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DNA Sequence Characterization and Molecular Evolution of *MAT1* and *MAT2* Mating-Type Loci of the Self-Compatible Ascomycete Mold *Neosartorya fischeri*▽

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Degenerate PCR and chromosome-walking approaches were used to identify mating-type (*MAT*) genes and flanking regions from the homothallic (sexually self-fertile) euascomycete fungus *Neosartorya fischeri*, a close relative of the opportunistic human pathogen *Aspergillus fumigatus*. Both putative alpha- and high-mobility-group-domain *MAT* genes were found within the same genome, providing a functional explanation for self-fertility. However, unlike those in many homothallic euascomycetes (Pezizomycotina), the genes were not found adjacent to each other and were termed *MAT1* and *MAT2* to recognize the presence of distinct loci. Complete copies of putative *APN1* (DNA lyase) and *SLA2* (cytoskeleton assembly control) genes were found bordering the *MAT1* locus. Partial copies of *APN1* and *SLA2* were also found bordering the *MAT2* locus, but these copies bore the genetic hallmarks of pseudogenes. Genome comparisons revealed synteny over at least 23,300 bp between the *N. fischeri MAT1* region and the *A. fumigatus MAT* locus region, but no such long-range conservation in the *N. fischeri MAT2* region was evident. The sequence upstream of *MAT2* contained numerous candidate transposase genes. These results demonstrate a novel means involving the segmental translocation of a chromosomal region by which the ability to undergo self-fertilization may be acquired. The results are also discussed in relation to their significance in indicating that heterothallism may be ancestral within the *Aspergillus* section *Fumigati*.

Aspergillus fumigatus and *Neosartorya fischeri* are two closely related euascomycete molds (Eurotiomycetes, Pezizomycotina) in the genus *Aspergillus*, subgenus *Fumigati*, section *Fumigati* (12, 33, 36, 42). *A. fumigatus* is the most common cause of invasive aspergillosis infection in humans (8, 22) and is only known to reproduce by asexual means involving the production of masses of mitotic conidiospores. *N. fischeri* has also been documented as an invasive opportunistic pathogen (4, 24). However, unlike *A. fumigatus*, *N. fischeri* has a known sexual cycle and thus may reproduce by both asexual and sexual means (14, 26). Close relationships between predominantly asexual and readily sexually recombining species such as these are common among fungi (1, 2, 5, 19, 23, 37). A key challenge in present research is to understand the genetic changes that underlie such shifts in reproductive modes. It is hoped that studies may provide insights into the genetic basis of asexuality and possibly allow mating to be realized by supposedly asexual species. The ability to perform sexual crosses with *A. fumigatus* would provide researchers with a powerful tool to analyze the genetics of pathogenicity in this prevalent mold and would be informative for an understanding of the population biology of the species and disease management (9).

One key factor governing the sexual reproductive mode in fungi is the presence or absence of so called mating-type (*MAT*) genes. These are located within the genome at mating-type (*MAT*) loci and determine mating compatibility and incompatibility (3, 7). In euascomycetes, there is most commonly a single *MAT* locus (termed *MAT1*) (39). In obligately outbreeding heterothallic species, which require a partner to mate, two nonallelic versions of the locus, termed idiomorphs, are found in isolates with complementary mating types (15, 27). By convention, the two versions are termed *MAT1-1* and *MAT1-2* (abbreviated to *MAT-1* and *MAT-2* for convenience; for all heterothallic species, the nomenclature is like that of *A. fumigatus*) and are distinguished by the presence of an open reading frame (ORF) encoding a MAT protein with an alpha domain and a single ORF encoding a MAT protein with a high-mobility group (HMG) domain (16), respectively (40). These MAT proteins act as transcription factors to enable the sexual cycle (3, 43). Other ORFs may also be present at the idiomorphic *MAT* loci. In contrast, self-fertile homothallic species usually contain a single *MAT* locus with the alpha and HMG genes either fused together (as in *Cochliobolus homomorphus* [45]) or located in close proximity (e.g., ~1 kb apart in *Mycosphaerella zeae-maydis* [44]) (7). These arrangements presumably originated from a recombinational event mediating a mating system shift from heterothallism to homothallism (44, 45). To date, only two exceptions to this arrangement are known among homothallic euascomycetes. In *Cochliobolus cymbopogonis* and *Emericella* (*Aspergillus*) *nidulans*, both alpha and HMG *MAT* genes are present within the same genome but are unlinked (45, 10, 11). More in-depth genome analyses of *E. nidulans* have revealed that the alpha and HMG *MAT* genes are on separate chromosomes in this

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species (these genes are termed *MAT1* and *MAT2*, respectively, to recognize the different loci [10]; for all homothallic species [e.g., *E. nidulans*], the nomenclature is like that of *N. fischeri*), and it has been suggested that *E. nidulans* was derived from a homothallic ancestor (which contained both alpha and HMG genes at the same original locus) as a result of a translocating break (13). Intriguingly, *MAT* genes in certain asexual euascomycetes (i.e., species with no known sexual state) have also been reported, and the *MAT* loci have been characterized in detail in some instances (7, 32, 44).

