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Complex multicellularity in fungi: evolutionary convergence, single origin, or both?

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ABSTRACT

Complex multicellularity represents the most advanced level of biological organization and it has evolved only a few times: in metazoans, green plants, brown and red algae and fungi. Compared to other lineages, the evolution of multicellularity in fungi follows different principles; both simple and complex multicellularity evolved *via* unique mechanisms not found in other lineages. Herein we review ecological, palaeontological, developmental and genomic aspects of complex multicellularity in fungi and discuss general principles of the evolution of complex multicellularity in light of its fungal manifestations. Fungi represent the only lineage in which complex multicellularity shows signatures of convergent evolution: it appears 8–11 times in distinct fungal lineages, which show a patchy phylogenetic distribution yet share some of the genetic mechanisms underlying complex multicellular development. To explain the patchy distribution of complex multicellularity across the fungal phylogeny we identify four key observations: the large number of apparently independent complex multicellular clades; the lack of documented phenotypic homology between these clades; the conservation of gene circuits regulating the onset of complex multicellular development; and the existence of clades in which the evolution of complex multicellularity is coupled with limited gene family diversification. We discuss how these patterns and known genetic aspects of fungal development can be reconciled with the genetic theory of convergent evolution to explain the pervasive occurrence of complex multicellularity across the fungal tree of life.

Key words: multicellularity, fruiting body, convergent evolution, development, phylogenetically patchy character, mushroom, fungal reproduction, cell adhesion, gene regulatory network, fruiting body initiation.

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I. INTRODUCTION: SIMPLE AND COMPLEX MULTICELLULARITY

Multicellularity comes in many forms and complexity levels, ranging from simple cell aggregations, colonies, films or filaments to the most complex organisms known (Szathmary & Smith, 1995; Rokas, 2008; Fairclough, Dayel, & King, 2010; Knoll, 2011; Niklas & Newman, 2013; Richter & King, 2013; Sebé-Pedrós *et al.*, 2013; Niklas, 2014; Rainey & de Monte, 2014; Umen, 2014; Aguilar, Eichwald, & Eberl, 2015; Herron & Nedelcu, 2015). While simple cell aggregations and colonies evolved at least 25 times in both pro- and eukaryotes (Grosberg & Strathmann, 2007; Rokas, 2008), complex multicellularity has evolved in up to five major groups: animals, embryophytes, red and brown algae (Knoll, 2011; Claessen *et al.*, 2014; Niklas, 2014; Umen, 2014; Cock *et al.*, 2015, 2010; Nagy, 2017; Niklas & Newman, 2016; Sebé-Pedrós, Degnan & Ruiz-Trillo, 2017), and fungi. While for many groups evolving simple multicellularity seems to be relatively easy, complex multicellularity probably represents a more difficult leap for organisms (Grosberg & Strathmann, 2007). Simple and complex multicellularity are distinguished based on the proportion of cells in direct contact with the environment (i.e. all *versus* some), the extent of cellular differentiation, cell adhesion, communication, a developmental program and programmed cell death (PCD) (Cock *et al.*, 2010; Knoll, 2011; Knoll & Hewitt, 2011; Herron & Nedelcu, 2015). Complex multicellularity is usually used with reference to three-dimensional differentiated structures, although how (and whether) it is defined varies widely across studies. Here, we focus on a genetically determined developmental program, determinate growth and three-dimensional organization as key traits for complex multicellularity. The rationale for this is that these traits represent major hurdles to evolving higher-level complex organization; in three-dimensional structures not all cells are in direct contact with the environment, necessitating mechanisms for overcoming limitations to diffusion and for cell–cell adhesion. Primitive mechanisms for cell adhesion, communication and differentiation exist also in simple colonial and even unicellular protists (King, Hittinger, & Carroll, 2003; Richter & King, 2013; Brunet & King, 2017; Sebé-Pedrós *et al.,* 2017), although they reach their highest complexity in complex multicellular organisms. Similarly, PCD also occurs in uni- and simple multicellular lineages (Herron & Nedelcu, 2015), so the more relevant question in the context of complex multicellularity is whether unprogrammed cell death is lethal to the multicellular individual or stalls its further development and reproduction (Knoll, 2011). It should be noted that, as is often the case in biology, discretely categorizing a continuum of evolved forms can be challenging, nevertheless, the distinction of simple and complex multicellularity is useful for comparing phyletic and genetic patterns across distantly related multicellular groups.

