Mating Type and Mating Strategies in *Neurospora*

Robert L. Metzenberg and N. Louise Glass

Summary

In the heterothallic species *Neurospora crassa*, strains of opposite mating type, *A* and *a*, must interact to give the series of events resulting in fruiting body formation, meiosis, and the generation of dormant ascospores. The mating type of a strain is specified by the DNA sequence it carries in the mating type region; strains that are otherwise isogenic can mate and produce ascospores. The DNA of the *A* and *a* regions have completely dissimilar sequences. Probing DNA from strains of each mating type with labelled sequences from the *A* and the *a* regions has shown that, unlike in *Saccharomyces cerevisiae*, only a single copy of a mating type sequence is present in a haploid genome. The failure to switch is explainable by the physical absence of DNA sequences characteristic of the opposite mating type. While the mating type sequences must be of the opposite kind for mating to occur in the sexual cycle, two strains of opposite mating type cannot form a stable heterokaryon during vegetative growth; instead, they fuse abortively to give a heterokaryon incompatibility reaction, which results in death of the cells along the fusion line. The DNA sequences responsible for this reaction are coextensive with those sequences in the *A* and *a* regions which are necessary to initiate fruiting body formation. The genus *Neurospora* also includes homothallic species – ones in which a single haploid nucleus carries all the information necessary to form fruiting bodies, undergo meiosis, and produce new haploid spores. One such species, *N. terricola*, contains one copy each of the *A* and the *a* sequences within each haploid genome. Another homothallic species, *N. africana*, contains a single copy of the *A* sequence in the haploid genome, but does not contain any sequences corresponding to the *a* sequence. We present a model for the role of the mating type-specific sequences in heterothallic and homothallic species of *Neurospora* and we speculate on the origin of the different modes of reproduction in the genus *Neurospora*.

Introduction

What is mating good for? After the obligatory round of facetious answers, we are still left with a dilemma: some organisms mate regularly as part of their life cycles, but others seem to be able to propagate themselves without limit by vegetative growth. Obviously, since both forms of reproduction exist, there must be advantages to each of them. The commonest rationalization for sexual reproduction (though far from universally accepted) is that sexual organisms can produce offspring with new, perhaps favorable, combinations of alleles from both parents by genetic recombination. A second possible benefit of biparental reproduction is that it affords the possibility of each partner repairing random epigenetic or conventional genetic damage by comparison with the genes of a partner with a different history, and therefore different sites of damage[1]. Organisms that grow only vegetatively lack these capacities, but they also avoid the construction costs of structures related to sex, the metabolic costs of courtship, and the risk of failing to find a mate. They also avoid the 'downside risk' of recombination, which can break up successful combinations of alleles as well as create them.

One might think, then, that no creature would make elaborate mating structures in order to mate with itself. Such a strategy would incur many of the costs of sexuality without conferring the widely proclaimed benefit of bringing together new allele combinations. Yet many organisms do exactly that. In the kingdom of fungi, such organisms are said to be *homothallic*. In some genera, homothallic species closely resemble their outbreeding, *heterothallic* relatives. Space constraints limit our discussion of heterothallism to species in which there are only two mating types. We will also consider only fungi that grow vegetatively as haploids. In the sexual cycle, fusion of two such haploid nuclei, whether from heterothallic or homothallic species, generates the diploid state of the life cycle. Meiosis ensues, either promptly or after an indefinite number of mitotic divisions as a diploid. In either case, haploid spores are generated. Commonly these spores are dormant and long-lived, and they may be quite resistant to killing by heat, desiccation, ultraviolet light, and inhospitable conditions in general[2]. The production of these long-lived dormant spores, often thought of as a minor ancillary benefit of mating, may be more important than is generally assumed – especially in the case of homothallic fungi, in which other benefits of sexuality are less obvious. An interesting question is: do homothallic fungi arise from heterothallic ancestors, or is it the other way around? While that question can not yet be answered, a combination of classical and molecular genetics has made it possible to ask the question much more precisely.