Although there is no known sexual cycle in *A. fumigatus*, genes involved in mating have been identified in the genome (13, 31, 32, 34, 41) and the expression of both *MAT-1* and *MAT-2* genes, together with pheromone signaling genes, has been observed (32). The *MAT* locus arrangement in *A. fumigatus* is characteristic of a heterothallic species, with isolates having either a *MAT-1* or a *MAT-2* idiomorph present (32), which would hypothetically make it necessary for an *A. fumigatus* isolate to outcross for a successful mating to proceed. It has been suggested that *A. fumigatus* has also evolved from a homothallic ancestor which contained both alpha and HMG genes at the same original *MAT* locus and that this evolution involved the loss of *MAT* genes through segregation (13, 14), but this hypothesis remains unproven.

The aim of the present study was to determine the organization of *MAT* loci within *N. fischeri* in order to gain insights both into the genetic basis of the reproductive mode of *N. fischeri* compared to that of *A. fumigatus* and into the evolution of *MAT* loci and sexual reproductive modes among the aspergilli as a whole. Evidence from phylogenetic and genomic studies, together with the predominance of homothallism among the aspergilli, has lead to the suggestion that homothallism is the ancestral state of the aspergilli, with heterothallism being a derived characteristic (13, 14). However, it is also possible that heterothallism is the ancestral state, as evidenced by the presence of an idiomorph arrangement in *A. fumigatus* and *Aspergillus oryzae* and other asexual aspergilli (13, 32; M. Paoletti and P. S. Dyer, unpublished results). Indeed, reports elsewhere have provided convincing evidence of the evolution of homothallism from heterothallism (21, 45). Thus, it was of interest to determine whether *N. fischeri* had a genetic arrangement with two distinct *MAT1* and *MAT2* loci, similar to that of the homothallic *E. nidulans*, or a single *MAT1* locus containing both alpha and HMG domain-encoding *MAT* genes, consistent with an ancestral homothallic state. Alternatively, some other novel organization might support the more recent evolution of homothallism and would not contradict the hypothesis that the heterothallic arrangement is ancient.

This study therefore involved the identification and sequencing of mating-type and flanking genes from *N. fischeri* and a comparison of the DNA sequences to those of homologous regions from *A. fumigatus* and *E. nidulans*. Sequence data were also obtained from an ongoing *N. fischeri* genome-sequencing project (conducted by The Institute for Genomic Research [TIGR] in the United States and the Wellcome Trust in the United Kingdom). Arising data allowed for an assessment of the polarity of mating system evolution in the section *Fumigati* and provided insights into events relating to the divergence and speciation of the *A. fumigatus* and *N. fischeri* lineages.

MATERIALS AND METHODS

Strain used and DNA isolation. *N. fischeri* strain NRRL4075 was used for experimental work; it forms a monophyletic group with the type strain NRRL181 (34). The type strain NRRL181 is presently the focus of a genome-sequencing project by TIGR (Rockville, MD) and the Wellcome Trust Sanger Institute (Hinxton, United Kingdom), and so another strain was intentionally chosen for ready comparison to the project data. NRRL4075 was originally isolated in the United Kingdom from garden soil.

Genomic DNA was prepared from mycelium grown in liquid potato dextrose medium (12 g/liter) by using a protocol modified from that of Zolan and Pukkila (46). Lyophilized fragments were transferred with forceps to 1.5-ml tubes containing 500 μl of 1% sodium dodecyl sulfate extraction buffer. Mycelium was ground with a polypropylene micropestle and incubated at room temperature for 1 h. DNA was purified with phenol-chloroform (1:1, vol/vol) as described by Raeder and Broda (35). Next, 300 μl of chloroform-isoamyl alcohol (24:1, vol/vol) was added, and the solution was emulsified and centrifuged for 15 min. The DNA was precipitated from the aqueous phase by the addition of an equal volume of cold isopropanol. The pellet was washed with 70% ethanol, and the DNA was resuspended in 50 μl of water.

