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The Genetics of *Aspergillus nidulans*; Past, Present and Future

A. John Clutterbuck
Division of Molecular Genetics, University of Glasgow, Scotland, UK

The earliest genetics, from Mendel onwards, was done on domesticated plants, for which many well-defined variants already existed, then came the use of Drosophila, in which the first step on the road to becoming a "genetical model organism" was the induction and understanding of mutants. Then in 1941 Beadle and Tatum introduced the first micro-organism, *Neurospora crassa* as an organism in which to attempt to answer biochemical questions first raised in Drosophila. Pontecorvo, in introducing a second fungus, *Aspergillus nidulans*, also following work with Drosophila, was less interested in biochemical pathways than in the definition of the functional gene as definedby complementation tests, and the relationship of this to the unit defined by recombination (Pontecorvo *et al.* 1953).

A peculiar feature of *A. nidulans*, as compared to *N. crassa*, is that it is homothallic. This has a number of consequences. Firstly, any strain can be crossed with any other, a source of scepticism for workers with rival organisms, since it was assumed that self-fertilization would heavily outweigh outcrossing. This proved not to be the case, a feature which Pontecorvo *et al.* (1953) named "relative heterothallism", although they could not explain its basis. The simplest explanation is that that some auxotrophic mutants are partially defective in mating, and if this is a general rule complementing pairs of nuclei become selected as mating partners.

However, more recent work has shown that nuclear behaviour in cleistothecia (sexual fruiting bodies) is quite complex. It has been known for some time that the body of the fruiting body is normally formed by only one of the parental strains (Zonneveld, 1988) so by this means a female role can be identified. This parent also determines the colour of the ascospores (Apirion, 1963), a disappointing feature since it was hoped that ascospore colour would be autonomously determined as in Neurospora and Sordaria where it is a valuable feature in tetrad analysis. In *A. nidulans* the strain taking the nest-building role ("nidulans" means "making small nests") is also the donor of mitochondria to the next generation (Rowlands & Turner, 1976) as in other bisexual systems. In arginine and tryptophan auxotrophs it is noticeable that aborted cleistothecia are formed in abundance on single strains, and these strains never self, but are effective hybridizers (*e.g.*

Bainbridge 1965). It is not known whether it is the male or female role that is unable to be incompleted in the aborted cleistothecia.

More recently, a quite different piece of evidence for divergence of sexual roles comes from an observation that certain strains on self-fertilization give progeny which show mutual heterokaryon incompatibility reactions (AJC, unpublished). This, of course, is quite unexpected since all progeny of a self-cross are expected to be identical. In *Neurospora crassa* heterokaryon incompatibility is a feature of differential mating types, so the possibility arises that these *A. nidulans* colonies are displaying two mating types. If so, opposite mating types must arise as a result of a mating-type switch, as in *Saccharomyces cerevisiae*. Since heterokaryon incompatibility reactions have never been observed before within the isogenic Glasgow stocks of *A. nidulans*, I assume that mating type is silenced during vegetative growth, or it could be re-switched to a uniform state in vegetative hyphae. In the strains in which incompatibility reactions are observed in young ascospore-generated colonies, it must be assumed that the mating type differences persist a little longer than usual.

At present the observation of mating type differentiation is repeatable, but not consistently so. However, if it can be confirmed, the interesting question will remain as to whether mating type and male/female differentiation are the same thing. It should be noted that in *N. crassa* this is not the case: either mating type can adopt the male or female role.

To return to the consequences of the homothallic status of *A. nidulans*: this has also allowed the accumulation of a complete set of genetic strains which are effectively isogenic, since they started from a single wild isolate. This has a number of advantages; isogenicity provides a uniformity of genetic background ideal for experimentation. A second valuable feature of isogenicity, not appreciated until its absence was proved important in other organism, is the high frequency of homologous integration of transforming DNA (see below). On the other, exploration of background variation has sometimes proved valuable in other organisms, as in the discovery of variable recombination frequencies in differing Neurospora stocks. It is also postulated that the isogenicity may be the cause of the absence of interference in meiotic crosses (Kafer, 1977), and possibly also the absence of a synaptinemal complex (Egel-Mitani *et al.* 1982.)

One of the earliest experiments on *Aspergillus* in Pontecorvo's laboratory illustrates the current thinking which he wished to clarify: Roper (1950) tested for recombination between what we now call biA1 alleles, but his use of the term "pseudoalleles" reflects the doubt at that time about the possibility of intragenic recombination. Only a few years later, however, Pritchard (1955) made it very clear that recombination between

non-complementing adenine mutants was the rule, not the exception, and it was only the far more extensive experiments of Benzer on bacteriophage T2 rII mutants which supplanted this as the definitive test of intragenic recombination.

