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Nuclear and Genome Dynamics in Multinucleate Ascomycete Fungi

Review

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Genetic variation between individuals is essential to evolution and adaptation. However, intra-organismic genetic variation also shapes the life histories of many organisms, including filamentous fungi. A single fungal syncytium can harbor thousands or millions of mobile and potentially genotypically different nuclei, each having the capacity to regenerate a new organism. Because the dispersal of asexual or sexual spores propagates individual nuclei in many of these species, selection acting at the level of nuclei creates the potential for competitive and cooperative genome dynamics. Recent work in *Neurospora crassa* and *Sclerotinia sclerotiorum* has illuminated how nuclear populations are coordinated for fungal growth and other behaviors and has revealed both molecular and physical mechanisms for preventing and policing inter-genomic conflict. Recent results from population-level genomic studies in a variety of filamentous fungi suggest that nuclear exchange between mycelia and recombination between heterospecific nuclei may be of more importance to fungal evolution, diversity and the emergence of newly virulent strains than has previously been recognized.

Introduction

Filamentous fungi are characterized by long, often multinucleate hyphae that grow by tip extension. However, in many species these hyphae are also capable of branching and fusing to create an interconnected network (Figure 1A) [1]. Like multicellular plants, but unlike multicellular animals, in which germ and somatic cell lines are segregated to prevent intergenerational transmission of somatic mutations, almost any hyphal fragment is capable of regenerating the entire organism. However, unlike multicellular plants, in which rigid cell walls prevent nuclear movement, nuclei of many species of filamentous fungi are capable of moving freely through septal pores to traverse the interconnected syncytium (Figure 1A,B; Supplemental Movie S1). Rates of nuclear migration can reach several microns per second (Table 1).

A single mycelium has the potential to harbor genetically different nuclei. For example, 2–3% of nuclei isolated from a laboratory strain of *Neurospora crassa* bore mutations that morphologically altered the mycelium phenotype [2]. Similarly, as many as 26% of wild *Fusarium moniliforme* isolates contain two or more populations of genetically diverse nuclei [3]. It is likely that mutation is the most common source of genotypic diversity, but genetically different nuclei may also be acquired via hyphal fusion and genetic exchange with other mycelia [4]. The mycelium's ability to harbor genetically diverse nuclei has been shown to enhance phenotypic plasticity [5] and is also thought to contribute to fungal virulence [6–8].

Recent and ongoing work reveals two fundamental challenges of multinucleate fungal lifestyles, both in the presence and absence of genotypic diversity — namely, the coordination of populations of nuclei for growth and other behaviors, and the suppression of nucleotypic competition during reproduction and dispersal. The potential for a mycelium to harbor fluctuating proportions and distributions of multiple genotypes led some 20th century mycologists to argue for life-history models that focused on nuclei as the unit of selection, and on the role of nuclear cooperation and competition in shaping mycelium growth and behavior [9,10]. In particular, nuclear totipotency creates potential for conflict between heterogeneous nuclear populations within a mycelium [11,12].

Phylogenomic analyses of fungal populations have revealed the evolutionary traces of gene flow between species. Although variability in filamentous fungal genomes can be generated by homospecific outcrossing (see Box 1 for a glossary), recent phylogenomic evidence also supports a role for introgression from other species of genomic regions that confer a selective advantage [13,14]. Although introgression can occur via occasional hybridization between different species, phylogenetic analyses of other fungal populations have revealed that gene or chromosome transfer via hyphal fusion may also play a role in generating genome diversity [7,8,15,16]. The tolerance of a multinucleate mycelium for even large genetic heterogeneity may allow rare exchange and recombination of nuclei between fungal colonies and may be an important contributor for fungal diversification and evolution.

In this review, we focus on the life history of multinucleate ascomycete fungi, and in particular on the handful of model organisms for which population genetics are well resolved and nuclear and genomic dynamics can be directly visualized by molecular labeling. We focus in particular on nuclear cooperation and conflict during vegetative growth as well as genome/gene conflict and cooperation during sexual reproduction and spore dispersal. Although the high growth rates of some of these species may put them at the extremes of nuclear dynamism in terms of multinuclearity and nuclear migration rates within and across hyphal compartments, they are useful models for all fungi because multinucleate hyphal compartments are ubiquitous in ascomycete fungi and common in other phyla (Table 1). For example, basidiomycete fungi have a sexual phase, during which dikaryotic mycelia are formed by fusion of two homokaryotic mycelia, followed by the rapid proliferation and dispersal of nuclei through each hyphal network [17]. Moreover, in more than 30% of basidiomycete species, the ratios of the nuclear populations are not fixed and may be persistently imbalanced by nuclear selection [18]. Although controversial [19], genetic heterogeneity may also contribute to the fitness and diversity of glomeromycete fungi [20], where it is maintained by propagating multinucleate mitotic spores [21,22].