Degenerate PCR. Degenerate primers were used to amplify the alpha domain-encoding sequence from *MAT1* family genes (MAT5-6, 5′GIMGICCI YTIAAYWSITTYATHGC3′, and MAT3-4, 5′ARRAAICKIARIATICCISW YTT3′ [32]) and the HMG domain-encoding sequence from *MAT2* family genes (MAT5-4, 5′AARRTICCIMGICCICCIAAYGC3′, and MAT3-2, 5′T TNCKIGGIGTRTAITGRTARTCNGG3′, as published here). The primers were designed from regions of conserved sequences identified in *E. nidulans* and other closely related taxa (10, 11; Paoletti and Dyer, unpublished). The 50-μl PCR amplification mixtures contained 100 ng of genomic DNA, 100 ng of MAT5-6 and MAT4-3 or MAT5-4 and MAT3-2, 200 μM (each) deoxynucleoside triphosphates, and 1.25 U of DNA polymerase. Cycle parameters were 5 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 55°C for *MAT2* and 50°C for *MAT1*, and 1 min at 72°C; and a final 5 min at 72°C. Following PCR, samples were resolved by electrophoresis on 1% Tris-acetate-EDTA agarose gels, which were subsequently stained with SYBR green. PCR products were purified with the QiaQuick PCR purification kit according to the protocol of the manufacturer (QIAGEN). Amplicons of predicted sizes for the *MAT1* alpha-domain region and the *MAT2* HMG domain were cloned using a pCRII-TOPO TA cloning kit (Invitrogen).

Sequencing reactions were performed in a 10-μl final reaction volume containing 1 μl of BigDye (BigDye Terminator cycle sequencing kit; ABI PRISM, Perkin-Elmer, and Applied Biosystems), 3 μl of BigDye buffer, 1 μl of 10 mM primer, 2 μl of distilled water, and 3 μl of purified PCR product. Sequencing primers were the same as those used for PCR, and sequencing reactions were analyzed on an ABI 3730 DNA analyzer. Sequence fragments were assembled by using Sequencher 3.0 (Gene Code Corporations). Contigs obtained were then compared to gene and genome sequences present in the National Center for Biotechnology Information (NCBI) GenBank database by using the basic local alignment search tool (BLAST), specifically tblastx and blastn.

PCR and sequencing of *MAT*-flanking regions. Genomic flanking sequences of isolate NRRL4075 were obtained using the BD GenomeWalker universal kit (BD Biosciences Clontech). DNA libraries were prepared as recommended by the manufacturer by using the blunt-end-cutting restriction enzymes EcoRV, PvuII, StuIV, and XmnI. The PCR amplification procedure used BD Advantage 2 polymerase mix (BD Biosciences Clontech) with gene-specific primers and adaptor-specific primers according to PCR protocols supplied with the BD GenomeWalker universal kit (BD Biosciences Clontech). PCR, the sequencing protocol, and contig assembly were performed as outlined above. The short (~200-bp) DNA sequences obtained from degenerate PCR were used for primer design for the first walk. For subsequent walks, primers were designed from the sequence acquired by the previous walk. Overlap between walks enabled the assembly of the sequences obtained from all walks possible by using Sequencher 3.0 (Gene Code Corporations).

DNA and amino acid sequence comparisons. The sequences obtained for the *MAT1* alpha domain- and *MAT2* HMG domain-encoding genes of *N. fischeri* and each of their flanking genes were compared to sequences from the NCBI database and posted fungal genome project results for the species *A. fumigatus* (NCBI), *E. nidulans* (Broad Institute; http://www.broad.mit.edu, accessed 21 March 2006), and *N. fischeri* (NCBI), and the sequences were aligned by using