Mating Type Sequences in Budding Yeast

The most detailed insight into mating type gene action is provided by findings with the budding yeast, *Saccharomyces cerevisiae*[3]. The haploid, vegetative cells of this yeast are found in two different cell types, *a* and α. The cell type depends on which mating type sequence is present at the mating type (*mat*) locus. The mating type sequences are, in every sense, regulatory elements.

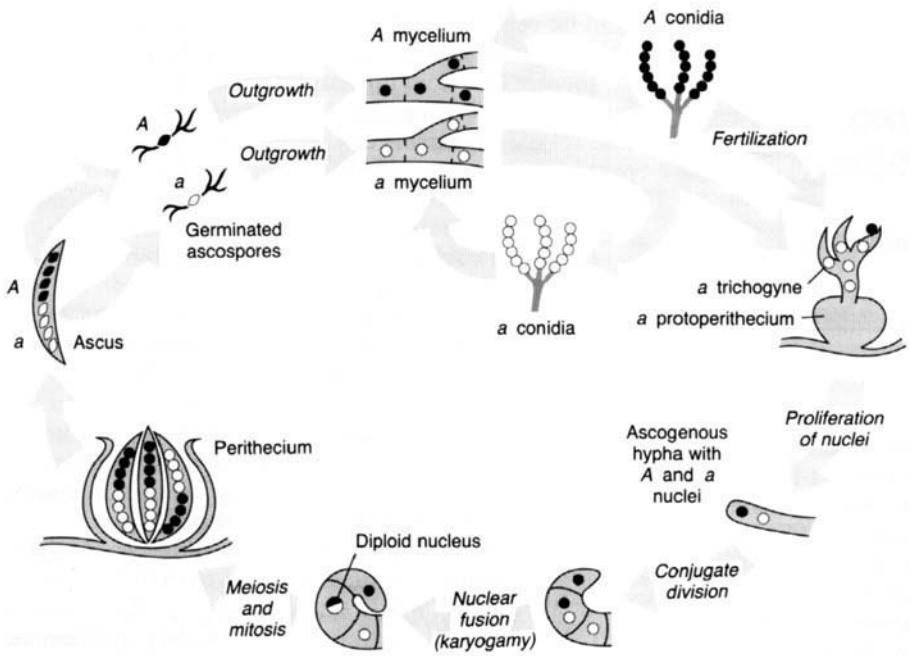

Fig. 1. The life cycle of *Neurospora crassa*.

Products of these sequences, including a heterodimer of *a* and α-encoded polypeptides, exert their effects by acting upon a number of target genes. Surprisingly, the yeast cell contains not only the expressed sequence at the *mat* locus, but also an unexpressed, archival copy of the *a* and α sequences on the same chromosome as the *mat* locus, but on opposite sides. Thus every haploid cell has three mating type genes, two of one sequence and one of the other. In the vegetative cells of most wild-type isolates, the DNA sequence information at the *mat* locus is very frequently replaced with information from one of the archival copies. This process, which does not result in any loss of information from the archival copy, often switches the mating type of the cell. If such a switched cell is still close to, or in contact with, a sister cell that has not switched its mating type, the two otherwise isogenic cells will mate to produce a diploid cell capable of meiosis and spore formation. *S. cerevisiae* is therefore 'functionally homothallic'. The strains most commonly used in genetic research are mutants that have lost the capacity for facile switching of the sequence at the *mat* locus, though molecular probing reveals that they retain three copies of the mating type sequences. These strains are 'functionally heterothallic', in that their mating type behaves as an ordinary genetic trait. Their loss of capacity to switch is not, however, absolute, and their very occasional switches of mating type hinted at the nature of the phenomenon long before molecular evidence became available.