The main focus of this review is the convergent evolution of complex multicellularity from a fungal perspective. We discuss how genetic and developmental information can be reconciled with the multiple origins of complex multicellularity in fungi to understand its evolutionary history. We first demonstrate that complex multicellularity is so widespread in fungi that it challenges our general view of its origins by convergent evolution. We then evaluate alternative hypotheses on the genetic mechanisms of the evolution of complex multicellularity in fungi and how emerging theories of convergent evolution can inform our understanding of the evolution of multicellularity. We start by introducing a concept for distinguishing simple and complex multicellular grades of fungal evolution, and then discuss phylogenetic, developmental and genetic aspects of complex multicellularity in the fungal world.

II. SIMPLE MULTICELLULARITY IN FUNGI

Multicellular organisms have diverse unicellular ancestry. Presumably, most multicellular eukaryotes evolved from aggregative or colony-forming ancestors, resembling extant choanoflagellates (Fairclough *et al.*, 2010; Richter & King, 2013; Hanschen *et al.*, 2016; Sebé-Pedrós *et al.*, 2017) and volvocine algae, among others (King, 2004; Rokas, 2008; Fairclough *et al.*, 2010; Richter & King, 2013; Niklas, 2014; Telford, Budd, & Philippe, 2015; Hanschen *et al.*, 2016; Niklas & Newman, 2016; Sebé-Pedrós *et al.*, 2017). Here, the evolution of sophisticated mechanisms for cell adhesion and cell–cell communication followed by functional and morphological differentiation defines the 'classic' route to multicellularity (Brunet & King, 2017). The evolution of multicellularity in fungi departs from this classic scheme in many aspects. Fungi develop multicellular thalli composed of hyphae that extend apically and grow and branch under rules similar to fractal geometry. Hyphae most likely evolved to optimize foraging efficiency; they direct growth and occupy space to maximize substrate utilization, resulting in a loosely arranged, interconnected, fractal-like network, called the mycelium. Hyphae are hypothesized to have evolved by the gradual elongation of substrate-anchoring rhizoids of unicellular ancestors resembling extant Chytridiomycota (Harris, 2011), although alternative routes may exist in convergently evolved hyphal forms [e.g. Monoblepharidomycetes (Dee *et al.*, 2015)]. Nevertheless, the evolution of fungal hyphae likely did not involve the modification of cell wall biogenesis for daughter cells to remain together, as seen in filamentous bacteria and algae (Claessen *et al.*, 2014; Niklas, 2014; Herrero, Stavans, & Flores, 2016) or snowflake yeast (Ratcliff *et al.*, 2012, 2013). The first hyphae were probably similar to those of extant Mucoromycota and gradually evolved sophisticated mechanisms for septum formation, nutrient and organelle trafficking, branch site selection, etc. (for recent reviews on hyphal morphogenesis see Harris, 2011, Lew, 2011, Lin *et al.*, 2014; Steinberg *et al.*, 2017). Primitive hyphae were uncompartmentarized coenocytic multinucleate structures where the free flow of cell content was probably little regulated. In modern hyphae, hyphal segments are closed

off from the growing tip by cross walls (septa) and by various septal occlusures, such as Woronin bodies, dolipores or simpler amorphous materials. It has been hypothesized that the complexity of the septal pore systems correlates with the complexity of the multicellular structures produced by the respective species (Jedd, 2011). It is also noteworthy that transitions to a primarily unicellular lifestyle occurred in several fungal lineages as an adaptation to liquid niches. These lineages, known as yeasts, have small, streamlined genomes resulting from the loss of 3000–4000 genes, coupled with limited novel gene duplicates (Nagy *et al.*, 2014; O'Malley, Wideman, & Ruiz-Trillo, 2016).