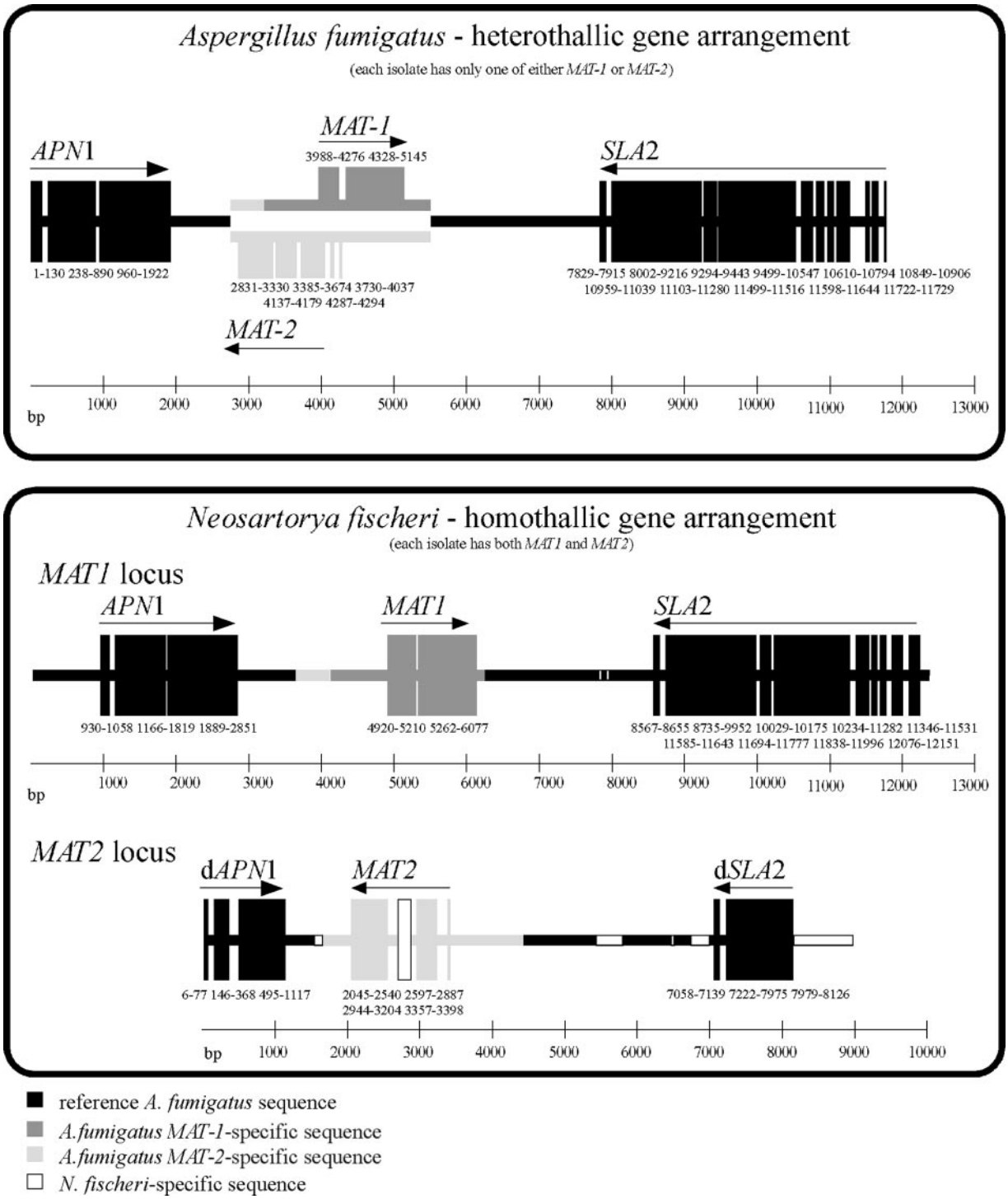

FIG. 1. DNA sequence arrangement of the *MAT* regions of *N. fischeri* and *A. fumigatus*, including *MAT* genes and their nearest flanking genes. The sequences obtained in this study for the *MAT1* and *MAT2* loci of *N. fischeri* were described and compared with sequences from *A. fumigatus*. Homologous sequences are indicated by different levels of shading, and coding regions are indicated below or above the corresponding boxes. Narrow sections of the maps represent noncoding sequences. The directions of the arrows indicate the predicted directions of gene translation.

MacClade 4.01 (25). Total DNA sequence similarity was estimated using the NCBI blast2seq tool. Sequence data for both *MAT1* and *MAT2* regions were extended by a further ~5,000 bp on either side, or as far as possible given limited data, by finding the regions of the genome project contigs corresponding to the regions sequenced in this study and extending the continuous sequence. For *A. fumigatus*, a 22,492-bp sequence from the strain Af293 (*MAT2*) from the genome project was compared individually to the *MAT1* and *MAT2* sequences of *N. fischeri*. For *E. nidulans*, a 16,280-bp sequence of *MAT1* and a 16,338-bp sequence of *MAT2* from the strain FGSCA4 from the genome project were compared to the *MAT1* and *MAT2* sequences of *N. fischeri* and *A. fumigatus*, respec-

tively. Dot plots for pairwise comparisons of the sequences were generated using the Colorado State University Web-based interactive nucleic acid dot plots program (http://www.vivo.colostate.edu/molkit/dnadot/).

Nucleotide sequence accession numbers. Sequence data obtained for *N. fischeri MAT1* and *MAT2* and their respective flanking regions have been deposited in GenBank under accession numbers EF363034 and EF370391.