Mating Behavior in *Neurospora crassa*, a Heterothallic Species

Unlike natural isolates of *S. cerevisiae*, those of the haploid filamentous fungus *Neurospora crassa* are invariably heterothallic[2], and none has ever been observed to switch to the opposite mating type[4]. The two mating types, called *A* and *a*, have, in the past, been defined purely functionally. Any two strains of opposite mating types, including ones isogenic at all other known loci, are competent to mate. For the events of the sexual cycle to be initiated, the two types must come together under suitable conditions (not too rich a medium, not too high a temperature). In this situation, the earlier-arriving strain, either *A* or *a*, produces a nearly spherical pre-fruiting body made of sterile, specialized hyphae. This structure, called a protoperithecium, is the female element (see Fig. 1). Specialized hyphae, called trichogynes, grow directionally from the protoperithecium toward a vegetative 'male' cell (typically a recently-arrived airborne asexual spore, or conidium) if this cell is of the opposite mating type. This directional growth is believed to be guided by uncharacterized pheromones[5,6], one pheromone being secreted by each mating type and acting exclusively on cells of the opposite mating type. When a trichogyne meets a conidium or hypha of the opposite mating type, the cytoplasms of the two fuse. One fertilizing, haploid nucleus from the male is conducted by the trichogyne into the protoperithecium, which becomes, by definition, a perithecium. The haploid nuclei from the male element proliferate along with the resident, haploid nuclei as a dikaryon in structures called ascogenous hyphae; in the final few nuclear divisions, nuclei of the opposite mating type are paired and divide synchronously (conjugate division), and a final round of premeiotic DNA synthesis occurs. This 'nuclear courtship' culminates in fusion of the paired nuclei (karyogamy) to give diploid cells. The diploid cells promptly undergo

the two meiotic divisions to give four haploid nuclei, followed by a mitotic division to give the eight nuclei, around which ascospores are soon delineated. Additional mitotic divisions occur in the ascospores during their maturation. The mature ascospores can be induced to germinate by a heat shock or exposure to certain ethers, returning the cells to the vegetative phase of the life cycle.

Mating Type Sequences in *Neurospora crassa*

Classical mutational analysis[7] resulted in the isolation of a number of strains of each mating type that failed to participate in sexual cycle events, from pheromone-mediated attraction to ascospore formation. The mutations were thought to be in the mating type genes. However, these strains were sterile and not complementable by heterokaryosis with fertile strains of the same mating type; hence the mutations could not be mapped. They did, however, provide null tester strains for molecular cloning. From libraries of *N. crassa* DNA that were prepared from strains of *A* and from *a* mating types, Vollmer and Yanofsky[8] isolated DNA sequences that restored the ability of these sterile mutants to form perithecia, and, occasionally, ascospores. Restriction fragment length polymorphism mapping showed that the complementing DNA sequences originated from the mating type region, supporting the notion that the original mutations conferring sterility were in the mating type sequences.

The cloning of the two mating type regions made it possible to answer some old questions. The two regions are flanked on both sides by DNA that is identical in sequence, or nearly so. However, the *A* region contains a stretch of 5301 bp that has little or no homology with the *a* region. The latter has 3235 bp with little or no sequence similarity with the *A* region (see Fig. 2). In a departure from previous practice, we will define the *A* and *a* regions structurally by their non-homology, rather than functionally. In the past, *A* and *a* have been referred to as 'alleles', but this now seems inappropriate and misleading. 'Alleles' implies similarity between two different forms of a gene, and 'members of a set of alleles are mutually exclusive genetic markers and arise by gene mutation'[9]. We do not know whether the DNA sequences of *A* and *a* arose from a common sequence by mutation, but certainly there is no evidence to support such an idea. Therefore we introduce the word 'idiomorphs' (see Footnote 1) to denote sequences of the *A* and *a* regions which occupy the same locus on their chromosome but are not obviously related by structure or common descent, and suggest that the word may be a useful appelation for the mating type 'alleles' of other organisms as well.

Footnote 1. 'Idiomorph' was suggested to us by Professor John Wyatt. It is a combination of the Greek '*morphos*' (form) and '*idio*' (singular, unique, private, apart from the community – cf. idiotype, idiosyncracy, etc.). It can be compared with 'allelomorph' (the original version of 'allele'[9]) which merely means 'different in form'.