Aspergillus, however is not the ideal organism for investigation of meiotic recombination, since it has small, unordered asci, in comparison with those of *Neurospora* and its relatives, and at this point the biology of the fungus led Pontecorvo's laboratory in another direction. Heterokaryons had always fascinated him (Pontecorvo 1946) and it may have been this that led to discovery of the parasexual cycle. In his search for a fungus suitable for genetic analysis, Pontecorvo must have been very aware of the fact that many fungi are apparently asexual, hence the importance in his eyes, of heterokaryotic variation. However, a complete "alternative to sex" would be of even greater interest. To this end, Roper sought and found rare vegetative diploids formed by fusion of unlike nuclei in heterokaryons (Roper 1952). A distinctive feature of *Aspergillus* is the green colour of its vegetative spores, genetic variants of which provide instantly recognizable markers which are useful in analysis of meiotic cross progeny, but crucial in the observation of vegetative segregants from heterozygous diploids. Further analysis showed that these segregants had arisen by either mitotic recombination or haploidisation. Mitotic recombination was predicted from previous experience in Drosophila (Lewis, 1945), and spontaneous haploidisation is perhaps not surprising given that diploidy is an unnatural state for *Aspergillus* vegetative nuclei. The parasexual cycle provides a most valuable mapping tool for A. nidulans, and the only available mapping method for many other fungi. Since A. nidulans chromosomes are long when measured in map units (300-700 units), a new mutant may often fail to show meiotic linkage to a standard marker on the same chromosome. Haploidisation, however, since it achieves chromosome reduction independently of crossing over, normally gives complete linkage of all markers on the same chromosome. Haploidisation of a diploid made with a master strain, which has markers one each chromosome, is a standard first step in mapping any mutant (McCully & Forbes, 1965). Mitotic recombination is more rarely used, since a mutant, once located to a chromosome can usually be mapped by meiotic linkage. However mitotic crossing over, which maps whole chromosome arms at a time, has been important in establishing the order of markers on all chromosomes, especially where there are gaps in the meiotic map (see below).

In other fungi, the most advanced parasexual cycle-based map is that of *Aspergillus niger* (Bos *et al.*, 1993), where 76 markers have been mapped on 8 chromosomes. This example, however is exceptional, and it appears that

where a genetical approach to a problem is important, many workers have chosen to switch to *A. nidulans* as a model organism, even if it is second best for the process under study. A prime example of this is study of penicillin biosynthesis, which is much less efficient in *A. nidulans* than in Penicillium chrysogenum, but has nevertheless been studied extensively in the two fungi in parallel (Luengo & Pe?lva, 1994).

Does the parasexual cycle operate in the wild? Pontecorvo considered the versatility of heterokaryons would confer a natural advantage on fungi that form them in the wild (Pontecorvo 1946). It is therefore ironic that wild strains were later found to carry a multitude of heterokaryon incompatibility alleles (e.g. Grindle, 1963) to the extent that hybrid vigour from fusion of strains with different capabilities will almost always be ruled out, and diploid formation will be equally deterred (Dales & Croft, 1977). The best explanation for the existence of these incompatibilities is they provide protection from the spread of viruses, as shown for *Aspergillus niger* strains (van Diepeningen *et al.* 1997). It leaves us, however, with the question why mycelia should fuse at all; perhaps it is an inherent property of hyphae, relating to their method of growth requiring an extensible, and therefore plastic and fusible hyphal tip.

Further studies of wild strains of *A. nidulans* suggest that this is a young species, which does not even show sufficient RFLP variation to be useful for map construction (Croft & Varga, 1994). There are, however, as stated above, many different heterokaryon incompatibility groups, whose differences are determined by at least eight loci, some of with multiple alleles (Dales *et al.*, 1993). Within each group, variation is even more restricted, and it is suggested that each group may be a vegetative clone (Geiser *et al.*, 1994). Surprisingly enough, heterokaryon incompatibility is apparently no barrier to sexual crossing (Dales *et al.*, 1993), and indeed it is suggested that new incompatibility groups arise regularly by this means (Geiser *et al.*, 1994). The picture of frequent reassortment of compatibility genes in the wild is consistent with the fact that strains compatible with the historic laboratory strains of A. nidulans have never been recovered again from the wild. What is more surprising is that the same is true of A. niger (Lhoas, 1967; Bos *et al.*, 1993): does this suggest frequent in recombination in the wild for this species group also?

Recent analysis of molecular characters in wild Australian populations of *A. flavus* (Geiser *et al*, 1998) came to two surprising conclusions: firstly that there appear to be two morphologically indistinguishable, but molecularly divergent species, and secondly that the species which was most represented in their samples, and which incidentally included type strains of *A. oryzae*, showed evidence of sexual or parasexual gene exchange in its ancestry.