Thus, although its genetic bases are incompletely known as yet, simple multicellularity in fungi likely evolved *via* a linear process that could have avoided some of the hurdles that should be overcome for establishing an evolutionarily stable multicellular organization (Brown *et al.*, 2012; Du *et al.*, 2015). Hyphae might not face group conflicts and could bypass the need for fitness alignment between individual cells to directly confer a higher exported organism-level fitness, or handle conflicts at the level of individual nuclei. Fractal-like filling of the available space might further minimize conflict among separate hyphae of the same individual. This is underpinned by negative autotropism observed at the growing edge of vegetative mycelia, whereas in other parts of the colony hyphae can form interconnections (positive autotropism) presumably to enable the flow of nutrients and/or signals across the individual (Leeder, Palma-Guerrero, & Glass, 2011; Fleissner & Herzog, 2016). Similar 'siphonous→multicellular' transformations can be found in certain algae (Niklas, Cobb, & Crawford, 2013; Niklas & Newman, 2013) and may represent a third way to evolve simple multicellularity in addition to the colonial and aggregative routes (Brown *et al.*, 2012; Sebé-Pedrós *et al.*, 2013; Brunet & King, 2017).

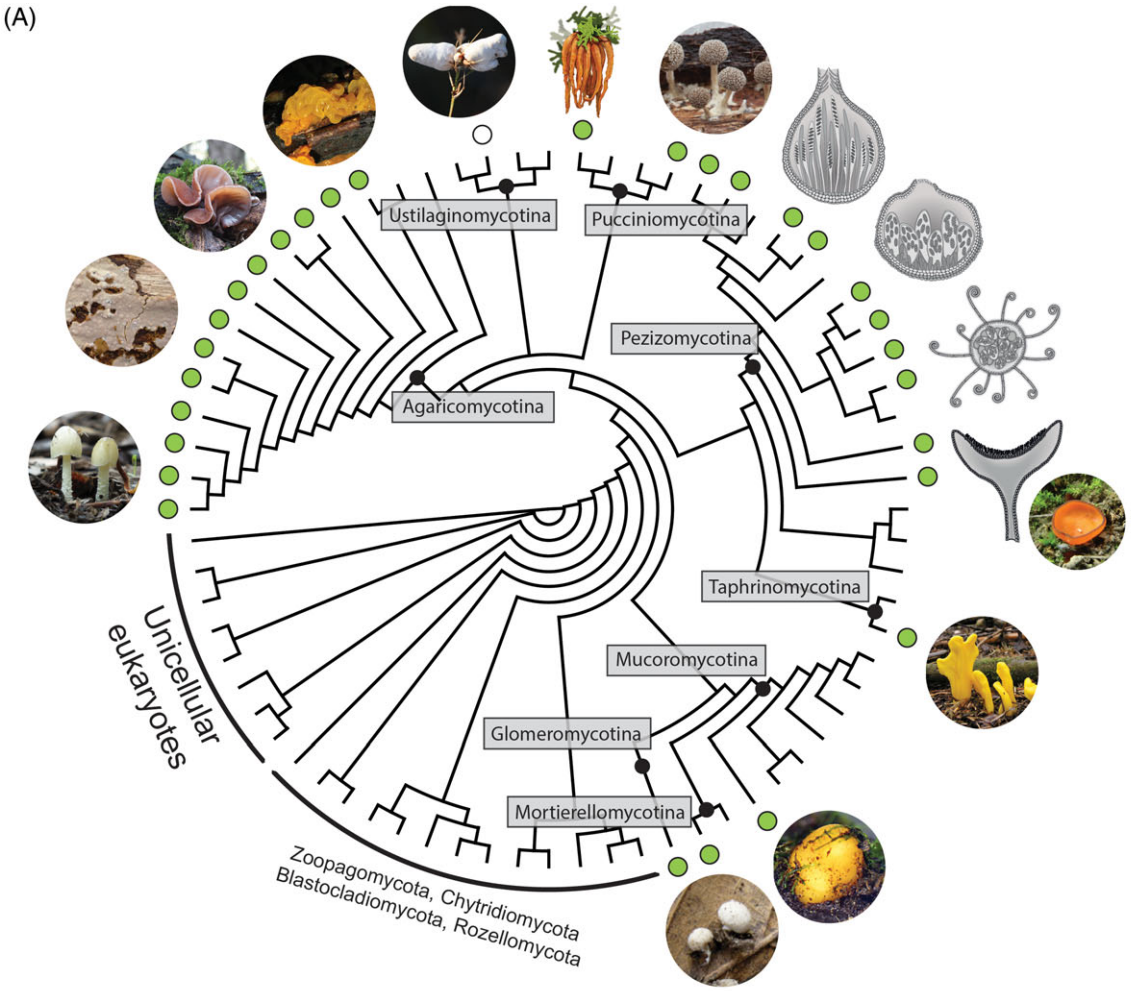
However, fungal mycelia do not show all characteristics of complex multicellularity. The growth of vegetative mycelia is indeterminate and cellular differentiation is mostly limited and not spatially or temporally integrated into a developmental program. Further, all cells are in direct contact with the external environment, which means that nutrient and O_2 uptake through diffusion is not impeded by a compact, three-dimensional organization. Although programmed cell death is widely observed, unprogrammed cell death is not lethal to the entire organism. Thus, we consider vegetative mycelia as a grade of simple multicellularity, while noting that in some cases vegetative mycelia are capable of complex functionalities and can differentiate several distinct cell types.