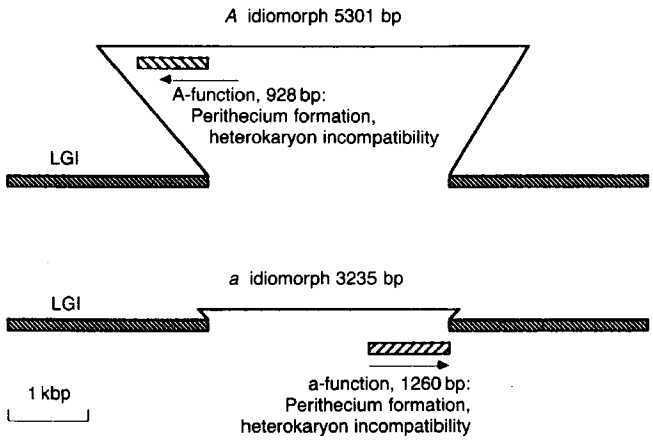

Fig. 2. Diagram of the region of Linkage Group I (LG I) carrying the non-homologous *A* and *a* mating type idiomorphs. The 928 bp sequence identified with *A* function includes the open reading frame for the protein and an internal intron, but not upstream promoter sequences; the minimum fragment which gives effective transformation is about 750 bp (see text). The 1260 bp sequence identified with *a* function includes the open reading frame and two internal introns, but effective transformation requires about 1600 bp (Staben and Yanofsky, in preparation).

The heterothallic *Neurospora* species have been examined for the possible presence of silent *A* or *a* idiomorphs. Unlike the case in *S. cerevisiae*, a given haploid genome of *N. crassa* contains idiomorphic sequences of only one kind. There is no archival idiomorph either of the same, or of the opposite mating type. The failure of *N. crassa* to switch mating types even rarely in the laboratory or to be found as homothallic variants among natural isolates seems easy to explain: information for the other mating type is physically absent[10].

Heterokaryon Incompatibility as an Aspect of Mating Type Sequences

The functions of the mating type regions discussed above are all restricted to courtship, mating, and the production of meiotic products. But these regions have a second role that is evident only during vegetative growth. If two essentially isogenic strains *of the same mating type* are placed together on minimal medium, hyphal fusion and mingling of nuclei will occur without any nuclear fusion. If the two strains contain complementing, non-allelic auxotrophic mutations, the resulting heterokaryon will grow vigorously on minimal medium. However, if the two auxotrophic strains are *of opposite mating types*, hyphal fusion results in death of the cells along the fusion line, and no stable heterokaryon will be formed. Strains of opposite mating type are said to be heterokaryon incompatible. Unsuccessful attempts to separate the incompatibility and fertility functions of the mating type region by high-resolution recombination suggested that the two might be different manifestations of the same sequences[11]. *A* and *a*

mutant strains selected by their ability to form stable heterokaryons with strains of the opposite mating type were found, with one exception, to be sexually sterile, and revertants that recovered their fertility also recovered heterokaryon incompatibility[7].

The availability of cloned idiomorphs allowed us to ask whether the DNA sequences that cause perithecium formation in the sexual cycle are coextensive with those that cause heterokaryon incompatibility in vegetative growth. This has been examined in detail in the *A* mating type idiomorph. A restriction fragment of about 1000 bp, inserted into a sterile, heterokaryon compatible mutant, restores both functions to transformants. Exonuclease digestion from one end or the other can define the functional region more closely, to a fragment of about 750 bp, but further digestion from either end results in loss of the ability to transform strains to either perithecium formation or heterokaryon incompatibility.

The sequences in the *A* idiomorph that are necessary for these functions also contain the only convincing open reading frame (ORF) within the 5301 bp region (Glass *et al.*, in preparation) and encodes the only mRNA species detected thus far (Fig. 2). This mRNA is the result of excision of a 59 base intron (deduced from the presence of intron consensus sequences and confirmed by cloning and sequencing of cDNA). The protein encoded by the *A* ORF contains 288 amino acids, and the carboxy terminus is not necessary for function. Sterile, heterokaryon compatible mutants of the *A* idiomorph all owe their properties to frameshift mutations within this open reading frame, as shown by sequence analysis of the mutant idiomorphs.