RESULTS AND DISCUSSION

DNA sequences of *N. fischeri MAT* genes and identification of flanking regions. *N. fischeri* is readily self-fertile in culture, which is common for homothallic aspergilli (36). A key aim of the present study was to determine the genetic basis of homothallism in *N. fischeri*. Experimental work provided a DNA-based confirmation that mating-type genes encoding both putative alpha- and HMG-domain proteins are present within a single isolate of *N. fischeri*, providing an explanation for the homothallic breeding system since both types of protein are normally required for the sexual development of euascomycete fungi to occur (3). Homothallic euascomycete species most frequently have both alpha- and HMG-domain *MAT* genes tightly linked at the same locus (7). In *N. fischeri*, however, these *MAT* genes were instead found at two distinct loci, here termed *MAT1* and *MAT2* for the alpha- and HMG-domain-encoding genes, respectively (Fig. 1) (40). Successive genome-walking steps were used to identify a 12,300-bp sequence comprising the *MAT1* gene and flanking regions and a 9,001-bp sequence comprising the *MAT2* gene and flanking regions (Fig. 1). *MAT1* encodes a putative 360-amino-acid protein in two exons and *MAT2* encodes a putative 363-amino-acid protein in four exons (Fig. 1). The DNA sequence of *MAT1* of *N. fischeri* is 92% similar to that of the homologue from *A. fumigatus* characterized by Paoletti et al. (32), and *MAT2* of *N. fischeri* shows 84 to 87% similarity to the *A. fumigatus* homologue identified by genome studies (13, 31) (Table 1).

Sequence data obtained for NRRL4075 were compared with genome project sequence data for strain NRRL181 of *N. fischeri*. The alignment of contigs allowed for the identification of further sequences flanking the *MAT* regions. A total of 22,301 bp of the sequence of the *MAT1* region and 14,988 bp of the sequence of the *MAT2* region was obtained in this manner.

Analysis of *N. fischeri MAT1*- and *MAT2*-flanking regions. BLAST analysis of the flanking regions of *N. fischeri MAT1* revealed the presence of putative DNA lyase (*APN1*) (38) and cytoskeleton assembly control (*SLA2*) (18) genes upstream and downstream of *MAT1*, respectively (Fig. 1). Both genes appeared to encode functional proteins. BLAST analysis of the flanking regions of *N. fischeri MAT2* also revealed the presence of fragments of DNA lyase and cytoskeleton assembly control genes adjacent to *MAT2*. However, these fragments of *APN1* and *SLA2* contained multiple stop codons and frameshift insertions and deletions (from 1 to 46 nucleotides in size), indicative of nonfunctional pseudogenes. Therefore, the loci were termed d*APN1* and d*SLA2* to recognize the disabled ORFs (17) (Fig. 1).

One of or both *APN1* and *SLA2* have been reported to neighbor *MAT* loci in lineages including the hemiascomycete yeasts (2), related *Aspergillus* species (13), and distant euascomycetes (39). A gene arrangement identical to the *N. fischeri MAT1* architecture is known to exist in *Neurospora crassa*, *Yarrowia lipolytica*, *Fusarium* and *Xanthoria* species, *A. fumigatus*, and *A. oryzae* (39, 13). This consistent gene arrangement suggests that *APN1* and *SLA2* were closely linked with the ancestral ascomycete *MAT* locus progenitor.

TABLE 1. DNA sequence similarity based on alignment and blastn data

Comparison[a]	% Similarity at:				% Overall similarity
	APN1	*MAT-1*	*MAT-2*	*SLA2*	
Nf1 vs *A. fumigatus*	94–98	92		95–100	94–100
Nf2 vs *A. fumigatus*	82–87		84–87	86–92	82–92
Nf1 vs Nf2	83–85			75–88	75–88

[a] *A. fumigatus* data are from the genome sequence of *A. fumigatus*, except for *MAT-1* data, which are from reference 32. Nf1 and Nf2 indicate the *N. fischeri* sequence at or flanking *MAT1* and *MAT2*, respectively.

The *APN1* and *SLA2* genes flanking *MAT1* in *N. fischeri* have greater sequence similarity to the homologous genes from *A. fumigatus* than to the d*APN1* and d*SLA2* pseudogenes flanking *N. fischeri MAT2*. The *MAT1*-flanking copy of *APN1* has 94 to 98% sequence similarity to *APN1* from *A. fumigatus*, whereas the two *N. fischeri APN1* sequences show only 83 to 85% similarity (Table 1). The *MAT1*-flanking copy of *SLA2* has 95 to 100% similarity to *SLA2* from *A. fumigatus*, whereas the two *N. fischeri SLA2* sequences have only 75 to 88% similarity (Table 1).