Nuclear Coordination during Vegetative Growth

A fungal mycelium grows by hyphal tip extension and flow of cytoplasm and nuclei to the spaces created at the extending

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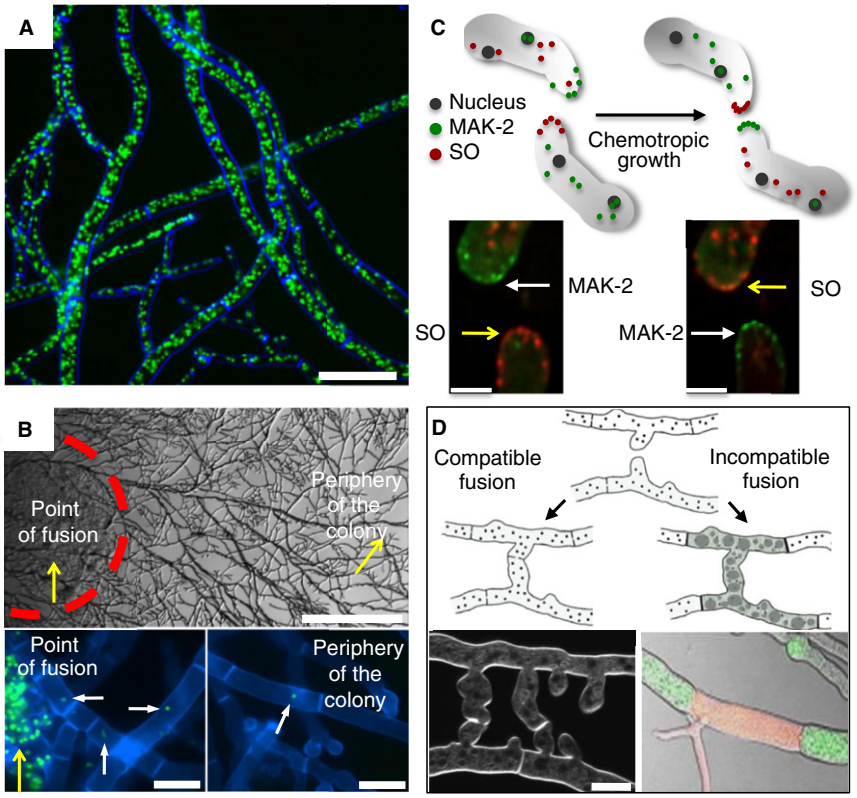

Figure 1. Nuclear coordination.

(A) Hyphal compartments of the ascomycete fungus *Neurospora crassa* are multinucleate. Nuclei can migrate between compartments through septal pores and between hyphae via hyphal fusion events (Table 1 and Supplemental Movie S1). Nuclei are visualized with histone H1–GFP (green) and cell walls/septa are visualized by staining with calcofluor (blue). Size bar = 50 μm. (B) H1–GFP-labeled conidia (green) were placed in the interior of an unlabelled *N. crassa* colony (points of fusion; yellow arrows and red circle). Germinating conidia fuse with hyphae and H1–GFP nuclei are transferred to hyphae within the colony (white arrows). Two hours later, H1–GFP labeled nuclei (white arrow) were observed at the periphery of the colony (periphery of the colony, yellow arrow). Nuclei were able to travel more than 1 cm over the two hour time period. Size bars = 1 mm (main panel), 20 μm (inset). (C) Chemotropic interactions during germling fusion in *N. crassa* are associated with MAK-2 and SO oscillation to the tips of conidial anastomosis tubes (CATs). Top panel, cartoon of oscillation of MAK-2–GFP and SO–dsRed during chemotropic interactions; switching occurs every ~4 minutes and is associated with chemotropic growth (panel C adapted from [41]). Bottom panels show deconvolution microscopy of CAT tips containing both MAK-2–GFP and SO–dsRed. Localization of MAK-2–GFP (white arrows) or SO–dsRed (yellow arrows) to CAT tips alternates between germlings undergoing chemotropic interactions. Size bar = 2 μm. (D) Compatible hyphal fusion events (between strains with identical alleles at all *het* loci) allow mycelia to create efficient networks for transport and to repair damage. The bottom left panel shows confocal microscopy of a compatible fusion event in *N. crassa* (Reproduced with permission from [83]). Size bar = 10 μm. Hyphal fusion events with another mycelium can lead to invasion by aggressive nucleotypes. However, productive heterokaryon formation with genotypically different mycelia can be limited if strains differ in *het* genotype. The incompatible fusion cartoon shows hyphal compartmentation and death, which occurs post-fusion between incompatible hyphae. The bottom right panel shows compartmentalization and degradation of H1-dsRed-labeled nuclei that have entered an incompatible cytoplasmic-GFP-labeled mycelium (Reproduced with permission from [84]).

tips. Production of new nuclei by mitosis must therefore match tip growth. However, balanced proportions of genetically different nuclei cannot be maintained when the hypha is populated with the descendants of a small dividing population of nuclei located, for example, at the hyphal tip. Even when all nucleotypes have equal division rates, models suggest that stochastic fluctuations alone would eventually eliminate diversity [23] and create a homokaryotic hypha. As an extreme example of this phenomenon, when *Fusarium oxysporum* germlings fuse, the resident, mitotically active nucleus in one germling may be replaced by an 'invading' nucleus translocated into the apical compartment from the other germling; subsequent growth propagates only the new nuclear lineage [24].

