An Overview of the Genus *Aspergillus*

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Abstract

Aspergillus is the name used for a genus of moulds that reproduce only by asexual means. The morphology of the conidiophore, the structure that bears asexual spores, is the most important taxonomic character used in *Aspergillus* taxonomy. *Aspergillus* species are common and widespread. They are among the most successful groups of moulds with important roles in natural ecosystems and the human economy.

Among scientists working on *Aspergillus,* there is a continuing fascination with their biotechnological potential. In addition to producing numerous useful extracellular enzymes and organic acids, these moulds also produce secondary metabolites of importance in biotechnology.

Some *Aspergillus* species function as plant and/or animal pathogens. Aspergillosis is the name given to all animal diseases caused by growth of any member of the genus on a living host. Immunosuppression is generally a prerequisite for systemic *Aspergillus* infections in humans. The incidence of systemic aspergillosis, the most serious form, is on the rise and imposes an increasing medical burden upon hospitals and physicians. Better antifungal drugs and diagnostic methods are needed.

Advances in *Aspergillus* genomics are giving us new tools for understanding this extremely diverse genus. Hitherto undiscovered sexual stages have been discovered based on findings from genomics. Molecular biologists are trying to understand the mechanisms by which pathogenicity and sexuality work and to deconstruct the physiological pathways that are central to these processes. Evolutionary biologists are focusing on the forces that drive variation within and among population. Economically important species are being re-tested for new capabilities using new screens developed with the aid of post genomic technologies.

Introduction

One of the oldest named genera of fungi, *Aspergillus* received its name from Micheli in 1729. In viewing the microscopic spore-bearing structure, Micheli was reminded of a device used by the Roman Catholic clergy to sprinkle holy water during a part of the liturgy called the *asperges* (Ainsworth, 1976). The *asperges* was described as follows in the 11th edition of *The Encyclopaedia Britannica*:

> **ASPERGES** *('thou wilt sprinkle,' from the Latin verb aspergere), the ceremony of the people with holy water before High Mass in the Roman Catholic Church … with which the priest begins the ceremony. The brush used for sprinkling is an aspergill (aspergillum)…'*
>
> (Anonymous, 1910)

By the time Thom and Church published the first major monograph on the genus in 1926, *Aspergillus* had become one of the best-known and most studied mould groups. Their prevalence in the natural environment, their ease of cultivation on laboratory media and the economic importance of several of its species ensured that many mycologists and industrial microbiologists were attracted to their study. Aspergilli grow

abundantly as saprophytes on decaying vegetation where have been found in large numbers from mouldy hay, organic compost piles, leaf litter and the like. Most species are adapted for the degradation of complex plant polymers, but they can also dine on substrates as diverse as dung, human tissues, and antique parchment (Polacheck *et al.,* 1989). There is even a report of an unidentified *Aspergillus* species being capable of the solubilization of low rank coal (Torzilli and Isbister, 1994).

Furthermore, this common mould is involved in many industrial processes including enzymes (e.g. amylases), commodity chemicals (e.g. citric acid) and food stuffs (e.g. soy sauce). In classical fungal genetics, one species *Aspergillus (Emericella) nidulans* has been used to elucidate the parasexual cycle, as well as to understand basic concepts in regulation of metabolic pathways, the cell cycle, intron splicing and hyphal polarity. Several species contaminate grains and other foods with toxic metabolites that are a threat to the health of humans and other animals. Certain *Aspergillus* species also can infect humans directly causing both localized and systemic infections, especially in immunocompromised individuals.

Physiology and ecology

Aspergillus spores are common components of aerosols where they drift on air currents, dispersing themselves both short and long distances depending on environmental conditions. When the spores come in contact with a solid or liquid surface, they are deposited and if conditions of moisture are right, they germinate (Kanaani *et al.,* 2008). The ability to disperse globally in air currents and to grow almost anywhere when appropriate food and water are available means that 'ubiquitous' is among the most common adjectives used to describe these moulds.

One of the defining characteristics of the entire fungal kingdom is its distinctive nutritional strategy. These organisms secrete acids and enzymes into the surrounding environment, breaking down polymeric molecules down into simpler ones that are then absorbed back into the fungal cell. Fungi, like animals, are heterotrophic. While animals eat their food and then digest it, fungi first digest their food and then 'eat' it. Gaining access to nutrients is aided by mechanical forces whereby fungal hyphal tips grow into and through their food substrates. Species of *Aspergillus* are typical examples of the fungal life style. They are most often found in terrestrial habitats and are commonly isolated from soil and associated plant litter. The decomposition process carried out by these moulds is important in driving natural cycling of chemical elements, particularly in the carbon cycle where they contribute to replenishment of the supply of carbon dioxide and other inorganic compounds (Carroll and Wicklow, 1992).

In the ecosystem, different substrates are attacked at different rates by consortia of organisms from different kingdoms. *Aspergillus* and other moulds play an important role in these consortia because they are adept at recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers. Some aspergilli are capable of degrading more refractory compounds such as fats, oils, chitin, and keratin. Maximum decomposition occurs when there is sufficient nitrogen, phosphorus and other essential inorganic nutrients. Fungi also provide food for many soil organisms (Carroll and Wicklow, 1992).

For *Aspergillus* the process of degradation is the means of obtaining nutrients. When these moulds degrade human-made substrates, the process usually is called biodeterioration. Both paper and textiles (cotton, jute, and linen) are particularly vulnerable to *Aspergillus* degradation. Our artistic heritage is also subject to *Aspergillus* assault. To give but one example, after Florence in Italy flooded in 1969, 74% of the isolates from a damaged Ghirlandaio fresco in the Ognissanti church were *Aspergillus versicolor* (Ciferri, 1999).

Similarly, foods utilized by humans and our domestic animals are good nutritional sources for *Aspergillus*. Words like 'decay,' 'rot' and 'spoilage' are used to describe such fungal utilization of our foodstuffs, which can occur in the field prior to harvest, during storage, and after commercial processing or cooking in the home. Although foods with an acidic pH, dried foods, and those with a high concentration of sugars such as jams and jellies normally do not support microbial growth many members of the *A. glaucus* group (*Eurotium*) are able to grow at low water activity. These species even have been isolated from salted, dry fish (Hocking, 1993). In the same way, grains, nuts and spices, all of which have relatively low

amounts of water, regularly are attacked by moderately xerophilic species of *Aspergillus* (Lacey, 1994).