The 3235 bp *a* idiomorph also contains a limited region that can encode a protein (Staben and Yanofsky, in preparation). The intactness of this region is necessary for mating and perithecium formation and for producing heterokaryon incompatibility during vegetative growth, and mutations that affect mating and vegetative incompatibility are due to base changes within this region. The protein-coding regions of the *A* and *a* idiomorphs are almost at opposite ends of the regions of non-homology, and are transcribed divergently. The relative placement of the ORFs within the *A* and the *a* idiomorphs is shown in Fig. 2.

Mating Type Idiomorphs in *N. tetrasperma*, a Pseudohomothallic Species

Other species within the genus *Neurospora* have other patterns of mating. The least fundamental variation is that seen in *N. tetrasperma*. The asci of this species have four spores instead of the eight present in asci of *N. crassa* and other heterothallic species. The four-spored condition is not due to failure of the first post-meiotic division. Instead, the configuration of the meiotic spindles is such that two nuclei of opposite mating type are usually included in each ascospore. On germination, such an ascospore gives rise to a self-fertile, heterokaryotic culture that is operationally homothallic. However, individual conidia from a self-fertile culture very often give rise to self-sterile, cross-fertile cultures. For this reason, *N. tetrasperma* is said to be 'pseudohomothallic'. Molecular characterization of the self-sterile cultures shows that each contains sequences that hybridize either with DNA of either the *A* or the *a* idiomorph of *N. crassa*, but never with both. Thus, the resolved vegetative strains are heterothallic both by functional and molecular criteria. Pseudohomothallism depends not only upon the presence of both mating type genes in separate nuclei of the same ascospore, but on their coexisting without provoking the heterokaryon incompatibility reaction. This does not reflect a difference in the functional mating type sequences themselves. When these mating type idiomorphs are introgressed into an *N. crassa* genetic background, they show heterokaryon incompatibility similar to that seen with the original *N. crassa* idiomorphs[12]. The simplest interpretation is that the expression of heterokaryon incompatibility due to mating type heterokaryosis requires the action of unlinked target genes that function in *N. crassa* but not in *N. tetrasperma*.

Mating Type Idiomorphs in *N. terricola* and *N. africana*, Two 'True' Homothallic Species

The genus *Neurospora* also contains examples of 'true' homothallism, in which self-fertile cultures arise from homokaryotic propagules. *N. terricola* has asci with eight haploid spores, and these are formed by a series of events that are cytologically indistinguishable from those of *N. crassa*[13]. Molecular characterization shows that the haploid genome contains one copy each of the sequences corresponding to each mating type idiomorph of *N. crassa*[10]. Hence each nucleus is structurally homothallic. Presumably both idiomorphs are functional, though, as in *N. tetrasperma*, incompatibility is absent. In contrast with the situation in heterothallic and pseudohomothallic species, the two premeiotic nuclei that fuse to form a diploid cell in the ascogenous hyphae of *N. terricola* are genetically identical. The *A* and *a* idiomorphs appear to be closely linked, and no third copy of the mating type sequences is present. This indicates that, if mating type switching occurs, its mechanism is different from that in *S. cerevisiae*.

A more bizarre form of 'true' homothallism is found in four other species, of which *N. africana* is representative. Like *N. terricola*, this organism forms eight-spored asci by processes essentially identical with those of other species in the genus, and no self-sterile forms can be isolated. However, the explanation for the homothallic behavior of *N. africana* does not lie in the presence of both mating type idiomorphs within a haploid genome. Hybridization studies show that only the *A* idiomorph is present[10]; no sequences similar to the *a* idiomorph can be detected by hybridization even under conditions of low stringency. The *A* idiomorph of *N. africana* has been cloned and sequenced. The

deduced protein encoded by it is the same length as that of *N. crassa*, and it is 95% identical in amino acid sequence. Transformation of sterile *N. crassa* by the *A* idiomorph of *N. africana* shows that the latter functions, though quite weakly, as an *A* idiomorph in *N. crassa*. However, it does not make the recipient strain detectably homothallic (Glass *et al.*, in preparation). Quite likely, the response of target genes in *N. africana*, rather than any peculiar properties of the mating type idiomorph, makes the species homothallic. Surprisingly, the *A* idiomorph of *N. africana* functions as a heterokaryon incompatibility idiomorph in *N. crassa*. The retention of this function in a 'true' homothallic species in which the *A* idiomorph presumably never sees an *a* idiomorph suggests that the *A* sequences which are necessary for mating also cause heterokaryon incompatibility in *N. crassa* as an inescapable aspect of their mating function.