III. COMPLEX MULTICELLULARITY IN FUNGI

We here define complex multicellularity as structures showing a three-dimensional differentiated organization with a spatially and temporally integrated developmental program that grows until reaching a genetically determined shape and size. Complex multicellularity in fungi is mostly discussed in the context of sexual fruiting bodies (Fig. 1), although fungi produce a plethora of other complex multicellular structures, such as asexual fruiting bodies, rhizomorphs, mycorrhizae or sclerotia (Fig. 2, see below). Fruiting bodies are three-dimensional structures that enclose reproductive cells and the developing spores in a protective environment and facilitate spore dispersal both passively and actively (Roper *et al.*, 2010; Dressaire *et al.*, 2016). This highlights the most crucial difference between fungi and other complex multicellular organisms. Whereas in other lineages the complex multicellular individual performs all organismal functionalities, in fungi it refers to specific structure(s) of the individual. Complex multicellularity in fungi fulfills mostly reproductive roles, whereas for feeding through osmotrophy, foraging for nutrients and exploration of the substrate simple multicellularity clearly represents a better adaptation. Simple and complex multicellularity coexist in the same species in fungi. Fruiting-body-forming fungi undergo a transition from simple to complex multicellularity as part of their life cycle, which not only makes them unique among complex multicellular organisms, but also a potentially useful model system to study the development of complex multicellularity. In spite of this, understanding of the genetics of fruiting body formation is still incomplete.

Another important difference is that growth remains polarized in fruiting bodies, i.e. complex multicellular structures and organs are formed by the aggregation, elongation and specialization of hyphae, which has implications for the evolution of complex multicellularity. For example, there is no need for a qualitatively new mechanism for long-distance distribution of nutrients or O_2 (Woolston *et al.*, 2011), as seen in complex animals and plants. It should be noted, however, that it has been hypothesized that in the most complex fruiting bodies of Basidiomycota air channels are formed by the deposition of hydrophobins along the cell walls.

What is the driving force for the evolution of complex multicellularity in fungi? Predation has been suggested as one factor driving the evolution of increasingly complex and larger animals (Kaiser, 2001; Rokas, 2008; Knoll, 2011). This is, however, unlikely to be a driving force in fungi, because of their osmotrophic lifestyle and because hyphal multicellularity is sufficient to avoid the entire individual being eaten by predators. Even if much of the thallus is destroyed, the individual can completely regenerate, as long as sufficient nutrients are available (Fricker *et al.*, 2017). The most obvious selective advantage for fruiting bodies is the promotion of spore dispersal and enclosure of developing sexual propagules into a three-dimensional structure. Remarkably, both major sporogenous cell types, asci and basidia, have evolved mechanisms for active spore discharge (Trail, 2007; Roper *et al.*, 2010; Dressaire *et al.*, 2016). Increasing the efficiency of spore dispersal could have driven the evolution of structures that enclose and raise asci and basidia above ground level. Fruiting bodies also provide protection against infection and predation of spores, through



(B)