Taxonomy

The great English bacteriologist, Samuel Cowan, once wrote of 'The Trinity that is Taxonomy': identification, nomenclature, and classification. The genus *Aspergillus* poses taxonomic problems in all three realms. Important manuals and monographs devoted to *Aspergillus* taxonomy are listed in Table 1.1.

Identification and intrageneric categories

The defining characteristic of the genus *Aspergillus* is the aspergillum-like spore-bearing structure. It is the most important microscopic character used in *Aspergillus* taxonomy. During mycelial differentiation certain cells enlarge, develop a heavy cell wall and form 'T' or 'L' shaped 'foot cells' (which are not separate cells) that produce a single conidiophore perpendicular to the long axis of the cell. Sometimes it is difficult to see the foot 'cell,' but when visible, morphologists take it as strong evidence that an isolate is an *Aspergillus* species. The erect hyphal branch developing from the foot cell is the conidiophore, which enlarges at its apex to form a rounded, elliptical or club shaped vesicle. The fertile area of the vesicle gives rise to a layer of cells called phialides (or steigmata in the older literature) that produce long chains of mitotic spores called conidia or conidiospores. The size and arrangement of the conidial heads as well as the colour of the spores they bear are important identifying characteristics. For example, species in the *A. niger* group bear black spores, the *A. ochraceus* group is yellow to brown, while *A. fumigatus*, *A. nidulans*, and *A. flavus* are green.

The major cultural features used in species identification are the colour of the colony, the growth rate and thermotolerance. Aspergilli have varying morphological and growth response to different nutrients so it is important to standardize conditions. Species identification depends upon pure cultures grown on known media. The early taxonomic micrographs used a defined medium adapted from Czapek by Dox, often called 'Czapek–Dox medium' which contains sucrose as the carbon source and nitrate as the nitrogen source (Raper and Fennell, 1965). Strain variation is quite extensive within species and a variety of subtle effects such as air exchange, light and volume of the medium can affect morphology

Table 1.1 Important taxonomic treatments and identification manuals for the genus *Aspergillus*

Year	Reference
1926	Thom, C., and Church, M. The Aspergilli (Baltimore: Williams & Wilkins)
1954	Thom, C., and Raper, K.B. A Manual of the Aspergilli (Baltimore: Williams & Wilkins)
1965	Raper, K.B., and Fennell, D.I. The Genus *Aspergillus* (Baltimore: Williams & Wilkins)
1985	Samson, R.A., and Pitt, J.I. Advances in *Penicillium* and *Aspergillus* Systematics (New York: Plenum)
1988	Klich, M.A., and Pitt, J.I. A Laboratory Guide to Common *Aspergillus* Species and Their Teleomorphs (North Ryde, Australia: Division of Food Processing).
1989	Kozakiewicz, A. *Aspergillus* Species on Stored Products (Wallingford: CAB International)
1990	Samson, R.A, and Pitt, J.I. eds. Modern Concepts in *Penicillium* and *Aspergillus* Classification (New York: Plenum Press)
2000	Samson, R.A, and J. I. Pitt, eds. Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* classification (Amsterdam: Harwood Academic Publications)
2002	Klich, M.A. Identification of Common *Aspergillus* Species (Utrecht: Centraalbureau voor Schimmelcultures).
2008	Samson, R.A., and Varga, J. *Aspergillus* systematics in the genomic era (Utrecht: CBS Fungal Biodiversity Centre)

(Okuda *et al.,* 2000). Contemporary taxonomists usually grow strains on several media, at several temperatures, to identify species (Klich, 2006).

In addition to the conidiophore, other morphological structures useful for identification include cleistothecia, Hűlle cells and sclerotia. Both cleistothecia and sclerotia are closed and usually round structures about the size of a poppy seed that may be so abundant as to dominate a colony. Cleistothecia are the sexual reproductive stage and contain the meiotic ascospores borne within asci. In some of the early monographs (e.g. Thom and Church, 1926; Thom and Raper, 1945) cleistothecia are called perithecia; this latter term is now limited to ascus-bearing bodies that are open at one end such as those produced by *Neurospora crassa*. Hűlle cells are thickened, often globose, cells that are associated with cleistothecia.. Sclerotia are rounded masses of mycelium with an outer melanized rind that macroscopically resemble cleistothecia but do not contain sexual spores. They are believed to serve as resting structures that allow species to survive adverse growth conditions. Finally, some taxonomists supplement these major characters with biochemical characteristics such as secondary metabolite production or ultramicroscopic traits such as spore ornamentation (Kozakiewicz, 1989).

During the 20th century, as mycologists isolated and identified increasing numbers of isolates, the number of named species of *Aspergillus* increased. These tended to fall into morphologically distinct clusters so in order to facilitate identification the genus was divided into intrageneric 'groups' by Thom and Church (1926), Thom and Raper (1945) and Raper and Fennell (1965). For example, the *Aspergillus glaucus* group was characterized by abundant, typically green conidial heads, with perithecia (*sic*) generally present while the *Aspergillus ochraceous* group had yellow conidia and abundant cream to purplish-coloured sclerotia (Thom and Raper, 1945). Unfortunately, the term 'group' does not have nomenclatural status within the formal rules of biological nomenclature. Gams *et al.* (1985) therefore reorganized the genus into a new subgeneric taxonomic hierarchy based on 'sections.' In this system, the subgenus '*Aspergillus*' consists of xerophilic species. *A. glaucus* is the type species, classified in the subgenus *Aspergillus* and the section *Aspergillus*. They introduced a new subgenus called Circumdata that encompassed seven sections, in which 'section circumdati' was the new rubric for the old '*A. ochraceous* group' (Gams *et al.,* 1985). The attempted imposition of subgeneric epithets, only one of which is called '*Aspergillus,*' is extremely confusing and has not caught on. On the other hand, most taxonomists now use the term 'section' rather than 'group' for *Aspergillus* intrageneric classifications and identifications.

Nomenclature

Moulds that produce a characteristic asexual spore head that looks like an aspergillum are placed together in the genus *Aspergillus*. There are approximately 250 named species of *Aspergillus* (Geiser *et al.,* 2008). This number is likely to increase significantly in the near future because of increasing application of the phylogenetic species concept based on DNA sequence data rather than on visible morphological characters. Many *Aspergillus* species and strains have industrial importance, so the delineation of species in this genus is frequently a matter of contention in the patent world. The widespread acceptance of 'phylogenetic species' undoubtedly will have future legal ramifications.