Geiser *et al.* speculate that the sterile sclerotia found on some *A. flavus* colonies, may be defective versions of the sexual fruiting bodies of the related fungus Petromyces alliaceus The implications are, of course, considerable: it is evident that the population genetics of fungi is still in its infancy, and we should look harder at variation in the wild, where we may also find traditionally asexual species doing things on the quiet that we knew nothing about. In addition, the distinctness of *A. oryzae* strains is once again thrown into doubt: they are evidently different from many wild strains, especially in not producing aflatoxin (or transcribing the *aflA* regulator gene - Kusumoto *et al.*, 1998), but they are not a distinct species, and if sex does occur in the wild, gene exchange is possible.

A. nidulans entered the molecular era when it became possible to transform it (Tilburn *et al.*, 1983). This opened the way to gene cloning by complementation of mutants, which comprise the wealth of this organism.

Transforming plasmids carrying wild type homologues of a mutant gene in the recipient were found, as in yeast integrative transformation, to have three possible fates: homologous or non-homologous integration, and conversion of the resident mutant copy of the selected gene by the plasmid version, without plasmid integration. Homologous integration may also be accompanied by gene conversion, resulting in tandem wild-type or mutant copies of the gene, rather than the expected one mutant and one wild-type (Clutterbuck, unpublished). Double crossing over at the ends of linear transforming molecules also occurs with sufficient frequency to be regularly used for gene replacement. Both homologous and non-homologous integration is often of multiple tandem copies, with frequencies depending on the selective marker, e.g. it is suspected that the amdS marker, conferring ability to grow on acetamide nitrogen source, is selected for integration in multiple copies, and often at non-homologous sites, because the native gene is poorly expressed in its normal position.

Since plasmid replication was not initially found, despite incorporation into plasmids of various sequences thought likely to promote this, it was originally thought that filamentous were unable to support plasmid replication (Turner & Ballance, 1985). However, Gems *et al.* (1991) found a gene bank component, later identified as a duplication of part of a transposable element, which was able to promote replication of plasmids in its original host, *A. nidulans*, as well as other *Aspergillus* and *Penicillium* species (reviewed in Aleksenko & Clutterbuck 1997).

Cotransformation with two plasmids, one selected and the other containing a gene of interest, has been regularly employed in *A. nidulans*. Work with replicating plasmids showed that cotransforming DNAs tended to recombine with each other during transformation, and that this would happen

irrespective of whether one or both of the DNAs were circular or linear, and if linear, whether they had cohesive ends or not. It is evident that A. nidulans can readily recombine or ligate any introduced DNA fragments.

This ability was exploited by Gems *et al.* (1994) to construct an in vivo ligated gene bank, a method that has since been used to isolate a number of genes, using either homologous or heterologous DNA.

The next step in the molecular development of *A. nidulans* was the construction, by Xiong *et al.* (1996) of a physical map, i.e. a cosmid contig map. This has been produced in various versions but is still incomplete and in too many areas, inconsistent with the linkage map. Of course the linkage map is not error-free, but is less error-prone than a contig map because each new marker added to a linkage map should confirm the relative positions of its neighbours (Clutterbuck, 1997). Moreover, *A. nidulans* has the special advantage that the meiotic map is strongly supported by mitotic recombination, due greatly to the tireless work of E. Kafer (e.g. Kafer, 1977). An unusual feature of this work was the use of translocations, which can cause confusion to a meiotic map, but in a mitotic map can be used either as markers or as dividers, splitting the chromosome into segments to which a series of markers can be readily assigned.

Sequencing of one cosmid (Kupfer *et al.*, 1997) showed a gene density of about 300 genes/Mb, implying, if this is typical of the rest of the genome, a total complement of approximately 9500 genes, roughly 50% more than that of *Saccharomyces cerevisiae.* The final stage of map-making is the full genome sequence, and after some years of indecision by the Aspergillus community, the sequence is now reported to have been completed by Cerion Ltd. (W.E. Timberlake and T.H. Adams, personal communication).

It should now be possible to exploit the excellent resources of this fungus for functional exploration of its genome. In terms of applications to a wider world, *A. nidulans* has already contributed widely to the human genome sequencing project by the cloningof many genes, ranging from the novel g-tubulin, encoded by *mipA*, to the historically important alkaptonuria gene hmgA (Oakley *et al.*, 1990, Fernandez-Ca?n *et al.*, 1995). This, surely, is just the start!

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A. John Clutterbuck

1960	Kingswood School Bath Shefield Univ.	(B.S.)
1964	"	(Ph.D.)
1966-1996	Glasgow Univ.	(Lecturer)
1996-present	" .	(Senior Lecturer)

Major research field - Genetics of *Aspergillus nidulans*