromone-response pathway [14], and pheromone-precursor and receptor genes are required for sex in homothallic *S. macrospora* [46]. The observed expression changes could also be a response to plate sealing unrelated to sex, although this is unlikely given the specificity of signaling to sexual induction and anastomosis [11, 37].

MAT Gene Deletion and Overexpression and Pheromone Signaling

Expression of pheromone precursors and most probably receptors is controlled by *MAT* genes in heterothallic species [23, 40]. Gene expression of the pheromone pathway was therefore investigated in the *MAT* gene deletion and overexpression strains (Figures S4 and S5). Greatly reduced expression of the remaining *MAT* gene was observed in Δ*MAT1* and Δ*MAT2* strains compared to controls. However, no other clear differences were apparent, except possibly increased *ppgA* expression in the Δ*MAT2* mutant (Figure S5). Meanwhile, no clear increase in pheromone-precursor/-receptor gene expression was observed after transfer to inducing media in any overexpression strains despite greatly increased *MAT* gene expression (Figure S4). The apparent lack of requirement for, and induction by, *MAT* genes suggests that other factors may also control pheromone signaling in *A. nidulans*. Thus, the pathway may be activated under environmental conditions favorable for sex, with such conditions leading to a pheromone-response signal similar to that seen in heterothallic species, but without the requirement for a compatible mate. This might in turn stimulate *MAT* expression, consistent with an *S. pombe* Ste11-binding site (a likely functional equivalent of *A. nidulans* SteA) [47] in the *MAT1* and *MAT2* promoter regions (Supplemental Data). *MAT* gene expression may therefore be primarily required for later stages of sexual development (e.g., nuclear identity [20, 35]), a possible adaptation for homothallism.

Conclusions

We have obtained results indicating that mating-type-gene activity is required for normal sexual development of *A. nidulans* and that sexual differentiation is correlated with greatly increased expression of pheromone-response-pathway genes known to be involved with mating in heterothallic species. We along with other researchers have shown that elements of this pathway (*gprA* [*preB*], *gprB* [*preA*], *fadA*, *sfaD*, *gpgA*, *steC*, *steA*, and *mpkB* [this work]) are essential for normal sexual fertility in *A. nidulans*, although pleiotropic effects are sometimes evident and a requirement may be overcome in forced heterokaryons [17, 42–45]. Taken as a whole, these results provide evidence that selfing in *A. nidulans* involves activation of the same mating pathways characteristic of sex in obligately outcrossing species, from which self-fertile species are thought to have evolved; i.e., self-fertilization does not bypass the normal requirements for sexual signaling between partners but instead requires activation of these pathways within a single individual isolate. Our data also suggest that modifications have occurred in the control of expression of genes involved in sexual signaling as a possible adaptation to homothallism. These results from a model fungal system may provide insights into signaling processes that occur in self-fertilizing plant and animal taxa. The precise cellular locations of these pathways