In 1854, early in the scientific history of this genus, deBary became the first person to notice that an *Aspergillus* mycelium could produce a cleistothecium as well as an aspergillum. DeBary's observation was significant because the cleistothecium-producing mould had been observed before and given its own name: *Eurotium herborarium*. When DeBary realized that *A. glaucum and E. herborarium* were different reproductive phases of the same organism, it was an important milestone in mycology and also the beginning of a nomenclatural predicament that persists until this day. Since deBary's original observation, many other fungal species both within and without the genus *Aspergillus* have been named on the basis of their asexual stage and then 'connected' ('linked') to a sexual stage with a different genus and species name. Which name should be used? Early taxonomists concluded that both names were valid and instituted a system of dual naming.

With the exception of fossils, fungi are the only organisms that depart from one of the basic rules of biological nomenclature, i.e. that each

taxonomic group can bear only one correct name. Since 1905, the Botanical Code (which governs the naming of plants and fungi) has allowed two different names to be applied to the same organism, depending on whether it is viewed in its sexual or asexual stage. Under this system of taxonomic governance, Article 59 permits dual nomenclature. When a sexual phase is known, the name for this phase takes precedence. According to these rules, *Aspergillus nidulans*, the well-known genetic model, should be called *Emericella nidulans*. Indeed, it is indexed as such within GenBank. However, most popular and professional usage continues to call it *Aspergillus*. A Google search in late 2008 under the entry '*Aspergillus nidulans*' yielded 725,000 hits while '*Emericella nidulans*' gave fewer than 60,000, a difference of more than an order of magnitude.

Some *Aspergillus* species regularly produce both sexual and asexual spores; in other species the sexual form is rare; for still others, sexual spores have never been seen – and perhaps never will be seen. The names used for currently accepted sexual genera with close phylogenetic relationship or known linkage to *Aspergillus* species (representative *Aspergillus* species given in parenthesis) are: *Chaetosartorya* (*A. wentii*); *Emericella* (*A. nidulans*); *Eurotium* (*A. glaucus*); *Fennellia* (*A. terreus*); *Hemicarpenteles* (*A. paradoxus*); *Neocarpenteles* (*A. clavatus*); *Neosartorya* (*A. fumigatus*); *Petromyces* (*A. flavus and* possibly *A. niger); Sclerocleista* (*A. ornatus*); and *Warcupiella* (*A. spinnulosus*) (Geiser, 2008). Rigid interpreters of nomenclatural rules believe that it is wrong to use the name *Aspergillus* to denote a fungus with a known sexual stage and that only the generic name associated with the sexual stage should be used. Nevertheless, outside the community of fungal taxonomy and 'strict interpreters' of the Botanical Code, the proliferation of names for a genus that most biologists know as *Aspergillus* causes bewilderment. Dual nomenclature is also a major problem for information retrieval.

The proliferation of arcane terminology is also problematic. For many years, mycologists called those fungi incapable of forming sexual spores 'imperfect' while the sexual forms were called 'perfect.' Another term often encountered was 'pleomorphic,' referring to that fact that these organisms could take more than one form. ('Pleomorphism' is particularly confusing because it is sometimes used to describe the yeast-mycelial phase transition characteristic of some medically important fungal, a transition that is more properly called 'dimorphism [Woods, 2002]). When an 'imperfect' fungus also was found to be 'perfect' additional problems of etymology developed surrounding the use of the terms 'asexual phase/stage/state' and 'sexual phase/stage/state.' All of these terms were used inconsistently and interpreted differently. For this reason, during the late 1970s, Hennebert and Weresub introduced a new set of terminology whereby *anamorph* refers to the asexual, mitotic-spore-bearing morphological phase; *teleomorph* refers to the sexual meiotic-spore-bearing morphological phase; while *holomorph* refers to the 'whole fungus' (Hennebert and Weresub, 1979; Weresub and Hennebert, 1979). In 1981, this terminology was accepted by the Botanical Congress. Both 'anamorph' and 'teleomorph' have gained widespread acceptance; however, 'holomorph' has had less linguistic success.

See Bennett (1985) for a more detailed description of the early history and jargon surrounding dual nomenclature and an analysis of the accompanying logical and practical dilemmas that is poses. The edited volume by Reynolds and Taylor (1993) contains a number of well referenced and pertinent essays about the 'fungal holomorph.' The quasi-legalistic challenges posed by the use of dual nomenclature is given a very detailed analysis in a 50+ page article by Hennebert and Gams (2002) entitled 'Possibilities to amend or delete Article 59 of the International Code of Botanical Nomenclature to achieve a unified nomenclature and classification of the fungi.'

In summary, many *Aspergillus* species have two names, an exception to most rules of scientific nomenclature that is nevertheless legal under the Botanical Code. This mycological exceptionalism has caused regular and heated debates ever since the Botanical Code was formulated. Different generations of taxonomists have revised the rules pertaining to fungal nomenclature as better data have come along that inform our understanding of fungal speciation. These revisions will continue to be made into the foreseeable future. Nevertheless, there are experts who seem to forget that rigid

rules and neat categories don't always mesh with the variability of living systems and the realities of human language usage. Sometimes nomenclatural decisions go against common sense. One rationale for biological nomenclature is to provide stability. Another is to aid communication. Proliferation of jargon and frequent changes of specific and generic epithets do neither.

Suprageneric classification

Aspergillus presents several classification difficulties that are intertwined with its nomenclatural intricacies.

A form taxon (pl. form taxa) is a group, used for convenience, whose evolutionary and other relationships are not known. Form genera are used in classifying fossil plants, but they also have had widespread application in the history of *Aspergillus* classification. For a long time, all asexual (anamorphic) fungal species were classified into a form group commonly called the 'fungi imperfecti' or, more technically, Fungi Imperfecti. Depending on the author, the group was ranked as a class (Deuteromycetes) or a subdivision/subphylum (Deuteromycotina). The fungi imperfecti sometimes were subdivided into form classes in which the moulds were grouped together as Hypomycetes (see for example, Ainsworth, 1973; Alexopoulos and Mim, 1979). These 'artificial' rubrics were used to encompass asexual groups, while other higher order 'natural' categories were used to categorize species that produce by sexual means. Since all taxonomic classification schemes are human constructs designed to catalogue living things, many philosophical and practical objections are inherent in the concept of 'natural categories (Hey, 2001).