It has long been known that in other multinucleate ascomycete fungi, e.g. *Aspergillus nidulans*, mitosis is synchronized, either to a single internal clock or to a wave that propagates along the hypha starting at its tip [25–27]. Although the signaling processes that maintain mitotic synchronicity are not understood [28], this phenomenon is thought to aid the preservation of nucleotype diversity by preventing stochastic fluctuations or differences in division rate from influencing nuclear proportions [27]. However, in many fungi, including *Ashbya gossypii*, nuclei divide asynchronously, perhaps to avoid the large fluctuations in nucleocytoplasmic ratio that are associated with the sudden doubling of nuclear numbers in synchronized mitoses [29]. Curiously, the conserved transcriptional network that rapidly desynchronizes mitotic re-entry in daughter nuclei in filaments of *A. gossypii* [30] ensures coherent cell cycle re-entry of daughter cells in the yeast *Saccharomyces cerevisiae*, perhaps reflecting the different requirements faced by hyphal and unicellular fungi. Together with forced, and perhaps fitness-independent, stochasticity of nuclear division, dynein-motor-mediated reordering and shuffling of nuclei between mitotically active and interphase populations [31] may help to preserve nuclear proportions and thereby genotypic diversity in these fungi.

Mycelium growth and behavior presumably require the coordination of transcription among individual nuclei. Filamentous fungi are able to sustain large and stable transcriptional differences across a colony [32,33] and even between adjacent hyphae [34]. However, it is unclear how transcription can be coordinated in rapidly growing species in which nuclei are capable of migrating through the colony (Table 1). For example, in *N. crassa*, nuclei in the apical compartment cannot divide quickly enough to fill the tip space. In fact, given a typical hyphal growth rate of 0.2 μm/s and mitotic rate of 80 min/nucleus, approximately 960 μm of hypha would be needed to produce enough nuclei to fill a single growing tip. However, rather than being localized to the growing periphery of the mycelium, nuclear division

Table 1. Multinuclearity and nuclear dynamics in representative filamentous fungi.

Species	Phylum	Rate of nuclear migration	Number of nuclei per hyphal compartment	References
Neurospora crassa	A	0.14 μm/s[a] (tips); 1–10 μm/s (interior)	1–~100[b]	[67,68] Supplemental Movie S1
Ashbya gossypii	A	~0.1 μm/s[a]	8–10[b]	[69]
Aspergillus nidulans	A	0.002–0.02 μm/s (tips) 0.07–0.7 μm/s (colony interior)	~10–~60[h]	[70,71]
Sclerotinia sclerotiorum	A	Unknown	1–~100	[72]
Magnaporthe grisea	A	0.04 μm/s[c]	1 (young hyphae) 1–3 (mature hyphae)	[73] [74]
Fusarium oxysporum	A	0.0004–0.004 μm/s[c] (germling)	1 (germling) 7–26 (apical compartment) 5–12 (subapical compartment)	[24,25]
Botrytis cinerea	A	Unknown	>10[b]	[75]
Ustilago maydis	B	0.02 μm/s (dikaryotic cell)[d]	1–2	[76]
Schizophyllum commune	B	0.7 μm/s (dikaryotization)[e]	1–2	[77]
Coprinus congregatus	B	11 μm/s (dikaryotization)[e]	1–2[f]	[78]
Coprinus lagopus	B	0.2–0.4 μm/s (dikaryotization)[e]	1–2[f]	[17]
Heterobasidion annosum	B	0.4 μm/s (dikaryotization)[e]	1–20[b]	[79,80]
Glomus caledonium	G	3.4 ± 0.3 μm/s[a]	~700–2,500[g,h]	[81]