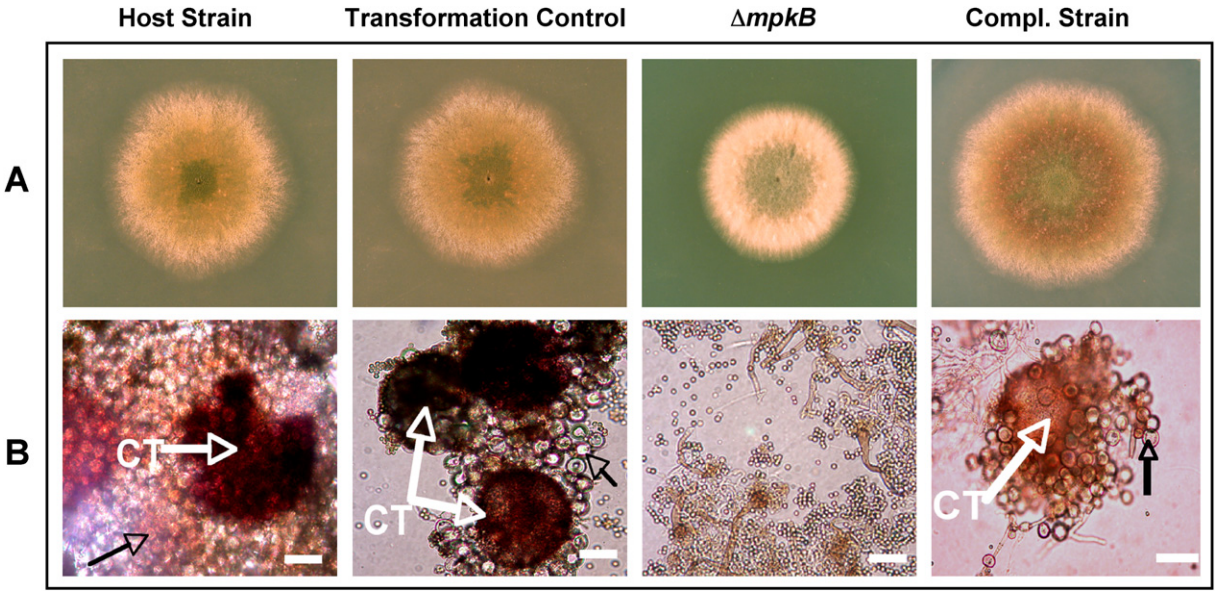

Figure 3. Role of *mpkB* in Sexual Development

The host (TNO2A7), transformation control (TNK7.3.7), Δ*mpkB* mutant (TNK7.3.6), and complementation strains were point inoculated on GMM in the dark at 37°C to demonstrate: (A) colony morphologies after 5 days' growth (unsealed plates) and (B) the presence or absence of cleistothecia ("CT," white arrows) and Hülle cells (black arrows) after 15 days' growth (scale bars indicate 30 μm).

and the nature of interaction(s) with proteins involved in regulating sexual reproduction in *A. nidulans* now remain to be determined.

Supplemental Data

Additional Discussion, Experimental Procedures, and five figures are available at http://www.current-biology.com/cgi/content/full/17/16/1384/DC1/.

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References

1. Nasrallah, M.E., Liu, P., Sherman-Broyles, S., Boggs, N.A., and Nasrallah, J.B. (2004). Natural variation in expression of self-incompatibility in *Arabadopsis thaliana*: Implications for the evolution of selfing. Proc. Natl. Acad. Sci. USA *101*, 16070–16074.
2. Shimizu, K.K., Cork, J.M., Caicedo, A.L., Mays, C.A., Moore, R.C., Olsen, K.M., Ruzsa, S., Coop, G., Bustamante, C.D., Awadalla, P., et al. (2004). Darwinian selection on a selfing locus. Science *306*, 2081–2084.
3. Burnett, J.H. (2003). Fungal populations and species (Oxford: Oxford University Press).
4. Murtagh, G.J., Dyer, P.S., and Crittenden, P.D. (2000). Sex and the single lichen. Nature *404*, 564.
5. Barriére, A., and Félix, M.A. (2005). High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. Curr. Biol. *15*, 1176–1184.
6. Debuchy, R., and Turgeon, B.G. (2006). Mating-type structure, evolution, and function in Euascomycetes. In The Mycota I: Growth, Differentiation and Sexuality, U. Kües and R. Fischer, eds. (Berlin: Springer-Verlag), pp. 293–323.
7. Yun, S.-H., Berbee, M.L., Yoder, O.C., and Turgeon, B.G. (1999). Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proc. Natl. Acad. Sci. USA *96*, 5592–5597.