Nevertheless, modern taxonomists seek a more 'natural' classification scheme based on evolutionary criteria. Such phylogenetic classifications seem more possible now that traditional morphological markers have been supplemented with nucleic acid sequences. In particular, DNA markers such as ITS regions or conserved genes such as calmodulin, tubulin or RNA polymerase are used to conduct molecular phylogenetic studies. Species with few nucleotide differences are considered more closely related than those with many. Taxonomists compare the sequence differences between species and use computer programs to come up with a hierarchy of evolutionary relationships. Despite the fact that such gene trees – especially those based on single genes – are error prone, despite the fact that different genes evolve at different rates, and despite the fact that horizontal gene transfer can muddy phylogenetic analysis, such analyses are largely robust and have revolutionized all taxonomic systems. It now seems likely that the aspergilli are all descended from one common ancestor, i.e. they constitute a monophyletic group. It is believed that that the ancestral form was sexually producing and that independent losses of sexual reproduction occurred repeatedly.

These molecular phylogenetic analyses have ramifications for both classification and nomenclature. It can be assumed that fungi called *Aspergillus* are descended from the same ancestral groups as their named teleomorphs. From the perspective of classification, it means that the old form taxa categories, (i.e. the fungi imperfecti and the Deuteromycotina) can be retired. In theory, mycologists have done so and these taxa are no longer formally accepted. *Aspergillus* species now are classified firmly within the Ascomycota (Geiser *et al.,* 2006). However, in practice the old terms for form taxa are still widely used and applied. At the time of this writing (late 2008), a Google search revealed 122,000 hits for 'fungi imperfecti' and 87,500 for 'Deuteromycotina.' '

The clear placement of all *Aspergillus* species within the Ascomycete lineage, along with the 10 named *Aspergillus* teleomorphs, has led some taxonomists to suggest that they all could be placed within a teleomorphic nomenclatural system, even without any sexual structure having been found. Nonetheless, the question remains: Are all *Aspergillus* species anamorphs of some kind of ascomycete? Should anamorphic *Aspergillus* species be classified within, and named for, a teleomorphic genus based on DNA evidence even when they lack a known teleomorph? These questions raise both theoretical and practical issues. Suppose the teleomorph does not exist? Suppose some large and successful groups of aspergilli have evolved a purely clonal and asexual form of reproduction? Does it make sense to rename them based on a morphological structure they do not possess? Caution and conservatism counsel the retention of both dual

nomenclature and the genus name *Aspergillus* (Peterson, 2008).

Commentary on taxonomy

Living things evolve. Natural systems are messy. Groups of closely related organisms do not always lend themselves to tidy classification schemes. The human need for neat categories coupled with an unfortunate tendency on the part of some scientists to view the Botanical Code as 'etched in stone,' means that over zealous interpretations of the Botanical Code can trump reasonableness and practicality. The Code is authored by human beings and necessarily is open to changes as new discoveries are made. For the sake of stability, these changes are made slowly and usually after considerable debate within the scientific community. Nonetheless it is important to remember that the debate mostly occurs between a relatively small number of people who are involved in policing the fine points of biological nomenclature. Even among this small group, it is not always easy to gain consensus. For example, when a assemblage of prominent mycologists gathered at an important meeting held in Newport, Oregon, in 1992, they discussed the provisions of Article 59 in detail. At the end of the discussion, 19 people voted to retain the dual system; 17 wanted to integrate mitotic and meiotic fungi into the same genera, and only five would accept the use of the teleomorph name for both morphs (Reynolds and Taylor, 1993; Hawksworth, 2004). Similarly, in 2007, an international workshop with 39 experts was held in Utrecht, the Netherlands on '*Aspergillus* systematics in the genomics era.' In the light of the overwhelming molecular data placing *Aspergillus* among the Ascomycetes, the question of dual nomenclature was revisited. In the final vote, the majority of participants decided that it was best to retain dual names 'where necessary' (Samson *et al.,* 2008). It is likely that naming and classifying *Aspergillus* species – and many other anamorphic fungi – will remain a contentious area within mycology for the foreseeable future.

Mating type loci and sex

A sexual stage typical of Ascomycetes is known for about a third of the described species of *Aspergillus*. The vast majority of these ascomycetous *Aspergillus* teleomorphs use a homothallic (self-fertile) system for crossing. Some scientists believe that all species of *Aspergillus* have the potential for forming a sexual stage and that heterothallism is common but occult. For example, one wide-ranging study of genetic loci in anamorphic populations yielded more variability than expected from clonal populations, indicating recombination and possible sexual reproduction (Geiser *et al.,* 1996).

In ascomycetes, sexual compatibility is determined by mating type loci designated MAT1-1 (or MAT-1) and MAT1-2 (or MAT-2). MAT-1 encodes a protein for an α-box motif and MAT-2 encodes a high mobility group (HMG) protein. In strains of heterothallic species bearing different mating types, the loci occupy the same chromosomal location, although they have different DNA sequences. Such genes have been termed idiomorphs (Metzenberg and Glass, 1990). The MAT proteins act as transcription factors that enable the formation of teleomorphic forms (Coppin *et al.,* 1997).

Until recently, the only way to connect asexual and sexual stages of a given fungus was to see the characteristic morphological structures in the same culture. Thus, a pure culture (which by definition would consist of a single mating type) of a heterothallic mould will never form a sexual stage. Genome studies have revealed MAT loci within several strictly anamorphic species of *Aspergillus.* Moreover, these genomes also posses the genes required for ascocarp development, meiosis and other aspects of sexual reproduction (Galagan *et al.,* 2005; Nierman *et al.,* 2005). The uncovering of these genes led to the hypothesis that *Aspergillus* species such as *A. fumigatus* are anamorphs of ascomycetes for which the teleomorph had not yet been seen because of cryptic heterothallism (Poggeler, 2002; Dyer and Paoletti, 2005). *Neosartorya fischeri*, a teleomorphic species that is closely related to *A. fumigatus*, is homothallic with both MAT1 and MAT2 loci within the same genome, but apparently on separate chromosomes (Rydholm *et al.,* 2007). The location of MAT genes on different chromosomes also is characteristic of *A. nidulans* (Galagan *et al.,* 2005).

Recent findings have supported the theory that heterothallism is more common among aspergilli than hitherto has been suspected.