[a]Nuclear migration is observed to produce resorting (i.e. change in relative ordering of nuclei along the hypha).
[b]Nuclei are observed to migrate between compartments. In many ascomycete species, nuclei are able to move, unobstructed, through pores in the septal walls that separate hyphal compartments. In the basidiomycete fungus *Heterobasidion annosum* fusion of two mycelia is sometimes followed by erosion of the septa, allowing free movement of nuclei.
[c]In *Magnaporthe grisea* hyphae and *Fusarium oxysporum* germlings, a single mitotically active nucleus sits at each hyphal tip, migrating at the hyphal growth rate and populating the hypha with successive descendants.
[d]In its infectious dikaryotic phase, *Ustilago maydis* mycelia consist of a single hypha, with an apical binucleate compartment. Protoplasm withdraws from the basal end of the hypha, leaving a succession of vacuolated and un-nucleated compartments behind.
[e]Nuclear migration rates for mushroom-forming fungi are rates of dikaryotization, proliferation and integration of a second nuclear population following plasmogamy with another mycelium. These rates are measured over entire colonies and migration along individual hyphae may be faster.
[f]Nuclear counts for *Coprinus* mycelia are for mature homokaryotic and dikaryotic mycelia. For immature *Coprinus ephmerus* colonies, Sass [82] counted up to 25 nuclei in apical compartments and 1–5 nuclei in sub-apical compartments.
[g]Glomeromycete fungi are aseptate — the entire mycelium is a single coenocytic cell.
[h]Number of nuclei per hyphal compartment for *Aspergillus nidulans* and *Glomus caledonium* is estimated from reported hyphal lengths and images showing nuclear distribution.

can be observed through the entire mycelium by monitoring expression rates of genes annotated with nuclear cycle (FunCat (http://mips.helmholtz-muenchen.de/proj/funcatDB) terms 10.03.01/04) and nucleogenesis functions (FunCat term 42.10) [32]. To populate the growing tip, nuclei produced in the mycelium migrate to the tips along complex multidirectional trajectories through the interconnected hyphal network (Figure 1B), reaching speeds of several microns per second (Table 1 and Supplemental Movie S1).

Although it has been hypothesized that transcriptional differences between nuclei are associated with shifts in function, i.e. permanent commitment of some nuclei to different expression profiles — somewhat akin to cell differentiation in plants and animals — this hypothesis is problematic because nuclei can move in a matter of hours across an entire mycelium, sampling many different cellular environments during this transit (Table 1 and Figure 1B). The alternative hypothesis, that nuclear expression is temporally plastic, is also problematic because it requires that nuclei jointly regulate their expression through cytoplasmic communication [35]. Nuclear communication is known to influence expression profiles in some fungi with relatively static cytoplasmic environments, as seen with hydrophobin expression in the dikaryotic basidiomycete *Schizophylum commune* [36]; however, cytoplasmic communication must be based on chemical gradients, and the flow of nuclei and cytoplasm in the syncytium would obliterate these signaling gradients. We highlight three recently discovered phenomena that hint at general principles for nuclear communication and coordination.

First, multinucleate fungi often have asynchronous nuclear division cycles, even though nuclei share a common cytoplasm, suggesting that nuclear behavior can be self-organized without communication across millimeters or centimeters of mycelium. For example, Gladfelter [28] found that mitotic rates increased after treating *A. gossypii* with a mitotic arrest agent that caused nuclei to become more widely spaced within hyphae. This response suggests that nuclei actively regulate their nucleocytoplasmic ratio. Although a true test of this form of regulation would require evidence that mitotic rates correlate with local nucleocytoplasmic ratio, these data provide a hint that the regulation of mitosis with mycelium growth rate can be self-organized at the level of individual nuclei.

Second, mRNA trafficking via cytoskeletal elements and localized translation can create localized expression patterns independent of nuclear movement. In *S. cerevisiae*, *ASH1* mRNA is preferentially trafficked via the actin cytoskeleton to daughter cells, where its translation results in the formation of a protein that represses mating type switching [37,38]. In the basidiomycete *Ustilago maydis*, microtubule-based mRNA transport is important for polar hyphal growth, suggesting that trafficking of mRNAs and localized translation can confer different developmental states, which can be at least somewhat independent of the location of the nuclei of origin [39].

Third, signaling mycelia can coordinate their behavior through the cycling of complementary physiological states. For example, many hyphal behaviors, such as avoidance and fusion, require communication between parts of the

Box 1.

Glossary of terms.

Homospecific: Individuals with the same species origins.
Heterospecific: Individuals with different species origins.
Apothecium: The disc-, cup- or saddle-shaped fruit body of a discomycete fungi (such as *Sclerotinia sclerotiorum*). The spore-bearing asci of these fungi decorate the inside of a cup-shaped structure, and many asci may discharge their spores nearly simultaneously.
Out-crossing/out-breeding: Sexual fusion of two different mycelia.
Allopatry: Geographic (or other physical) isolation of populations leading to speciation.
Sympatry: The absence of geographic separation of physical barriers between populations or species.
Hydrodynamic policing: A special case of the physical targeting of benefits to cooperating agents rather than cheating agents. In the case of forcibly launched spores of an apothecial fungus, the policing mechanism is hydrodynamic, i.e. derived from the geometry of the flow of air collectively mobilized by the spores.
Introgression: The transfer of genetic information from one species to another as a result of hybridization between them and subsequent, repeated crossing of mycelia from the hybridized lineage with mycelia from one of the original parental species.