From considerations of symmetry, we might have expected that the genus *Neurospora* or closely related genera would include 'true' homothallic species that carry only the *a* mating type idiomorph. These have not been found in available stock collections or among strains freshly isolated from nature (Glass, Metzenberg and N. B. Raju, in preparation). While it is obviously risky to argue that something that has not yet been found does not exist, the failure to find such a strain suggests that target genes may be incapable of mutating so that they can be controlled by the *a* idiomorph alone.

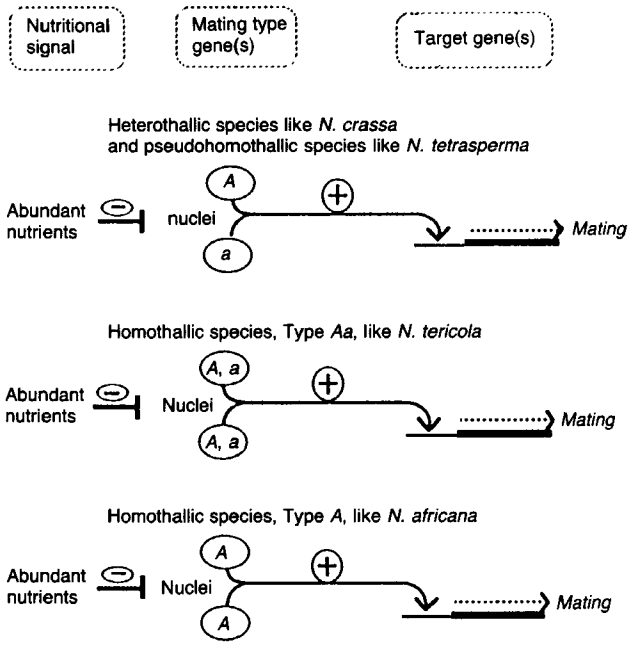

Fig. 3. Proposed model for action of mating type idiomorphs under conditions appropriate for mating. The mating type idiomorphs are shown as activating one or more target genes. Products of these genes are needed for perithecium formation. Transcription of the mating type idiomorphs is only partly repressed in the presence of abundant nutrients. The incompleteness of repression allows heterokaryon incompatibility between the two mating types to occur during the vegetative part of the life cycle.

Models for Action of the Mating Type Idiomorphs in the Genus *Neurospora*

What models can be made of the mechanisms by which mating type genes exert their actions? The similarities in structure and meiotic behavior between species in the genus *Neurospora* suggest that there should be underlying similarities in mechanisms that give rise to these different modes of mating. According to the model we favor, the protein products of the *A* and *a* idiomorphs are made to a limited extent during vegetative growth of heterothallic and pseudohomothallic species, and that they may act upon target genes in heterothallic strains to produce heterokaryon incompatibility. Under conditions appropriate for mating (deprivation of nutrients), the mating type idiomorphs are more highly expressed, and activate other target genes, perhaps including those coding for pheromone receptors and features on the nuclear surface that mark each nucleus as *A* or *a* (Fig. 3). Fusion to produce the diploid premeiotic cell occurs only between nuclei of unlike mating type. It is possible, but unproven, that this diploid cell contains a novel regulatory product specified by cooperation of the *A* and *a* idiomorphs analogous to that in a/α diploids of *S. cerevisiae*[3]. If so, the novel product probably functions both in nuclear recognition and in preparing the cell for immediate entry into meiosis.