O'Gorman *et al* (2009) studied an environmental population of *A. fumigatus* isolates from air samples taken from 91 locations in Dublin, Ireland. They found that there was a 1:1 ratio of MAT-1 to MAT-2 isolates within these strains. A subset of six MAT-1 and six MAT-2 isolates was crossed in all possible combinations on a variety of media and incubated at several temperatures. Cleistothecia were observed in pairings grown on Parafilm-sealed oatmeal agar plates and incubated at 30°C in the dark. The teleomorph was named *Neosartorya fumigata* (O'Gormon *et al.,* 2008).

Similar results were reported almost simultaneously for *A. parasiticus*, a highly aflatoxingenic species. Guided by the discovery of opposite mating type genes within a population of *A. parasiticus* from Georgia (Ramirez-Prado *et al.,* 2008) strains with opposite mating types were crossed. Following 'a lengthy incubation' (no details about media or incubation conditions for the crosses were given) indehiscent ascocarps were found embedded in the matrix of 'sclerotia.' (Such sclerotia that bear ascospores are better called ascocarps.) The sexual stage of *A. parasiticus* was placed in the teleomorph genus *Petromyces* (Horn *et al.,* 2008).

A. fumigatus and *A. parasiticus* are species that have been studied intensely for decades. The discovery of sexuality for both species within such a short period of time makes it likely that other reports of heterothallism within *Aspergillus* will be forthcoming. The ability to identify MAT genes means that in the future it will be much easier set up crosses between strains of opposite mating type and then to design appropriate environmental conditions for inducing the sexual stage.

Industrial impact and biotechnology

Primary and secondary metabolites

Species within the genus *Aspergillus* have a large chemical repertoire. Commodity products produced in *Aspergillus* cell 'factories' include citric, gluconic, itaconic and kojic acid. The use of *Aspergillus niger* in citric acid production dates back to Currie (1917); the history of the development of the fermentation process from stationary cultures to submerged fermentation has been reviewed by Bentley and Bennett (2008). Citric acid is one of the most widely used food ingredients. It also has found use in the pharmaceutical and cosmetic industries as an acidulant and for aiding in the dissolution of active ingredients. Other technical applications of citric acid are as a hardener in adhesive and for retarding the setting of concrete (Brooke, 1994). Citric acid is a true 'bulk chemical' with an estimated production approximating more than 1.6 billion kg each year (Dodds and Gross, 2007) *A. niger* also has found use in the industrial production of gluconic acid, which is used as an additive in certain metal cleaning applications, as well as for the therapy for calcium and iron deficiencies. *Aspergillus terreus* is used for itaconic acid production, a synthetic polymer. *A. oryzae* is fermented for kojic acid production which is used for skin whitening and as a precursor for synthesis of flavour enhancers (Ruijter *et al.*, 2002).

Several *Aspergillus* secondary metabolites also have major economic importance of which the statins and their derivatives are most profitable (Tobert, 2003). These cholesterol lowering drugs are now among the mostly widely used medicines. The first statin, mevastatin from *Penicillium citrinum*, was discovered by Endo and colleagues in Japan; for his finding, Endo won the Lasker Prize in 2008. A group of scientists at Merck Inc. in the USA developed the first statin approved for human use, lovastatin, a secondary metabolite isolated from *Aspergillus terreus* (Alberts, 1998). Lovastatin was sold under the brand named Mevacor™ and became a blockbuster drug for Merck (Shu, 1998). The statins are merely one family of useful, biologically-active secondary metabolites isolated from *Aspergillus*. Other compounds with pharmacological activities include cholecystokinin and neurokinin antagonists, ion channel ligands, antifungal drugs and a host of other compounds. A useful comprehensive review of fungal drug discovery is provided by An (2005) in *Handbook of Industrial Mycology*.

Food fermentations and enzymes

Food fermentations are ancient technologies that harness microorganisms and their enzymes to improve the human diet. Fermented foods keep better, have enhanced flavours, textures and aromas, and may also possess certain health benefits including superior digestibility. For Buddhists

and other vegetarians, fermented foods serve as palatable, protein-rich meat substitutes.

Asian cuisines rely on a large repertoire of fermented foods. In particular, *Aspergillus oryzae* and *A. sojae*, sometimes called koji moulds, are employed in many ways (Abe and Gomi, 2008). Their hydrolytic enzymes suit them for growth on starch and other carbohydrate-rich substrates (Bennett, 2001a). In the koji process, fungal enzymes perform the same function as the malting enzymes used in the beer fermentations of western cultures. The koji moulds release amylases that break down rice starch which in turn can be fermented to make rice wine. Fermented rice beverages have numerous local variations and names depending on country and region. Rice wine is called *shaoshing* in parts of China, *sake* in Japan, *takj* or *yakju* in Korea, as well as by many other names across Asia (Nout and Aidoo, 2002).

The koji moulds are also effective in a variety of legume fermentations of which miso and soy sauce are best known (Reddy *et al.*, 1986). Miso is a mixture of soybeans and cereals usually used to flavour soups (Shibasaki and Hesseltine, 1962). Soy sauce is a flavourful, salty liquid sauce made from soybeans that have been fermented by koji moulds, yeasts, as well as several halophilic bacteria. Other names for soy sauce include jiangyou (China), *makjang* and *kaniang* (Korea), *toyo* (Philippines) and *si-iu* (Thailand) (Yong and Wood, 1974; Steinkraus, 1983).

The first patent on a purified microbial enzyme was awarded to Jokichi Takamine in 1894. A Japanese-born chemist and entrepreneur who spent most of his adult life in the USA, Takamine eventually established companies in New Jersey and New York to produce bulk enzymes from *Aspergillus* and other microbes (Bennett, 2001b). Modern commercial enzymology is a thriving bio-based business in which *A. niger* and *A. oryzae* are among the major producers for hydrolytic enzymes (Godfrey and West, 1996). As high capacity secretors, their extracellular enzymes easily can be exploited for the production of enzymes used in the baking, beverage and brewing industries; in making animals feeds; and in the paper pulping industry. *A. niger* has been developed as an efficient host for the production of heterologous proteins using genetic engineering techniques (Archer and Turner, 2006). *A. oryzae* also has been extensively engineered (Kitamoto, 2002).

New and more extensive uses for fungal enzymes are envisioned in contemporary biotechnology because experts on energy policy are focusing on 'green' methods of biomass transformation. Plant-derived biomass theoretically could replace petrochemical feedstocks for certain chemical processes. Moulds have numerous enzymes that can turn complex polymers into sugars, lipids and other simpler molecules that can be used for fuels and chemical synthesis. Although much of the research has focused on the genus *Trichoderma*, *Aspergillus* represents a huge potential for finding new enzymes that could be used to convert plant biomass into fuels and other industrially useful products (Baker *et al.*, 2008).