mycelium that are not physically connected at small spatial scales [40]. These multinucleate mycelia must communicate across the substrate that separates them. However, to avoid self-stimulation, each partner must distinguish between its own signals and those of its partner despite the fact that the partners are genetically identical. In *N. crassa*, pairs of germinated asexual spores that are preparing to fuse with each other alternate between a 'broadcasting' and 'receiving' mode of extracellular signaling to coordinate chemotropic interaction and cell fusion. The alternation of physiological states was visualized by examining the localization of a MAP kinase (MAK-2) and a protein of unknown function (SO) to conidial anastomosis tubes (CATs) [41]. These two proteins oscillate perfectly out of phase at the tips of interacting conidial anastomosis tubes (Figure 1C); oscillation cycles of MAK-2 and SO are sustained by protein trafficking, rather than *de novo* protein synthesis.

Nuclear Competition and Cooperation

Hyphal fusion enables a multinucleate filamentous fungus to produce a multiply connected cytoplasmic network for resource transport and to reconnect damaged hyphae (Figure 1A), while fusion between germinated conidia has been hypothesized to be essential for colony establishment in nature [42]. However, fusion with other genetically different mycelia or, in the case of conidia, with genetically different partners, introduces the risk of infection by pathogenic elements, including mycoviruses [43], selfish genetic elements [44], and parasitization by aggressive genotypes [11] (for review, see [45]). In response to these challenges, fungi have evolved genetic systems to discriminate between 'self' and 'other', and thereby regulate the entry of new genetic material into the mycelium. For example, different *N. crassa* strains will form viable heterokaryons only if the resulting heterokaryon is homozygous at 11 heterokaryon incompatibility (*het*) loci. *N. crassa het* loci typically have two to three alleles or haplotypes [46], a feature that they share with other species of filamentous ascomycetes, where *het* loci may also be known as *vic* (vegetative incompatibility) loci. Thus, in a segregating population of *N. crassa*, there are at least 2^{11} (i.e. 2,048) distinguishable genotypes based on *het* loci alone. Fusion compartments between strains that are heterozygous at any of these loci undergo rapid nuclear degradation and programmed cell death (Figure 1D) [47]. Although heterokaryon incompatibility retards genetic transfer between incompatible strains in filamentous ascomycete fungi, it is not absolute and transfer of mycoviruses [43] as well as supernumerary chromosomes [48] has been observed between incompatible strains.

During mating and sexual reproduction, the tight genetic controls on heterokaryon incompatibility must relax so that out-breeding can occur. In *Neurospora*, the trichogyne, a specialized mating hypha, is able to fuse and accept nuclei from a hypha of opposite mating type, of apparently any *het* genotype [49], and can even fuse with multiple nearby hyphae [50–52]. Nuclei from all parents proliferate after fusion, with the eventual sequestration of two nuclei of opposite mating type within each ascus initial. The nuclei in each ascus initial fuse to produce a transient diploid nucleus that undergoes meiosis to form haploid nuclei that are sequestered into spores (Figure 2A). Forcible ejection of these spores allows the fungus to disperse to new substrates or hosts [53] and even to travel between continents [54]. Because of their small size, spores are rapidly decelerated by air resistance [55]. Synchronized spore ejection in many pezizomycete fungi, including *Sclerotinia sclerotiorum,* increases spore range by cooperatively creating flows of air as a microscopic and more potent analog of the drafting that reduces the drag on flocking birds or on the members of a cycling peloton (Figure 2B). For *S. sclerotiorum*, the dispersive wind can increase the range of individual spores from 3 mm to up to 20 cm [56]. Numerical simulations that track the fate of every spore show that only the last spores to be ejected enjoy the full benefit of this cooperation, and the first spores are sacrificed to set the air into motion. This situation is akin to *Dictyostelium discoideum* sporulation, in which some 20% of amoebae are sacrificed to form stalk cells that support a sporangium created by the remaining amoebae. Because of the genetic diversity of nuclei within ascogonial tissue, there is potential for intergenomic conflict [12], namely for progeny nuclei to manipulate the time of their ejection to avoid being sacrificed, similar to the identification of 'cheaters' in *Dictyostelium* that do not contribute to stalk formation [57]. In fact, hydrodynamic policing prevents spores from cheating: high-speed imaging and simulations show that in *Ascobolus furfuraceus* spores are ejected in a wave that travels over the fruit body to produce a sheet-like jet [56]. To be entrained into the jet, and to enjoy the benefits of hydrodynamic cooperation, spores must eject with or close to their neighbors, even if this leads to their sacrifice (Figure 2C).