The situation in the 'true' homothallic species, *N. terricola* might well be similar, in that the products of the *A* and *a* idiomorphs may interact to turn on a battery of target genes concerned with mating. The marking of nuclei for fusion is more problematic, since all the nuclei are genetically identical. It is possible that nuclei may fuse indiscriminately with any other nucleus in the same ascogenous hypha, but this begs the question of why fusion fails to occur in vegetative hyphae. An alternative is that the nuclei are *functionally* different, in that the *A* idiomorph is expressed in one nucleus while the *a* idiomorph is expressed in another, and only functionally unlike nuclei can fuse (Fig. 3). Thus, while the species is certainly genetically homothallic, it may be functionally heterothallic at the level of nuclei in the ascogenous hyphae.

Finally, 'true' homothallic species like *N. africana* must be considered – in particular, the question of whether paired nuclei preparing to fuse are functionally different. As in *N. terricola*, fusion of genetically identical nuclei must occur, but in this case it is not even possible that one is functionally of one mating type and the other is functionally of the other mating type. It is possible that one nucleus expresses the *A* mating type, but that this idiomorph is silent in the other nucleus of a pair. The analogy to the situation in other *Neurospora* species is, at best, far-fetched. The *a* idiomorph is not explainable as the absence of *A* function, because mutations in *A* (now characterized as frameshift mu-

tations – Glass *et al.*, in preparation) give rise to sterile strains of *N. crassa* that do not mate as if they were *a*, and mutations in *a* also cause sterility[7]. It is also formally possible that, in addition to the *A* mating type idiomorph (which is highly homologous with other *A* idiomorphs in the genus and in related genera), *N. africana* also contains a second mating type idiomorph, which we could call α. This idiomorph would be postulated to act in a manner similar to the *a* idiomorph in *N. terricola* and other species. The hypothetical α idiomorph is invisible to our existing probes, and thus would have to be completely non-homologous to *a*. It would be accessible only to mutational analysis. Our own intuition, based on Occam's Razor, suggests that more reasonable mechanisms are to be found without positing the existence of undetected idiomorphs. One can imagine that regions upstream of the target genes have been altered so that they are responsive to the product of the *A* mating type idiomorph, without there being any requirement for an *a* mating type product (Fig. 3). If the number of target genes is large, changing all of them to allow a mating strategy like that of *N. africana* might be, at best, an extremely rare event. However, if there is a single master target gene which, in turn, controls a retinue of effector genes, fertile strains containing only the *A* mating type idiomorph might appear with reasonable frequency over a geological time scale, though they have not been detected by mutational studies in the laboratory. An obvious feature of this model, which seems at odds with what we know about heterothallic species, is that fusion to form a diploid premeiotic cell would involve pairs of nuclei that are both genetically and transcriptionally identical. In short, no single model for action of the mating type genes suggests the kind of deep mechanistic unity that we might hope to see between the mating systems of various species.

Was the Common Ancestor of *Neurospora* Species Heterothallic or Homothallic?

What, then, is the value of mating? We still cannot answer this question. However, it is useful to rephrase it by asking whether homothallic species, which enjoy the benefits of ascospore production but not of biparental inheritance, evolved from heterothallic species, or whether heterothallic species evolved from homothallic ones. We feel that the most economical hypothesis holds that heterothallic species, with their mating type idiomorphs in separate genomes, were ancestral. Pseudohomothallic organisms like *N. tetrasperma* could arise from these readily by one or a few mutations; indeed, a single dominant mutation in this organism results in asci with eight homokaryotic, self-sterile spores that are effectively heterothallic[14,15]. Pseudohomothallic organisms retain the capacity for outbreeding because they at least occasionally produce self-sterile, cross-fertile cultures from conidia. Yet they keep the benefit of being able to produce resistant ascospores without (usually) having to encounter a mate. One can imagine that they would be at an advantage over obligate heterothallic species in an environment that supports only a low population density. 'True' homothallic *Neurospora* species are invariably non-conidiating[2]. While heterothallic and pseudohomothallic species are usually found on burned plant substrates, the non-conidiating types have only been found in soil. They may be indigenous to the soil or possibly to dung deposited on it, but in either medium, mobility is slight and the opportunities for finding a mate may be limited. We can imagine the evolution of *N. terricola* as follows. A haploid nucleus of a pseudohomothallic strain acquired a second mating type idiomorph, perhaps as a result of aberrant meiosis; such a variant would enjoy an advantage because its ability to produce resistant ascospores would be independent of the vagaries of nuclear ratios. The final stage of evolution of homothallism, represented by *N. africana*, would be the loss of dependence for ascospore formation on the presence of the *a* mating type idiomorph. When this idiomorph was deleted, any residual deleterious effects of heterokaryon incompatibility between the *A* and the *a* idiomorphs would also have been lost. While, in principle, it is possible to run this speculative scene backwards, we feel that such an order of events is very implausible. It would require the sudden appearance of the *a* mating type idiomorph, not only in *N. terricola*, but also in a number of related genera (Glass, Metzenberg, and Raju, in preparation). A more believable history is one in which the earliest members of the genus *Neurospora* were heterothallic. Subsequently, some species gave up on biparental sex because, in their habitat, it was difficult or impossible for two potential parents to get together. However, they retained the ancillary benefit of making resistant spores.