Aspergillus and animal disease

Aerosolized *Aspergillus* spores are found nearly everywhere so we are routinely and almost constantly exposed to them. Such exposure is a normal part of the human condition and generally poses no adverse health effects. Nevertheless, *Aspergillus* can and does cause animal disease in three major ways: through the production of mycotoxins; through induction of allergenic responses; and through localized or systemic infections. With the latter two categories, the immune status of the host is pivotal. Allergies and asthma are thought to be caused by an active host immune response against the presence of fungal spores or hyphae. In contrast, with invasive aspergillosis, the immune system has collapsed and little or no defence can be mounted.

Allergies and asthma

Atopy is a genetic predisposition to developing certain hypersensitivity reactions such as asthma, hay fever (allergic rhinitis), and food allergies. Allergic reactions to *Aspergillus* in atopic individuals can be caused by fungal spores in the air and from fungi ingested in food. Airborne spores are readily inhaled when we breathe; they also come into contact with the eyes and other exposed parts of the body. Moulds are involved in the initiation and exacerbation of lower airway diseases such as asthma, although the specific aetiology is poorly understood (Deltino *et al.*, 1996; Bush *et al.*, 2006). The literature on the relationship between

indoor moulds and asthma was reviewed by Jaakola and Jaakola (2004).

The level of spore inhalation varies enormously with local environmental conditions. Certain environments like compost heaps and barns have unusually high concentrations. Massive exposure, even among individuals who are not allergic or asthmatic, should be avoided because repeated contact with large doses of fungal spores may induce allergic alveolitis, in which a lymphocyte-directed hypersensitivity reaction occurs. Extrinsic allergic alveolitis is not limited to *Aspergillus* or even fungal spores, but is caused by the inhalation of antigenic dusts. These dusts are rarely if ever composed of one organic substance but tend to be a variable mixture of fungal and actinomycete spores, animal proteins and other organic matter. 'Occupational mycoses' are forms of extrinsic allergic alveolitis They are all inflammatory reactions caused by breathing high concentrations of mould spores and other antigenic organic matter. Some forms of these ill-defined human diseases have been associated particularly with exposure to high concentrations of *Aspergillus* spores, including farmer's lung, malt worker's lung, compost lung and bird fancier's lung. Malt worker's lung, one of the best known of these, is an occupational mycosis encountered during beer manufacture correlated with inhalation of high concentrations of *Aspergillus clavatus* and *A. fumigatus* spores from contaminated barley (Salvaggio, 2006). The *Aspergillus* website (http://www.aspergillus.org.uk/) is an excellent source of information on these and other *Aspergillus*-related diseases, and posts many of the original historical publications.

Mycotoxins

In agriculture, *Aspergillus* originally was considered a serious problem largely because of its prevalence in the biodeterioration of stored crops and as an opportunistic pathogen of field crops, particularly under high moisture conditions (Christensen and Kaufman, 1969). During the early 1960s, the discovery of aflatoxins associated with massive deaths of poultry, trout and other domesticated animals species worldwide raised new awareness that these fungi posed threats to foods and feeds beyond their ability to rot plant materials (Goldblatt, 1969). Research on aflatoxins led to a so-called 'golden age' of mycotoxin research during which many new fungal toxins were discovered from species of *Aspergillus* and other common moulds. In addition to aflatoxins, other important *Aspergillus* mycotoxins include ochratoxin, patulin and fumigillin (Cole and Cox, 1981; Bennett and Klich, 2005).

Aflatoxins are still recognized as the most important mycotoxins. They are synthesized by only a few *Aspergillus* species of which *A. flavus* and *A. parasiticus* are the most problematic. The expression of aflatoxin-related diseases is influenced by factors such as age, nutrition, sex, species and the possibility of concurrent exposure to other toxins. The main target organ in mammals is the liver so aflatoxicosis is primarily a hepatic disease. Conditions increasing the likelihood of aflatoxicosis in humans include limited availability of food, environmental conditions that favour mould growth on foodstuffs, and lack of regulatory systems for aflatoxin monitoring and control (Henry *et al.,* 1999; Williams *et al.,* 2004).

A. flavus and *A. parasiticus* are weedy moulds that grow on a large number of substrates, particularly under high moisture conditions. Aflatoxins have been isolated from all major cereal crops, and from sources as diverse as peanut butter and marijuana. The staple commodities regularly contaminated with aflatoxins include cassava, chillies, corn, cotton seed, millet, peanuts, rice, sorghum, sunflower seeds, tree nuts, wheat, and a variety of spices intended for human or animal food use (Weidenborner, 2001). When processed, aflatoxins get into the general food supply where they have been found in both pet and human foods as well as in feedstocks for agricultural animals. Aflatoxin transformation products are sometimes found in eggs, milk products and meat when animals are fed contaminated grains (Eaton and Groopmen, 2003; Bennett and Klich, 2005).

Human exposure to aflatoxins exposure is difficult to avoid because *A. flavus* grows aggressively in many foods at all stages of the food chain: in the field, in storage and in the home (deVries *et al.,* 2002). Evidence for acute human aflatoxicosis has been reported from several underdeveloped countries such as India and Thailand. The symptoms of severe aflatoxicosis include oedema, hemorrhagic necrosis of the liver and profound lethargy (Williams *et al.,* 2004). Further, aflatox-

ins are potent carcinogens, especially aflatoxin B_1. Based on epidemiological studies done in Asian and Africa, in 1988 the International Agency for Research on Cancer, part of the World Health Organization, placed aflatoxin B_1 on the list of human carcinogens (Moy, 1998). In developed countries, the emphasis on keeping aflatoxin out of the food chain concerns its carcinogenic potential. Strong regulatory limits (4–30 ppb) have been established for many commodities (Henry *et al.,* 2002).

Infections. Aspergillus fumigatus and other species capable of growing at 37°C are opportunistic pathogens. About 40 of the 250 species of *Aspergillus* have been reported as human pathogens (Klich, 2006) but the majority of cases are associated with just one species: *A. fumigatus* (Latge, 1999; Latge and Steinbach, 2008). The respiratory system is the normal portal of entry.