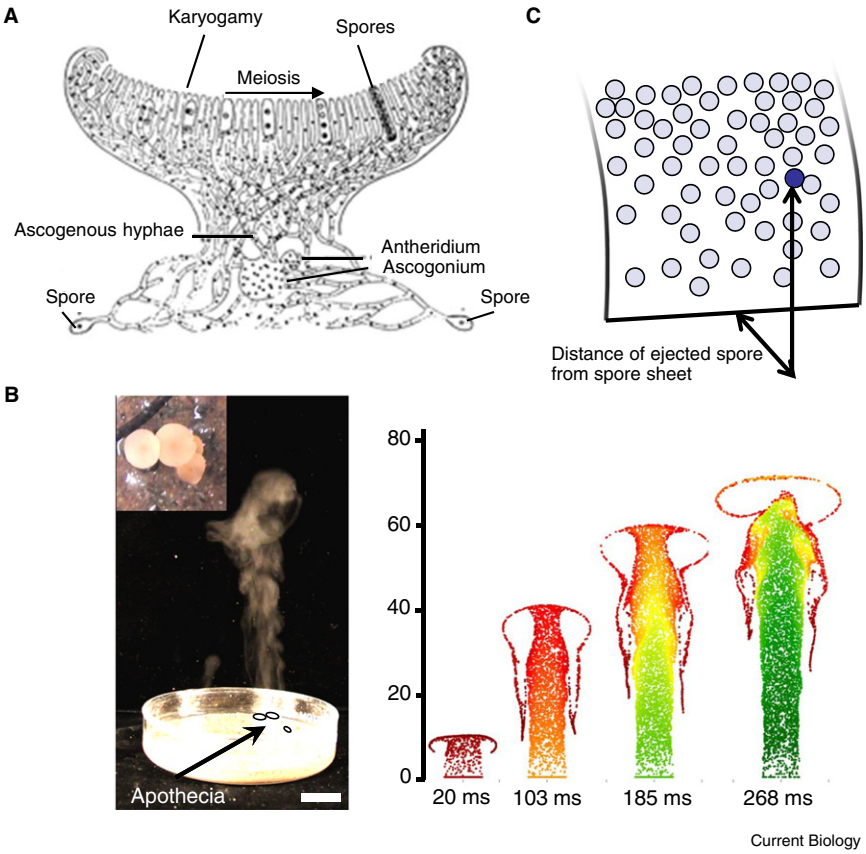

Figure 2. Competition and cooperation between genetically diverse nuclei.

(A) Olive's 1953 [85] drawing of nuclear migration and karyogamy following mating between heterothallic ascomycete fungi (with an apothecial fruiting structure). Multiple nuclei migrate into the mycelium following fusion of 'female' and 'male' multinucleate gametangia (the ascogonium and antheridium). Male nuclei proliferate within the ascogonium and conjugate into pairs, which are sequestered into ascogenous hyphae to undergo karyogamy and meiosis. Other forms of mating also exist, including between a multinucleate ascogonium and uninucleate spermatia. Multiple nuclear transfers are still possible in these mating events because of the ascogonium's ability to fuse with multiple spermatia [51,52]. (B) Synchronously ejected discomycete spores collectively create a flow of air that enhances spore range (shown: spore jets created by spores of *Sclerotinia sclerotiorum*, fruit bodies shown in inset image, scale bar = 2 cm) (adapted from [56]). The apothecia from which these jets originate are circled. Direct numerical simulations of spore jets show that range enhancement is shared unequally between progeny nuclei. On a slice through a simulated spore jet, we color spores according to their ejection (red, earliest spores; green, late spores). Only the late-ejected spores remain in the jet by the time that it attains its maximum height. (C) Cooperation between progeny nuclei is policed hydrodynamically. Close to the apothecium, cooperating spores form a sheet that moves across the apothecium as the spore jet develops. The range of spores that eject with and contribute to the sheet ('cooperators') is enhanced more than spores that eject at other times ('cheaters'). We discriminate spores that cooperate or cheat in the creation of the spore jet by the distance of the spore from the sheet at its moment of ejection.

Nuclear Exchange as a Motor for Fungal Evolution

Historically, many groups of fungi were believed to be asexual because fruiting structures had never been observed in Nature or in the laboratory. However, where population genetics has been used to assess recombination, evidence of outbreeding has been found in putatively asexual groups of fungi [58]. While sexual reproduction plays a crucial role in the evolution and adaptation of many populations of fungi, the role of other means of genetic exchange in the origin of adaptive alleles remains a topic of great interest, particularly given constraints on genome architecture, including the repeat-induced point mutation (RIP) mechanism that prevents gene duplication by hyper-mutation of repetitive DNA [59]. Although sexual fusions can produce viable hybrid offspring between recently diverged fungal lineages, extrinsic barriers such as allopatry or intrinsic barriers that may be reinforced in sympatry [60] make long-diverged lineages sexually incompatible. In the light of such constraints, one intriguing mechanism of evolutionary innovation in the fungi is the transfer of genes between genetically divergent lineages via vegetative fusion, nuclear exchange and recombination (Figure 3A).