It was also observed that the sequence upstream of the *N. fischeri MAT2* locus contains numerous regions with sequence similarity to both named and candidate transposase genes from *A. fumigatus* and other fungi, including the two candidate transposase genes *TAF1* and *TAF3*, a *Nectria* species *pep5* DNA transposase gene, and a putative *A. fumigatus* reverse transcriptase gene (31).

Comparison of *N. fischeri MAT* regions to those of other aspergilli and the evolution of homothallism. Dot plot comparison of the *MAT1* region of *N. fischeri* to the *MAT* locus region of *A. fumigatus* revealed sequence similarity and synteny over a region of at least 22,300 bp (Fig. 2). The *MAT1* region of *N. fischeri* also showed sequence similarity to and synteny with regions upstream and downstream of the *E. nidulans MAT1* and *MAT2* loci (10, 11, 13). In contrast, the *MAT2* region of *N. fischeri* exhibited limited sequence similarity to the *MAT* locus of *A. fumigatus* over a region of about 8,000 bp directly adjoining the *MAT* locus, with no similarity beyond this (Fig. 2A and B). The *MAT2* product from *N. fischeri* also showed amino acid homology to the *MAT2* product from *E. nidulans*, but fewer than 150 bp of the DNA sequences could be unambiguously and continuously aligned (11), and this small fragment is not perceptible in the dot plot alignments presented here for *N. fischeri MAT2* and *E. nidulans MAT1* or *MAT2* (Fig. 2B). A ca. 420-bp fragment of *N. fischeri MAT2* was found upstream of *MAT1* in *N. fischeri* (Fig. 1); a similar fragment is also found in the *A. fumigatus MAT-1* region (32). This *MAT2* fragment in *N. fischeri* has one midcodon insertion found in the 3′ end and presumably is not transcribed. This fragment may have arisen through meiotic recombination at some point prior to the divergence of the *A. fumigatus* and *N. fischeri* lineages. Recombination at either end of a *MAT* locus has been invoked to explain variations in gene orientations in