Acknowledgements

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References

1 Maynard Smith, J. (1978). *The Evolution of Sex*. Cambridge University Press, London and New York, 232 pp.
2 Perkins, D. D. and Turner, B. C. (1988). *Neurospora* from natural populations: toward the population biology of a haploid eukaryote. *Exptl. Mycol.* **12**, 91–131.

3 HERSKOWITZ, I. (1988). Life cycle of the budding yeast *Saccharomyces cerevisiae*. *Microbiol. Revs.* **52**, 536–553.
4 PERKINS, D. D. (1987). Mating type switching in filamentous ascomycetes. *Genetics* **115**, 215–216.
5 BISTIS, G. N. (1981). Chemotropic interactions between trichogynes and conidia of opposite mating-type in *Neurospora crassa*. *Mycologia* **73**, 959–975.
6 BISTIS, G. N. (1983). Evidence for diffusible, mating-type-specific trichogyne attractants in *Neurospora crassa*. *Exptl. Mycol.* **7**, 292–295.
7 GRIFFITHS, A. J. F. (1982). Null alleles of the *A* and *a* mating type alleles of *Neurospora crassa*. *Can. J. Genet. Cytol.* **24**, 167–176.
8 VOLLMER, S. J. AND YANOFSKY, C. (1986). Efficient cloning of genes of *Neurospora crassa*. *Proc. Natl Acad. Sci. USA* **83**, 4869–4873.
9 RIEGER, R., MICHAELIS, A. AND GREEN, M. M. (1976). *Glossary of Genetics and Cytogenetics*. Fourth edition. Springer-Verlag, Berlin, Heidelberg and New York. 647 pp.
10 GLASS, N. L., VOLLMER, S. J., STABEN, C., GROTELUESCHEN, J., METZENBERG, R. L. AND YANOFSKY, C. (1988). DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. *Science* **241**, 570–573.
11 NEWMEYER, D., HOWE, H. B., JR. AND GALEAZZI, D. R. (1973). A search for complexity at the mating-type locus of *Neurospora crassa*. *Can. J. Genet. Cytol.* **15**, 577–585.
12 METZENBERG, R. L. AND AHLGREN, S. K. (1973). Behaviour of *Neurospora tetrasperma* mating-type genes introgressed into *N. crassa*. *Can. J. Genet. Cytol.* **15**, 571–576.
13 RAJU, N. B. (1978). Meiotic nuclear behavior and ascospore formation in five homothallic species of Neurospora. *Can. J. Genet. Cytol.* **56**, 754–763.
14 DODGE, B. O. (1939). A new dominant lethal in Neurospora. The *E* locus in *N. tetrasperma*. *J. Heredity* **30**, 467–474.
15 CALHOUN, F. AND HOWE, H. B., JR. (1968). Genetic analysis of eight-spored asci produced by gene *E* in *Neurospora tetrasperma*. *Genetics* **60**, 449–459.

N. Louise Glass is a joint appointee of the Botany Department and the Biotechnology Center, University of British Columbia, Vancouver B.C., Canada.
Robert L. Metzenberg is in the Department of Physiological Chemistry, University of Wisconsin, Madison WI, U.S.A.