data will further accelerate our understanding of the biology of *Wolbachia*.

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Fungal Genomics: Forensic Evidence of Sexual Activity

The genome sequence of the 'asexual' human pathogenic fungus *Aspergillus fumigatus* suggests it has the capability to undergo mating and meiosis. That this organism engages in clandestine sexual activity is also suggested by observations of two equally distributed complementary mating types in nature, the expression of mating type genes and evidence of recent genome recombination events.

Neil A.R. Gow

The completion of the genome sequences of three *Aspergillus* species provides a resource to explore new avenues of fungal biology and evolution. Three genome centres — the Institute for Genome Research (TIGR), The Wellcome Sanger Genome Centre and The National Institute of Technology and Evaluation (NITE) in Japan — have collaborated with three rather separate scientific communities to sequence and annotate the genomes of three filamentous fungi with contrasting claims to fame. *Aspergillus nidulans* has a long tradition as a model eukaryote, and was the organism in which many basic findings about growth and nuclear division were discovered; *A. oryzae* is an important fungus in industrial fermentation of sake, soy sauce and miso; and *A. fumigatus* is infamous as a pernicious pathogen of the immunocompromised.

On the heels of the publication of the *Aspergillus* genome sequences comes an important new biological advance, reported in this issue of *Current Biology*: Dyer and colleagues [1] have used the sequence of *A. fumigatus* to obtain evidence that this fungus, traditionally classified amongst the asexual or so-called 'imperfect' fungi, may have been holding back the truth about its sexuality. Rather than being asexual, it seems to have the genetic machinery to mate, develop a sexual fruiting body and undergo a full meiotic cycle.

The significance of this relates to the importance of *A. fumigatus* as a pathogen and difficulties in its genetic analysis. It is appropriate for this fungus to be named after its ability to produce a smoke trail, or fumus, of conidial spores, as these are the agents of infection and route of infection into the lung. The fungus is a common mould of compost and plant surfaces and it is estimated that a cubic metre of air contains 1-10 conidia, which most people will inhale with no ill effect [2]. But in the immunocompromised patient, who lacks the normally efficient surveillance of pulmonary macrophages and circulating monocytes and neutrophils, the spores germinate. The ensuing invasive filamentous growth in the lung and other body sites results in a disease which 80–90% of patients will not survive, even with best available antifungal treatments [3,4].

The severity of systemic aspergillosis has fuelled efforts to understand the nature of the virulence traits of this pathogen

and to develop new chemotherapeutic drugs. The way forward has not been easy as *A. fumigatus* is not welcoming to the molecular geneticist. Although its nuclei are haploid, transformation frequencies are poor and homologous integration for targeted gene inactivation is less efficient than in many fungi [4]. The lack of a sexual cycle prevents assessment of the influence of strain background on the phenotypes of mutants and complicates analysis of potential virulence factors. If sexual genetics were available in *A. fumigatus*, then such analyses would certainly be easier.

Consequently, Paoletti *et al.* [1] were immediately drawn to emerging sequence data that indicated *A. fumigatus* has a mating-type locus with a structure characteristic of sexual ascomycete species. The sequenced strain had a *MAT-2* genotype and contained a gene apparently coding for a high mobility group (HMG) protein. It also had homologues of other genes known in other species to be required for sexual pheromone production and detection. Some sexual species of fungal pathogens, such as *Cryptococcus neoformans*, exist predominantly in one mating-type in the environment [5]. Was the mating-type of the sequenced strain of *A. fumigatus* representative of the population structure as a whole?

Paoletti *et al.* [1] addressed this issue and found, by screening over 290 isolates from around the world, that the *MAT-2* and *MAT-1* mating-types are almost equally distributed amongst natural and clinical isolates. The *MAT-1* allele encodes the complementary α box domain required for compatible matings between sexual outbreeding — heterothallic — species. Only in France was any statistical departure from a 1:1 ratio of *MAT1-2* and *MAT1-1* strains observed.

Such a distribution is very suggestive that mating either still occurs in this species or was lost so recently that there has not yet been a change to the structure and distribution of the mating loci of wild-type strains. Further evidence for mating came from an analysis of the distribution of polymorphisms, which showed evidence for recent recombination due to sexual or parasexual mechanisms. The genome sequence also reveals the presence of a non-functional, transposable element that would require sexual activity for its propagation.

The cumulative forensic evidence for sexual activity spelt out in the genome sequence was also reinforced by the demonstration of expression in mycelial cells of the *MAT-2* and *MAT-1* genes as well as of homologues of genes known in other species to encode α-factor pheromone precursor and the pheromone receptor. Clearly, there is at least some sexual posturing in extant *A. fumigatus* strains.

There is an interesting parallel between all this and the recent history of research into another major fungal pathogen of humans, *Candida albicans*. The genome sequence again showed that this 'asexual' fungus had a mating-type locus, this time with individual fungi having the heterozygous *MAT* genotype typical of mated diploid strains of *Saccharomyces cerevisiae* [6]. Strains hemizygous for the mating locus on chromosome 5 were then selected by induced chromosome loss, or created by reverse genetics, to generate compatible strains that mated to form tetraploids [7,8].