8. Inderbitzin, P., Harkness, J., Turgeon, B.G., and Berbee, M.L. (2005). Lateral transfer of mating system in *Stemphylium*. Proc. Natl. Acad. Sci. USA *102*, 11390–11395.
9. Hiscock, S.J., and Kües, U. (1999). Cellular and molecular mechanisms of sexual incompatibility in plants and fungi. Int. Rev. Cytol. *193*, 165–295.
10. Casselton, L.A. (2002). Mate recognition in fungi. Heredity *88*, 142–147.
11. Lengeler, K.B., Davidson, R.C., D'Souza, C., Harashima, T., Shen, W.C., Wang, G.P., Pan, X., Waugh, M., and Heitman, J. (2000). Signal transduction cascades regulating fungal development and virulence. Microbiol. Mol. Biol. Rev. *64*, 746–785.
12. Haber, J.E. (1998). Mating-type gene switching in *Saccharomyces cerevisiae*. Annu. Rev. Genet. *32*, 561–599.
13. Butler, G., Kenny, C., Fagan, A., Kurischko, C., Gaillardin, C., and Wolfe, K.H. (2004). Evolution of the MAT locus and its Ho endonuclease in yeast species. Proc. Natl. Acad. Sci. USA *101*, 1632–1637.
14. Lin, X., Hull, C.M., and Heitman, J. (2005). Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. Nature *434*, 1017–1021.
15. Braus, G.H., Krappman, S., and Eckert, S.E. (2002). Sexual development in ascomycetes. Fruit body formation of *Aspergillus nidulans*. In Molecular Biology of Fungal Development, H.D. Osiewacz, ed. (New York: Marcel Dekker), pp. 215–244.
16. Blumenstein, A., Vienken, K., Tasler, R., Purschwitz, J., Veith, D., Frankenberg-Dinkel, N., and Fischer, R. (2005). The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. Curr. Biol. *15*, 1833–1838.
17. Dyer, P.S. (2007). Sexual reproduction and significance of MAT in the Aspergilli. In Sex in Fungi: Molecular Determination and Evolutionary Implications, J. Heitman, J. Kronstad, J. Taylor, and L. Casselton, eds. (Washington, D.C.: ASM Press), in press.
18. Pontecorvo, G. (1953). The genetics of *Aspergillus nidulans*. Adv. Genet. *5*, 142–238.