The animal diseases caused by *Aspergillus* infection are all lumped together under the term 'aspergillosis.' Aspergillosis is a 'big umbrella' term, with an accompanying enormous literature. It refers to all infections caused by *Aspergillus*, including both veterinary and human disease, and ranging from localized and minor maladies to those that are systemic and life threatening. Although not contagious, human aspergillosis is of growing importance in modern medical care (Latge, 1999; Latge and Steinbach, 2008). Aspergillosis has been reported from all species of domestic animals and many wild species. Birds show a particularly high susceptibility; historically, aspergillosis was first recognized as an avian disease (Ainsworth and Austwick, 1959).

Different forms of aspergillosis include severe asthma with fungal sensitization; 'fungus ball,' (aspergilloma) whereby the mould colonizes usually without spreading, causing a gramulomatous disease of the lung; and systemic (invasive) aspergillosis, in which the fungus disseminates throughout the body. Aspergilloma and invasive aspergillosis both involve direct growth of the fungus inside of host tissues; the former is often found associated with tuberculosis and is 'semi-invasive.' Invasive aspergillosis, also called systemic aspergillosis, is the most life threatening form of *Aspergillus* infection. The clinical categorization of these conditions defies neat classification but the definitions used by Kwon and Bennett (1992) provide a good introductory framework for understanding aspergillosis. Despite advances in antifungal drug therapy, the invasive forms of aspergillosis have extremely high mortality rates (Vanden Bossche *et al.,* 1988; Denning, 1998; Latge, 1999; Calderone and Cihler, 2002; Latge and Steinbach, 2008).

Clinical manifestations and the severity of aspergillosis reflect the immunological status of the patient. The best protection is a robust immune system. Dissemination of *Aspergillus* in the body indicates a break of, or deficiency in, host defences. Immunosuppressive agents and other medical developments have created a new ecological niche for aspergilli to grow on people with impaired immune systems, where they can cause serious and often fatal infections. Invasive aspergillosis, with an attendant high mortality rate, has become increasingly common as the number of susceptible hosts is increased. Bone marrow recipients constitute particularly vulnerable populations. Unfortunately, drug resistance to known antifungal drugs is becoming more common. As the disease has become more prevalent, there is a great need for expanding the number of safe and more reliable anti fungal drugs (Denning, 1996; Latge and Steinbach, 2008).

The early diagnosis of invasive *Aspergillus* infections is still difficult. It is usually based on the isolation and subsequent identification of species from appropriate clinical specimens and/or the detection of characteristic septate hyphae in sections of tissue following biopsies. Unfortunately, disseminated aspergillosis is frequently not diagnosed until necropsy. Another growing problem is the increasing number species causing invasive aspergillosis in organ transplant patients. Many of these 'non-*fumigatus*' aspergilli are resistant to the drugs commonly usually used to treat aspergillosis (Torres *et al.,* 2003). *A. flavus*, the second most common species involved in invasive aspergillosis, is the most common cause of superficial infection (Hedayati *et al.,* 2007).

A good definition of human pathogen is 'a microbial or parasitic species that can infect and is capable of causing disease in humans under natural transmission conditions' (Woolhouse, 2006, p. 511). The best-known bacterial pathogens have co-evolved with their hosts to subvert host functions and they possess special virulence

factors that have indispensable functions in mediating host–pathogen interactions. *Aspergillus* infections do not resemble classical bacterial diseases. *A. fumigatus* has no obvious need for passage in humans or other animals. The fact that *Aspergillus* can become a deadly pathogen may be a biological accident associated with it extreme opportunism. With invasive aspergillosis, the immunological status of the host – not the virulence of the fungus – is the single most significant element. Despite intensive study, the molecular basis of the pathogenic potential of *A. fumigatus* remains elusive (Brakhage and Langfelder, 2002).

In summary, as disease categories go, aspergillosis is an extremely capacious rubric. Occupational mycoses, allergies, localized mycoses, and invasive mycoses are all assembled together under the same name. 'Aspergillosis' encompasses any and all medical and veterinary diseases caused by any and all members of the genus *Aspergillus.* The classic text in medical mycology by Kwon-Chung and Bennett (1992) points out that 'the word 'aspergillosis' describes so many different clinical entities that, without qualifications, the term is almost meaningless' (p. 203). It would be useful if we did a better job of stipulating definitions for the various specific diseases grouped together under the aspergillosis rubric. Hypersensitivity reactions are not the same as fungus ball. Invasive aspergillosis is profoundly different from both. Further, there is growing evidence that 'non-*fumigatus*' *Aspergillus* infections have different manifestations other than their patterns of differential drug resistance. One of our greatest challenges in studying aspergillosis may be to break conventional mindsets about the nature of pathogenicity. As the numbers of immune suppressed individuals increase within the human population, cases of invasive aspergillosis are bound to increase too and they will become a mounting burden on our health care systems.

Genetics and genomics

The parasexual cycle was discovered and then developed as a genetic system in *Aspergillus nidulans* (=*Emericella nidulans*) by Pontecorvo and coworkers during the 1950s (Pontecorvo, 1956). Although discovered in a teleomorphic species, the parasexual soon became an important alternative to sex for doing genetics in anamorphic aspergilli. During the decades before recombinant DNA approaches became available, the parasexual cycle was exploited to recombine genetic markers in such economically important anamorphic species as *A. flavus, A. fumigatus,* and *A. niger* (Ball, 1984; Demain and Solomon, 1985).

During the 1950s and 1960s, fungal geneticists developed A. *nidulans* into a highly sophisticated model for genetics, joining *Neurospora crassa* as a premier system for providing elegant mechanistic insights on recombination and other aspects of eukaryotic biology (Todd *et al.,* 2007). Carbon and nitrogen repression, pH regulation, polar growth, signal transduction, hyphal morphogenesis and the cell cycle were all fundamental research areas that were significantly advanced using *A. nidulans* as a model. To give but one example, gamma tubulin was discovered using blocked mitotic mutants of *A. nidulans* (Oakley and Oakley, 1989). The golden age of *A. nidulans* genetics has been reviewed in Smith and Pateman (1977) and Martinelli and Kinghorn (1994). More recent comprehensive reviews include Goldman and Osmani (2008) and Scazzocchio (2009).

