The genus *Aspergillus* includes fungi of importance in the food and biotechnology industries, and pathogens such as *Aspergillus fumigatus*. It would therefore be of major economic and medical advantage to be able to study the inheritance of genes of interest and to bring together desirable genetic traits in the aspergilli. Unfortunately, such efforts have been impeded because most *Aspergillus* species are only known to reproduce asexually, thus the sexual cycle cannot be used for strain improvement and inheritance studies. However, the ‘genomics revolution’ is now beginning to reveal the sexual secrets of *Aspergillus*, thereby offering the prospect of understanding reasons for sexuality and asexuality, and the basis of homothalic (selfing) or heterothalic (obligate outcrossing) modes of sexual reproduction in this group of fungi.

The genomes of two species, *A. fumigatus* and *Aspergillus nidulans*, have been sequenced recently and the data have been made available to the research community. A subsequent article by Varga (2003) noted that genome analysis revealed the presence of an ORF encoding a fungal mating-type (MAT) gene in *A. fumigatus*. This was highly significant because *A. fumigatus* is only known to reproduce by asexual means, yet MAT genes are involved with controlling sexual compatibility. This suggests either that *A. fumigatus* may have a latent potential for sexuality, as described elsewhere for the supposedly asexual pathogen *Candida albicans* (Gow et al., 2000), or that *A. fumigatus* is derived from a sexual ancestor. The same observation was made by Pöggeler (2002), who also used genome analysis to identify other elements of fungal sexual pathways including ORFs encoding putative pheromones and pheromone receptors. In both reports, a MAT-2 family gene was identified encoding a regulatory protein with a high mobility group (HMG) DNA-binding domain (Turgeon & Yoder, 2000).

We now further explore sexuality in *Aspergillus* by making observations of sexual pathway genes in the homothallic fungus *A. nidulans*. Prior to the *Aspergillus* genome studies, we had already characterized a MAT-2 gene from *A. nidulans* using ‘classic’ molecular biological techniques (Dyer, 2002; Dyer et al., 2003). However, these investigations failed to reveal any MAT-1 family gene encoding a protein with an alpha-box motif (Turgeon & Yoder, 2000). In heterothallic ascomycete species, complementary MAT-1 and MAT-2 isolates are required for sexual reproduction. The MAT-1 and MAT-2 genes have also been found in homothallic ascomycetes, suggesting that MAT genes may have a role in regulating sex even in ‘selfing’ fungi. A MAT-1 homologue has been detected in all homothallic fungi investigated so far, with an additional MAT-2 homologue present in many species – often linked directly to a MAT-1 gene (Coppin et al., 1997; Pöggeler, 2001). Thus, the failure to detect a MAT-1 homologue suggested a novel situation in *A. nidulans* (Dyer, 2002; Dyer et al., 2003). However, by BLAST searching the newly available *A. nidulans* genome sequence (*Aspergillus nidulans* Database: http://www-genome.wi.mit.edu/annotation/fungi/aspergillus/), we now report the presence of a MAT-1 z-domain homologue in *A. nidulans*, the first identification of a MAT-1 homologue from a plectomycete fungus (Fig. 1). The *A. nidulans* MAT-1 ORF encodes a putative 361 aa polypeptide with at least one intron and a conserved z-domain core which exhibits 59, 52, 59 and 43% identity to the z-domains of the ascomycetes *Pyrenopeziza brassicae, Gibberella fujikuroi, Tapesia yallundae* and *Podospora anserina*, respectively, over a 54 aa region.

Intriguingly, the MAT-1 ORF is not linked to the previously identified MAT-2 gene, but is present elsewhere in the genome (on chromosomes 6 and 3, respectively), an unusual organization of MAT genes in homothallic euascomycetes, so far only reported from one other filamentous species, *Cochliobolus cymbopogonis* (Yun et al., 1999).

Further BLAST analyses of the *A. nidulans* genome using gene sequences from *Saccharomyces cerevisiae* revealed the presence of other putative genes involved in mating. These include an ORF encoding a precursor gene which is predicted to yield two copies of hydrophilic pheromones similar to the *S. cerevisiae* a-factor, following cleavage by internal proteases [gene termed *ppgA* (Pöggeler, 2000), GenBank accession no. BK001308; Fig. 2a]. Genes involved in the processing of pheromone precursors were also identified (GenBank accession nos BK001295 to BK001301). A putative efflux pump for an a-factor-like pheromone and a Kex2 endoprotease have been identified already (Kwon et al., 2001). However, despite
extensive searches, no precursor gene for a hydrophobic pheromone similar to \textit{S. cerevisiae} \textalpha-factor could be detected; such \textsc{blast} analyses also failed in \textit{A. fumigatus} (Pöggeler, 2002). Two further ORFs are predicted to encode pheromone receptors with similarity to either the \textit{S. cerevisiae} Ste3 \textalpha-receptor family [gene termed \textit{preA} (Pöggeler, 2002), GenBank accession no. BK001309; 57\% identity in a 287 aa overlap to \textit{A. fumigatus} \textit{PREA}] or the \textit{S. cerevisiae} Ste2 \textalpha-receptor family [gene termed \textit{preB} (Pöggeler, 2002), GenBank accession no. BK001310; 48\% similarity in a 246 aa overlap to \textit{A. fumigatus} \textit{PREB}] (Fig. 2b). Thus, a complement of genes producing pheromones and pheromone receptors is present.

These observations are of significance for a number of reasons. The discovery of a \textit{MAT}-1 homologue from the aspergilli means that it is now possible to screen for the presence of \textit{MAT}-1 genes in supposedly asexual \textit{Aspergillus} species using methods such as homologous hybridization or degenerate PCR. If complementary isolates containing \textit{MAT}-1 and \textit{MAT}-2 genes were identified then sexual crosses could be attempted as an initial step to determine whether \textit{MAT} compatibility alone is required for sexuality, or whether there are other reasons for asexuality (Sharon \textit{et al}., 1996). Indeed, some 'asexual' aspergilli have population structures consistent with recombination (Geiser \textit{et al}., 1998).

Meanwhile, the detection of a series of genes (mating type, pheromone precursor and pheromone receptors, etc.) involved with sexual signalling between compatible heterothallic isolates, yet present in a 'selfing' fungus, is noteworthy. This observation may be interpreted in various ways, with both the evolution of homothallic species from heterothallic ancestors and vice versa possible. Discoveries from \textit{Cochliobolus} (Yun \textit{et al}., 1999) suggest that it is most likely that heterothallic ancestors have given rise to extant homothallics, which now retain the earlier 'sexual machinery'. Given that homothallism is predominant in sexual aspergilli (Samson, 1992), it is interesting to speculate what factors may have favoured the evolution of selfing in this group of fungi. Ecological reasons such as the ability to sporulate without the need for a mate and preservation of well-adapted genotypes have been suggested elsewhere (Murtagh \textit{et al}., 2000).

Finally, there is now the opportunity to study the expression of \textit{MAT} genes in asexual and homothallic aspergilli, and compare this with heterothallic species in which they have more clearly defined roles.
Ongoing research within the Sordariaceae indicates that similar signalling processes are required for sexual development in homothallic as in heterothallic fungi (Pöggeler, 2000). We, and other researchers (Han et al., 2003), already have functional studies underway assessing sexual pathway genes in A. nidulans. As a whole, these ‘sexual insights’ emphasize how genomics may be of use in elucidating biological processes in tandem with post-genomic functional analyses.

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