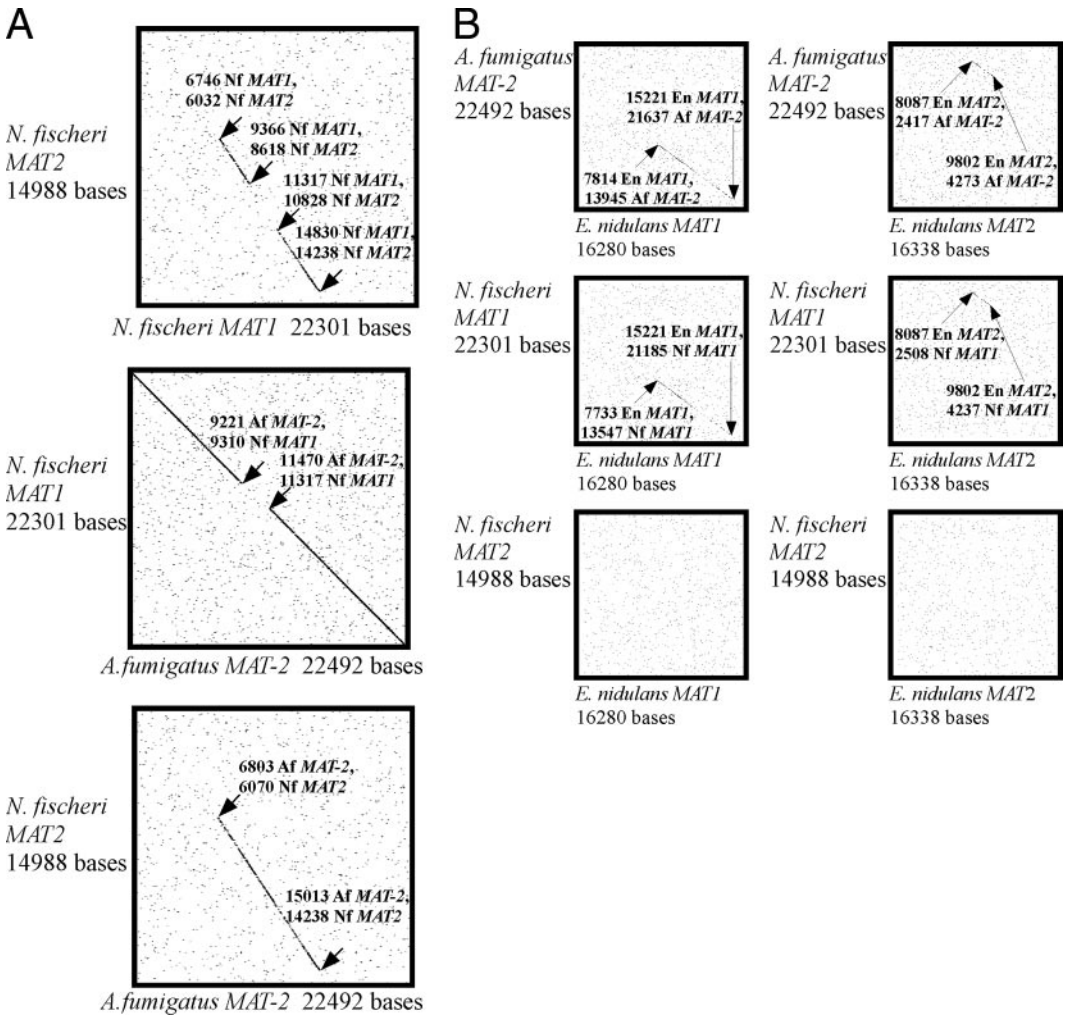

FIG. 2. Dot plot comparisons of pairwise alignments of DNA sequence data for *N. fischeri* (Nf), *A. fumigatus* (Af), and *E. nidulans* (En). Sequence lengths are given along the axes near the sequence identifications. (A) Three dot plot alignments compare *MAT* loci and flanking regions, including coding regions and ~5,000 bp upstream and downstream, obtained from the preliminary assembly of sequence contigs from the TIGR *N. fischeri* genome project for strain NRRL181. In the top dot plot, the *MAT1* and *MAT2* regions from *N. fischeri* are compared. In the middle dot plot, the *MAT* locus region of *A. fumigatus* (*MAT-2* in the genome project strain Af293) is compared to the *MAT1* region of *N. fischeri*. In the bottom dot plot, the *MAT* locus region of *A. fumigatus* (*MAT-2* in the genome project strain Af293) is compared to the *MAT2* region of *N. fischeri*. (B) The three dot plots on the left show comparisons of the *MAT1* region of *E. nidulans* with *A. fumigatus* Af293 *MAT-2* (top), *N. fischeri MAT1* (middle), and *N. fischeri MAT2* (bottom). The three dot plots on the right show a comparison of the *MAT2* region of *E. nidulans* with *A. fumigatus* Af293 *MAT-2* (top), *N. fischeri MAT1* (middle), and *N. fischeri MAT2* (bottom).

other fungal groups. Yun et al. (45) observed a fragment of *MAT1* in the 5′ end of *MAT2* in the homothallic species *Cochliobolus kusanoi*.

In the context of the evolution of reproductive modes, these observations lead us to speculate that *N. fischeri* arose from an ancestor with a *MAT* locus arrangement similar to that seen in extant *A. fumigatus* (32). The *MAT2* region of *N. fischeri* was then incorporated into the same genome containing the *MAT1* region, resulting in a homothallic breeding system. This incorporation is hypothesized to have arisen from a segmental break and translocation of a chromosomal region (Fig. 3), of significance in highlighting a novel means by which homothallism may evolve in euascomycete fungi. Furthermore, it is possible that incorporation was mediated by transposon activity. DNA transposons of euascomycete fungi are well documented (6). The possible transposon-mediated mobility of a *MAT* locus has not been reported before. The reasons why homothallism in the Eurotiales *N. fischeri* and *E. nidulans* has arisen in a form (the presence of two unlinked *MAT* loci apparently on separate chromosomes) different from that most commonly seen in other euascomycetes to date (the presence of alpha and HMG genes linked at the same single *MAT* locus) are unclear; it has been suggested that the separation of the *MAT* loci may provide a more genetically stable form of homothallism (D. M. Geiser, personal communication).

Evolution of reproductive modes in the section *Fumigati* and the genus *Aspergillus*. This study also aimed to assess whether homo- or heterothallism is the ancestral mating state of (i) the most recent common ancestor of *A. fumigatus* and *N. fischeri* and (ii) the section *Fumigati* as a whole. Previous evidence