As the phylogenetic breadth and depth of fungal taxa with sequenced genomes has increased, many putative gene transfer events have been discovered. For example, an extremely divergent genomic region has been identified between two populations of *N. crassa* that are recently diverged (~0.5 million years ago (MYA)) and quite similar across the rest of their genomes (Figure 3B) [14]. This region contains the major circadian oscillator gene *frequency* and a gene encoding a PAC10-like prefoldin that is involved in growth at low temperature. The pattern of sequence variation within this region is consistent with introgression from a more divergent *Neurospora* lineage and local adaptation to low temperature or day length or both. Similarly, Neafsey *et al.* [13] found evidence of asymmetrical introgression of ~800 genes, many related to immune evasion, into the human pathogenic fungus *Coccidiodes immitis* from the related, and also pathogenic, species *C. posadasii*, from which it diverged 5 MYA. In several independent regions of the globe, the Dutch Elm disease fungus, *Ophiostoma novo-ulmi*, colonized new territory as a clonal lineage with a single mating type, but subsequently acquired the opposite mating type from *O. ulmi* [61]. The mechanisms for these introgressions are not known, but in all three cases the donor and recipient species may be sufficiently closely related to hybridize sexually.

Gene transfer has also been detected between asexual species, or between species that are too divergent to be sexually compatible, indicative of introgression following vegetative transfer of nuclei. As was first shown in the yeast *S. cerevisiae,* genetic transfer between nuclei can occur even without recombination, by the donation or exchange of chromosomes [62]. Chromoduction, or chomosome transfer, seems to be widespread among several plant pathogenic fungi in which genes required for virulence are carried

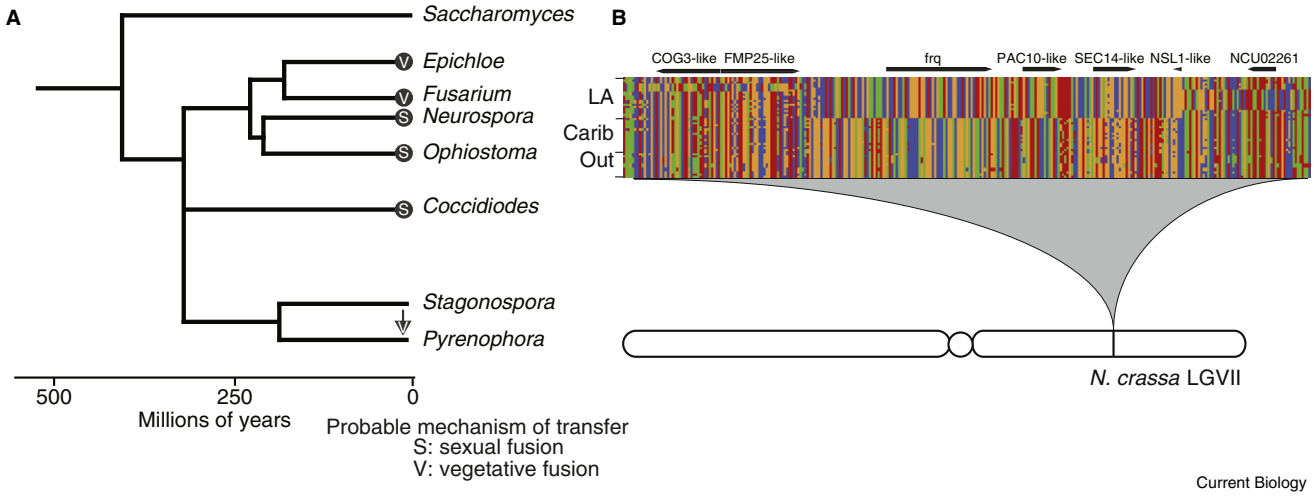

Figure 3. Transfer of genes between lineages of fungi.