Some of the most interesting biochemistry carried out by *Aspergillus* and other filamentous fungi has not been amenable to traditional genetic analysis. The elegant genetics available to model fungi such as *Aspergillus nidulans and Neurospora* crassa was not an option for the study of 'non-model' species such as *A. flavus* and *A. oryzae,* even with application of the parasexual cycle and recombinant DNA approaches. For this reason genomics promises a radical improvement for gaining a new level of understanding about the genetics and the theoretical protein coding genes of these anamorphic organisms. The genomic revolution has been brought about by improved methodologies for sequencing, generating libraries, annotation and so forth. After DNA sequencing, automated annotation uses bioinformatic gene finding programmes to locate the protein coding regions of genomes. These programmes work best when 'trained' on the appropriate genome. Automated annotation can provide a good first draft of the gene content and arrangement of a genome. Nevertheless, automatic annotation is notoriously imprecise and therefore must be

subject to continuous revisions as more experimentally based information becomes available. After annotation, deduced genes can be classified as enzymes, receptors, transcription factors and so forth. Annotated genomes allow us to compare genes descended from the same ancestor across many different organisms. Sometimes this comparative genomic approach allows us to assign putative functions to unknown predicted genes.

The simultaneous publication of three *Aspergillus* genome manuscripts in *Nature* in December 2005 established *Aspergillus* as the leading filamentous fungal genus for comparative genomic studies (Nierman *et al.,* 2005; Machida *et al.,* 2005; Galagan *et al.,* 2005). Like most major genome projects, these *Aspergillus* efforts were collaborations between a large sequencing centre and the respective community of scientists. For example, the Institute for Genome Research (TIGR) worked with the *Aspergillus fumigatus* community. *A. nidulans* was sequenced at the Broad Institute. *A. oryzae* was sequenced in Japan at the National Institute of Advanced Industrial Science and Technology. The Joint Genome Institute (JGI) of the Department of Energy has released sequence date for a citric acid-producing strain of *A. niger* (Baker, 2006). TIGR, now re-named the Venter Institute is currently spearheading a project on the *A. flavus* genome (Payne *et al.,* 2006; Payne *et al.,* 2007).

Aspergillus genomics was reviewed by Jones (2007) and her review provides URLs for major *Aspergillus* genome projects listing genes, availability of other resources, links to relevant data bases, and literature citations. Genome sizes for sequenced species of *Aspergillus* range from approximately 29.3 Mb for *A. fumigatus* to 37.1 Mb for *A. oryzae* while the numbers of predicted genes vary from approximately 9926 for *A. fumigatus* to approximately 12,071 for *A. oryzae* (Machida *et al.,* 2005; Nierman *et al.,* 2005). The genome size of an enzyme producing strain of *A. niger* is of intermediate size at 33.9 Mb (Pel *et al.,* 2007). Up-to-date listings of *Aspergillus* genome projects are available at the Genomes on Line Database (GOLD) at http://www.genomesonline.org/.

Aspergillus species are only one group among a large number of eukaryotes now catalogued in databanks. There are currently well over 100 fungal genome projects in various stages of completion (see http://www.genomesonline.org/). In addition to full-fledged genome projects, various EST (expressed sequence tag) projects identify expressed genes by sequencing cDNA copies of mRNA. This approach provides a 'poor man's' strategy for genomics and provides valuable information about the coding regions of a genome expressed under different environmental conditions. ESTs also guide later annotation of full genomes. Furthermore, DNA microarrays are available for an increasing number of *Aspergillus* genomes and their use allows targeted functional analyses.

Our expectations for genome projects have become higher than they were just a few years ago. It is no longer enough to determine the DNA sequence and catalogue the predicted genes. Now we hope to become genomic detectives using sequence similarities to find new enzymes, secondary metabolites and other biologically important gene products. There is a high expectation that such 'genomic mining' will uncover new natural products and other interesting discoveries (Archer and Turner, 2008; Bok *et al.,* 2006).

Comparative genomics is a growing field in its own right. Using molecular sequence alignment, evolutionary relationships can be inferred. For example, one of the most salient finding coming out of the *A. flavus* genome project (Payne *et al.,* 2006; 2007) is its close sequence similarity and genomic architecture to that of *A. oryzae* genome (Machida *et al* 2005). Since visible phenotype is a manifestation of many genes and pathways acting together, the high genomic identity merely confirms what taxonomists have known since they first described the *A. flavus–oryzae* group of yellow–green aspergilli. When Thom and Church published the first major monograph on the genus *Aspergillus* in 1926 they pointed out that although the type cultures of *A. flavus* and *A. oryzae* were morphologically distinct but if 'a whole series of organisms occurring in nature and especially including those utilized in the soy and sake fermentations is brought together and the members compared, this distinctness disappears' (Thom and Church, 1926, p. 198). The morphological, physiological and genomic correspondence between the species is all the more remarkable because of their differing

economic repercussions in human society. *A. flavus* produces aflatoxin and is a pan-kingdom pathogen capable of causing serious disease in plants, insects and vertebrates. *A. oryzae* is both non-toxigenic and non-pathogenic and is widely used in human food and beverage preparations.

DNA data permit us to make strong inferences about the comparative biology of these and other *Aspergillus* species so as to reconstruct possible scenarios for the evolution of mating types, secondary metabolite clusters, enzymes involved in biomass degradation and other important pathways. Comparative genomics data can be leveraged to characterize biosynthetic processes. Using micro arrays and proteomics technology, we can study expression levels. Together with advanced bioinformatics and data analysis tools, we are gaining new insights into the functional properties and activities of *Aspergillus* fungal genomes. However, many important questions remain unanswered. Large numbers of deduced genes still cannot be assigned to functional classes. Our ability to acquire genome-wide data has not enlightened us about the mechanics of pathogenicity and competitiveness, and at the broadest ecological level we are still a long way from understanding why some species are common whereas others are rare.

It is becoming clear that the 'easy' part of the research has been obtaining the DNA sequence. Interpreting these sequences and understanding the ways in which DNA sequences direct metabolism are much more complex undertakings than many molecular biologists predicted. Experimental characterization and functional analysis remain the rate limiting steps in translating genomics data into the drug discovery pipeline as well as for harnessing other aspects of *Aspergillus* metabolism. Nevertheless, opportunities for exploiting genomic data are already apparent. New ways to connect traditional biology, gene function and evolution are on the horizon. *Aspergillus* species remain resilient models for studying basic questions in eukaryotic biology. Undoubtedly, *Aspergillus* genomics will enlighten fundamental insights into cell biology as well as have important implications for agriculture, industry and medicine. The forthcoming chapters in this volume review the current state of knowledge and provide a blue print for building future knowledge.

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