Further work showed that the novel regulatory circuits that regulate the phenotype plasticity of *C. albicans* are controlled by the mating locus, and that this generated mating-competent 'opaque' phase cells in which *MAT*a–*MAT*α couplings occurred with equivalent efficiency to matings in *S. cerevisiae* [9–12]. Like *A. fumigatus*, *C. albicans* has the molecular machinery for meiosis, but again it has not yet been caught in the act [13].

These studies raise a number of interesting questions. Might mating and meiosis be observed between appropriate strains of *A. fumigatus* or could mating be reconstituted with the aid of some *in vitro* genetic manipulation? What are the implications of sex for virulence and molecular epidemiological studies of species of pathogenic fungi that are assumed to be clonally distributed? How many 'imperfect' fungi are really asexual?

The benefits of sexuality in generating combinations of genes fit enough to survive the stresses of a natural environment are obvious. Fungi that are self-fertile — homothallic — do exist, particularly those either highly adapted to a specialised niche or geographically isolated from potential sexual partners [14]. Neither situation would seem to fit *A. fumigatus*, and so any discovery of sex might be a surprise only to the laboratory voyeur but less so to the population ecologist. We must keep in mind that evolution has ensured the survival of *A. fumigatus* as a primary plant saprophyte and not as a human pathogen. The availability of three *Aspergillus* genomes with differing properties and life styles will, however, be a powerful resource to address questions about the ecological and sexual evolution of fungi.

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Arousal Mechanisms: Speedy Flies Don't Sleep at Night

Alertness and behavioral performance depend on an animal's level of arousal. In vertebrates, reinforcement and maintenance of arousal in the cortex are ensured by diffuse inputs from neurons releasing biogenic amine neuromodulators. Fruit flies similarly use dopamine for arousal control, indicating an ancient evolutionary origin of this essential feature of the functioning brain.

Serge Birman

The idea that the performance of complex behaviors depends on arousal levels was formulated fifty years ago by Donald Hebb [1] in a landmark paper of behavioral neuroscience. As Hebb wrote: "Physiologically, we may assume that cortical synaptic function is facilitated by the diffuse bombardment of the arousal system. When this bombardment is at a low level an increase will tend to strengthen or maintain the concurrent cortical activity. But when arousal is at a high level, the greater bombardment may interfere with the delicate adjustments involved in cue function, perhaps by facilitating irrelevant responses. Thus there will be an optimal level of arousal for effective behavior, as Schlosberg has suggested" (Figure 1A).

In the vertebrate cortex, such a 'bombardment' is ensured by diffuse afferent inputs from dopaminergic and other biogenic amine neuromodulatory neurons [2]. A new study in *Drosophila melanogaster* by Ralph Greenspan and colleagues [3], reported in this issue of *Current Biology*, shows that dopamine transmission similarly controls arousal states in the insect brain. They present evidence that, in fruit flies as in humans, an optimal level of arousal and dopamine release is required to perform complex behaviors (Figure 1B). This suggests that general features of brain functioning appeared very early in the course of animal evolution.

The starting point for this study [2] was a pharmacological analysis of the lowest state of arousal — sleep. Sleep appears to be a common feature in brain-endowed animals. But nobody knows for sure why this unconscious state of rest is essential for life, and what exactly its physiological function is at the cellular level. Even smaller animals, like the fruit fly, sleep for many hours in a day [4–6], principally during the night for this species. Drugs that reduce sleep need in humans also affect sleep in *Drosophila*. This is the case for caffeine [4,5] and the wake-promoting agents modafinil [7] and, as shown now by Andretic *et al.* [3], methamphetamine.

Methamphetamine is a potent psychostimulant, commonly named 'speed' and too widely used among college students. Long-term use has dramatic consequences, including addiction, tolerance, dementia-like behavior and brain damage. Methamphetamine acts principally by increasing dopamine release and preventing its reuptake into cells, thus strongly enhancing the effects of the neurotransmitter on target cells. Although the mechanism of action of modafinil is less clearly known, this drug may also interfere with dopamine transmission [8].

Feeding methamphetamine [3] or modafinil [7] to flies decreased both their sleep bout and cumulative sleep durations. Conversely, pharmacological inhibition of dopamine biosynthesis led to a narcoleptic-like behavior, with the flies spending a lot more time than normal sleeping during the day [3]. These observations point to an important role for dopaminergic signaling in fly arousal.

Sleep-deprived flies feel increased need for rest and tend to sleep more the next day [4,5,9]. This homeostatic regulation is characteristic of sleep in all animals. They also show reduced performance in behavior tests and heightened arousal thresholds. Andretic *et al.* [3] found that, in flies as in humans, sleep deprivation did not abolish the wake-promoting effect of methamphetamine: instead, methamphetamine feeding partially suppressed the need for sleep and rebound following rest deprivation.

A recent mutagenesis study in *Drosophila* [10] identified several short-sleeper lines as well as lines that show no sleep rebound after rest deprivation. One of the strongest phenotypes turned out to be caused by a loss-of-function mutation in *Shaker*, a gene encoding a voltage-dependent potassium channel. Lack of *Shaker* is known to increase neuronal excitability. In light of the