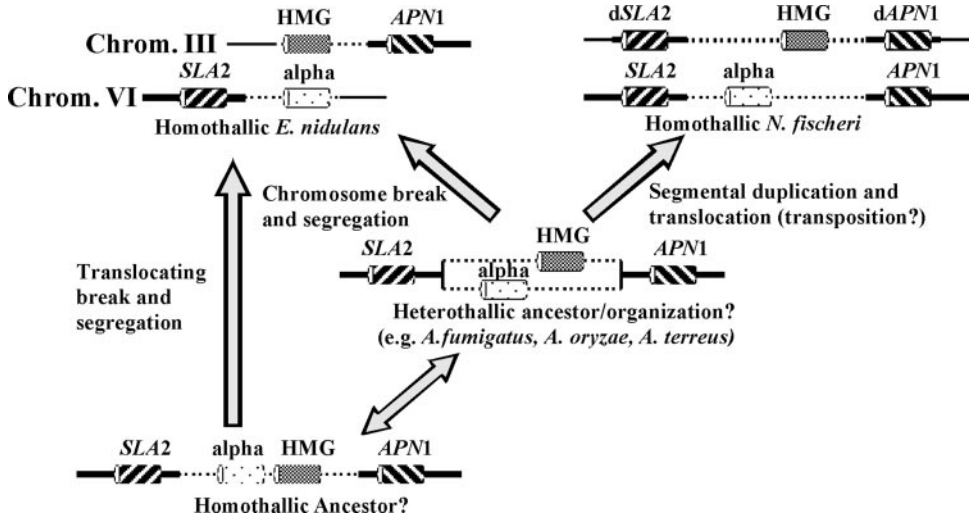

FIG. 3. Overall gene arrangements of *MAT* loci, *APN1*, and *SLA2* in the species *A. fumigatus*, *N. fischeri*, and *E. nidulans*. In *A. fumigatus*, each isolate has either a *MAT-1* (encoding a MAT protein with an alpha domain) or a *MAT-2* (encoding a MAT protein with an HMG domain) idiomorph located between a single genome copy each of *APN1* and *SLA2*. In *N. fischeri*, *MAT1* is located between *APN1* and *SLA2*, whereas *MAT2* is located between the d*APN1* and d*SLA2* pseudogenes. In *E. nidulans*, *MAT1* and *MAT2* are located on different chromosomes, bordered by only one of *SLA2* and *APN1*, respectively. Chrom., chromosome.

from phylogenetic studies of the section *Fumigati*, combined with results from later genome analyses, suggest that homothallism is the ancestral state of the section *Fumigati* and the aspergilli as a whole (13, 14). However, data obtained in the present study instead suggest that the ancestral state was heterothallism, at least in the section *Fumigati*, based on the following grounds. Firstly, *N. fischeri* and *A. fumigatus* appear to share a conserved ancestral *MAT* locus, flanked by *APN1* and *SLA2* genes, which contains either an alpha or an HMG domain-encoding *MAT* gene, i.e., a heterothallic idiomorph organization (Fig. 3). A similar heterothallic organization of either the *MAT-1* or *MAT-2* locus has also been detected in *A. oryzae*, a member of the genus *Aspergillus*, subgenus *Circumdati*, section *Flavi* (13, 32; P. S. Dyer, M. Paoletti, K. Kitamoto, and D. B. Archer, unpublished results). The conservation of a heterothallic idiomorph organization supports the hypothesis that the shared gene arrangement (genotypic heterothallism) precedes the origins of both subgenera *Fumigati* and *Circumdati*. Given that *A. fumigatus* and *A. oryzae* are taxonomically divergent within the genus *Aspergillu*s, their shared *MAT* sequence arrangement potentially represents the ancestral state.

Secondly, the organization of *MAT* loci seen in *N. fischeri* differs from that seen in *E. nidulans* (Fig. 3), in which homothallism is likely to have arisen from a translocating break, and that proposed in relation to an ancestral homothallic state of the aspergilli, in which the *MAT* genes are linked at a single *MAT* locus (13). Thus, there is little evidence for a consistent organization of *MAT* loci in homothallic aspergilli, which might be expected if homothallism was the ancestral state.

Thirdly, evidence for an ancestral heterothallic state comes from empirical studies of many genera of eusascomycetes, which have shown the evolution of a variety of *MAT* locus arrangements accompanying the divergence of species (21, 45). Theoretically, there is more support for the transition from heterothallism to homothallism among a fungal population (29, 30) and for a general unidirectional breeding system shift from obligate outcrossing to partial or predominant self-fertilization (20, 28). In the subsection *Fumigati*, there are nearly 20 additional species with a described sexual cycle, 3 of which are functionally heterothallic while the rest are homothallic. This reduced proportion of known heterothallic species in the subgenus makes an ancestral state of homothallism most parsimonious (14). However, a reconstruction of the ancestral mode of reproduction has never been evaluated using a maximum-likelihood framework to account for rate changes within the group's evolutionary history, the recent description of other taxa known to be either functionally or genetically heterothallic (32, 41), or the use of genetic confirmation of each species' mating system, including those of taxa for which no mating has yet been observed. The frequency of the heterothallic mating-type locus arrangement has been underestimated for other groups of euascomycetes in which numerous putatively asexual (anamorphic) taxa are present (21, 45). Anamorphic taxa have often been discovered to have an idiomorph arrangement characteristic of a heterothallic mating system (21, 32, 45).

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