(A) Phylogeny showing relationships of the exemplar Ascomycete genera where introgression or lateral gene transfer has been observed. In cases where the two lineages involved are genetically distinct but still capable of sexual reproduction, we presume introgression was mediated via mating. In contrast, transfer must have been mediated by vegetative fusion in cases where the two lineages are too distantly related for sexual reproduction to be possible, such as *Stagonospora* and *Pyrenophora* [15], or where transfer has been observed in the absence of sex, such as in *F. oxysporum* [7]. (B) Genomic signature of introgression. Shown here is a genomic region that is extremely divergent between two closely related populations of *Neurospora* (LA, Louisiana population; Carib, Caribbean; Out, strains from Central America, South America, and Africa) (Reproduced with permission from [14]). Each row represents an individual and each column represents a polymorphic site, with different colors representing the four different nucleotides. Introgression of this region into the Louisiana population from a more distantly related *Neurospora* species or strain explains the large number of fixed nucleotide differences between populations. It is possible that this region came into the Louisiana population as a larger genomic segment with recombination subsequently paring it down, which would explain the uneven boundaries present in isolates from the Louisiana population.

on dispensable chromosomes that show variable conservation in population samples. In both *F. oxysporum* and *Colletotrichum gleosporides*, chromoduction has been shown to occur following vegetative fusion, even between strains that are heterokaryon incompatible [7,48]. The endophytic symbionts *Acremonium coenophialum* and LpTG-2 have repeatedly acquired genes from pathogenic species of *Epichloe* [63]. Although these isolates are relatively closely related, the observation that sexual crosses are incompatible in the laboratory and the isolation of apparently trikaryotic hybrid strains suggest that gene transfer was initiated from a vegetative fusion event. Vegetative gene transfer between lineages is also believed to contribute to the emergence and virulence of new pathogens, for example the recent emergence of *Pyrenophora tritici-repentis* (tan spot disease) as a new disease in wheat [15] has been traced to the transfer of the *toxA* gene from *Stagonospora nodorum* (also known as *Phaeosphaeria nodorum*) to *P. tritici-repentis* (Figure 3A).

Conclusions and Perspectives

Recent advances in molecular labeling and high-speed imaging now allow direct observation of nuclear movement and policing both within syncytial mycelia and during the ejection of spores. At the same time, a wealth of new phylogenomic data has revealed evolutionary and ecological traces of gene transfer. The time is ripe for an integrative understanding of the mechanisms and evolutionary impact of nuclear exchange between mycelia. However, three outstanding questions remain.

Firstly, how common is nuclear transfer between mycelia from divergent lineages in Nature? For example, the relatively small colony sizes of ascomycete endophytes [64] means that a single 5 x 5 cm leaf contains a minimum of 5,000 pairs of contiguous mycelia. Combinatorial interactions and occasional failure of heterokaryon incompatibility mechanisms increase the likelihood of nuclear transfer.

Secondly, how are chimeric nucleotypes created and selected for following hyphal fusion? In a genetically heterogeneous mycelium, new nucleotypes may be generated by mitotic recombination [10], or by transfer of genetic elements [8]. Although the heterokaryon incompatibility response prevents the creation of heterokaryotic mycelia, it may yet allow transfer of genes by the degradation of invading nuclei and liberation of nuclear DNA [65]. Nonetheless, without strong selection and additional sexual recombination, we would expect chimeric nuclei to be less fit than the other nuclei in the mycelium. Subsequent selection seems to reduce introgression to genomic islands carrying a few fitness-enhancing genes (Figure 3B). For new genotypes produced by vegetative recombination, this selection can occur either between nuclei within the same host mycelium, or between mycelia, but only if chimeric nuclei can find new hyphal lineages within a mycelium or are packaged as spores to initiate new homokaryotic individuals. Experiments with auxotrophically marked nuclear populations show that nucleotype ratios can vary within a mycelium, suggestive of subcellular selection [2,66].

Thirdly, what are the relative contributions of sexual versus vegetative recombination to the generation of diversity in fungal populations? Reconstruction of historical recombination events and the presence of opposite mating types in fungal populations both underline the importance of meiotic recombination to fungal diversity. However, the multinucleate mycelium's tolerance for genetic heterogeneity and the evidence for horizontal gene transfer between long

divergent lineages suggest that vegetative recombination also contributes to the success and immense diversity of fungi.

In this review we have emphasized how the dynamic, multinucleate and even multi-genomic nature of fungal cells fundamentally distinguishes the evolutionary and life histories of fungi from animals or plants. However, fungal multinuclearity and chimerism may yet provide a useful paradigm for understanding how other organisms recognize and suppress or cope with internal genetic variation introduced at one extreme by the accumulation of silent somatic mutations and at the other by the uncontrolled proliferation of cancerous cells. For many animals, embryogenesis includes a multinucleate, syncytial stage as seen in the blastula of fish, reptiles and insects and in the peripheral trophoblasts of mammalian blastocysts. The syncytial state appears again in a handful of human tissues such as muscle (myocytes), bone marrow (megakaryocytes) and in alveoli during the early stages of infection by *Mycobacterium tuberculosis*, while abnormal cells produced during tumorigenesis can be both multinucleate and multigenomic. Insights emerging from studies of fungal syncytia may illuminate the mechanisms underlying nuclear communication, coordination and competition in these cells.

Supplemental Information

Supplemental Information includes one movie and can be found with this article online at doi:10.1016/j.cub.2011.06.042.

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