19. Sohn, K.T., and Yoon, K.S. (2002). Ultrastructural study on the cleistothecium development in *Aspergillus nidulans*. Mycobiology *30*, 117–127.
20. Shiu, P.K.T., and Glass, N.L. (2000). Cell and nuclear recognition mechanisms mediated by mating type in filamentous ascomycetes. Curr. Opin. Microbiol. *3*, 183–188.
21. Fraser, J.A., Diezmann, S., Subaran, R.L., Allen, A., Lengeler, K.B., Dietrich, F.S., and Heitman, J. (2004). Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. PLoS Biol. *2*, 2243–2255.
22. Elion, E.A. (2000). Pheromone response, mating and cell biology. Curr. Opin. Microbiol. *3*, 573–581.
23. Coppin, E., de Renty, C., and Debuchy, R. (2005). The function of the coding sequences for the putative pheromone precursor in *Podospora anserina* is restricted to fertilization. Eukaryot. Cell *4*, 407–420.
24. Turgeon, B.G., and Yoder, O.C. (2000). Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet. Biol. *31*, 1–5.
25. Rydholm, C., Dyer, P.S., and Lutzoni, F. (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.
26. Lee, J., Lee, T., Lee, Y.W., Yun, S.H., and Turgeon, B.G. (2003). Shifting fungal reproductive mode by manipulation of mating type genes: Obligatory heterothallism of *Gibberella zeae*. Mol Microbiol. *50*, 145–152.
27. Pöggeler, S., Nowrousian, M., Ringelberg, C., Loros, J.J., Dunlap, J.C., and Kück, U. (2006). Microarray and real-time PCR analyses reveal mating type-dependent gene expression in a homothallic fungus. Mol. Genet. Genomics. *275*, 492–503.
28. Galagan, J., Calvo, S.E., Cuomo, C., Ma, L.-J., Wortman, J., Batzoglou, S., Lee, S.-I., Bastürkmen, M., Spevak, C.C., Clutterbuck, J., et al. (2005). Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature *438*, 1105–1115.
29. Dyer, P.S., Paoletti, M., and Archer, D.B. (2003). Genomics reveals sexual secrets of *Aspergillus*. Microbiology *149*, 2301–2303.
30. Jacobsen, S., Wittig, M., and Pöggeler, S. (2002). Interaction between mating-type proteins from the homothallic fungus *Sordaria macrospora*. Curr. Genet. *41*, 150–158.
31. Nayak, T., Szewczyk, E., Oakley, C.E., Osmani, A., Ukil, L., Murray, S.L., Hynes, M.J., Osmani, S.A., and Oakley, B.R. (2006). A versatile and efficient gene-targeting system for *Aspergillus nidulans*. Genetics *172*, 1557–1566.
32. Agrawal, S., and Kandimalla, E.R. (2004). Role of Toll-like receptors in antisense and siRNA. Nat. Biotechnol. *22*, 1533–1537.
33. Miller, K.Y., Nowell, A., and Miller, B.L. (2005). Differential regulation of fruitbody development and meiosis by the unlinked *Aspergillus nidulans* mating type loci. Fungal Genetics Newsletter Supplement *52* (XXIII Fungal Genetics Conference), 184.
34. Lin, X., Huang, J.C., Mitchell, T.G., and Heitman, J. (2006). Virulence attributes and hyphal growth of *C. neoformans* are quantitative traits and the *MAT*α allele enhances filamentation. PLoS Genet. *2*, 1801–1814.
35. Coppin, E., and Debuchy, R. (2000). Co-expression of the mating-type genes involved in internuclear recognition is lethal in *Podospora anserina*. Genetics *155*, 657–669.
36. Hoffmann, B., Eckert, S.E., Krappman, S., and Braus, G.H. (2001). Sexual diploids of *Aspergillus nidulans* do not form by random fusion of nuclei in the heterokaryon. Genetics *157*, 141–147.
37. Banuett, F. (1998). Signalling in the yeasts: An informational cascade with links to the filamentous fungi. Microbiol. Mol. Biol. Rev. *62*, 249–274.
38. Beh, C.T., Brizzio, V., and Rose, M.D. (1997). KAR5 encodes a novel pheromone-inducible protein required for homotypic nuclear fusion. J. Cell Biol. *139*, 1063–1076.
39. Paoletti, M., Rydholm, C., Schwier, E.U., Anderson, M.J., Szakacs, G., Lutzoni, F., Debeaupuis, J.P., Latgé, J.P., Denning, D.W., and Dyer, P.S. (2005). Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr. Biol. *15*, 1242–1248.
40. Kim, H., and Borkovich, K.A. (2006). Pheromones are essential for male fertility and sufficient to direct chemotropic polarized growth of trichogynes during mating in *Neurospora crassa*. Eukaryot. Cell *5*, 544–554.
41. Krystova, S., and Borkovich, K.A. (2005). The heterotrimeric G-protein subunits GNG-1 and GNB-1 form a G$\beta\gamma$ dimer required for normal female fertility, asexual development, and Gα protein levels in *Neurospora crassa*. Eukaryot. Cell *4*, 365–378.
42. Seo, J.A., Han, K.H., and Yu, J.H. (2004). The *gprA* and *gprB* genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. Mol. Microbiol. *53*, 1611–1623.
43. Yu, J.H. (2006). Heterotrimeric G-protein signaling and RGSs in *Aspergillus nidulans*. J. Microbiol. *44*, 145–154.
44. Wei, H., Requena, N., and Fischer, R. (2003). The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol. Microbiol. *47*, 1577–1588.
45. Vallim, M.A., Miller, K.Y., and Miller, B.L. (2000). *Aspergillus* SteA (sterile12-like) is a homeodomain-C_2/H_2-Zn^{+2} finger transcription factor required for sexual reproduction. Mol. Microbiol. *36*, 290–301.
46. Mayrhofer, S., Weber, J.M., and Pöggeler, S. (2006). Pheromones and pheromone receptors are required for proper sexual development in the homothallic ascomycete *Sordaria macrospora*. Genetics *172*, 1521–1533.
47. Van Beest, M., Dooijes, D., Van de Wetering, M., Kjaerulff, S., Bonvin, A., Nielsen, O., and Clevers, H. (2000). Sequence-specific high mobility group box factors recognise 10–12 base pair minor groove motifs. J. Biol. Chem. *275*, 27266–27273.

Accession Numbers

DNA sequences have been deposited in GenBank under accessions DQ354692 and DQ354693 (*MAT1* 5′ and 3′ RACE-PCR products, respectively) and AF508279 (*MAT2*).