Complex multicellular lineage	Classification	Type of complex multicellular structures	Estimated # CMC species
Agaricomycotina	Basidiomycota	Sexual fruiting bodies, sclerotia	>20,000
Pezizomycotina	Ascomycota	Sexual fruiting bodies, asexual fruiting bodies (coremium, sporodochium, pycnidium, acervulus), sclerotia	>32,000
Pucciniomycotina	Basidiomycota	Sexual and asexual fruiting bodies (pycnium, aecium)	8,400[1]
Ustilaginomycotina	Basidiomycota	Fungal galls on host plants	1,700[1]
Endogonales	Mucoromycota/Mucoromycotina	Underground truffle-like sexual fruiting bodies	25
Neolecta	Ascomycota/Taphrinomycotina	Tongue-like sexual fruiting bodies	3
Glomus spp.	Mucoromycota/Glomeromycotina	Underground truffle-like sexual fruiting bodies	1-10
Modicella	Mucoromycota/Mortierellomycotina	Stalked/globular sexual fruiting bodies	2

Fig. 1. The phylogenetic distribution of complex multicellularity in fungi. (A) Typical complex multicellular morphologies of sexual fruiting bodies are shown for each major clade of fungi. Species pictured from left to right are: *Bolbitius titubans*, *Gloeocystidiellum* sp., *Auricularia auricula-judae*, *Tremella mesenterica*, *Testicularia cyperi*, *Gymnosporangium clavariiforme*, *Phleogena faginea*, *Podospora anserina* (perithecium), *Mycosphaerella* sp. (pseudothecium), *Microspheara* sp. (cleisthothecium), *Peziza* sp. (apothecium), *Neolecta irregularis*, *Endogone flammirocona*, *Modicella reniformis*. Green dots indicate lineages with known complex multicellular representatives; an empty circle at the Ustilaginomycotina refers to the uncertain status of the galls produced by *Testicularia* and allies. (B) Classification, types of complex multicellular structures produced, and estimated number of complex multicellular species (CMCs) for each major lineage of complex multicellular fungi. Images courtesy of Renee Lebeuf, Cimon Jules, George Rogers, Bálint Dima and László G. Nagy. Pictures of *Modicella* and *Endogone* (bottom right) are from Smith *et al.* (2013) and Yamamoto *et al.* (2015). [1]The number of described species is given, the actual number of complex multicellular representatives is much smaller.

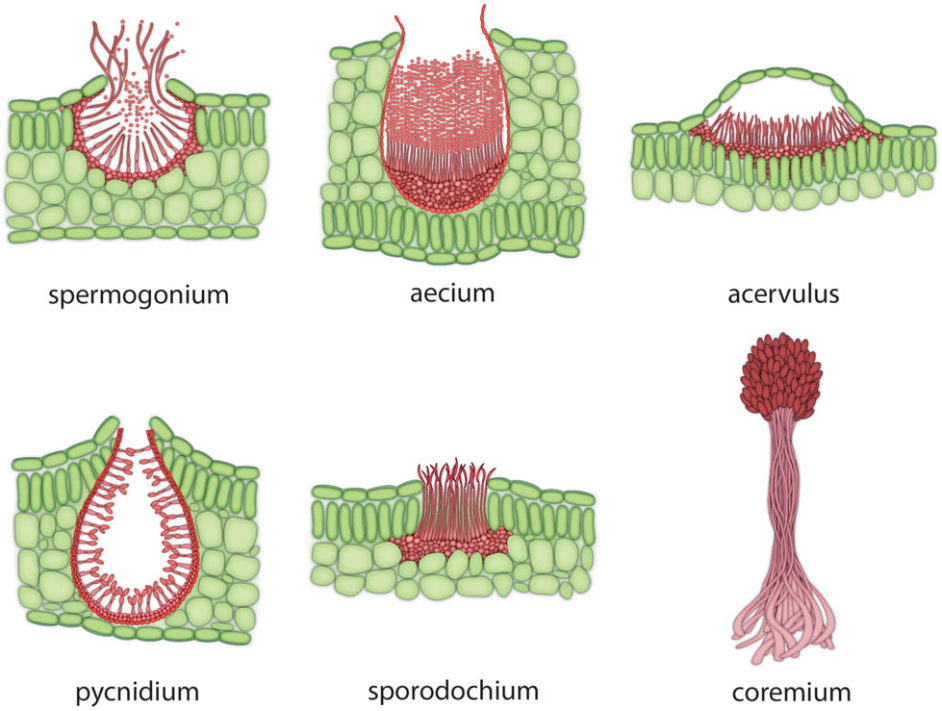


Fig. 2. Asexual complex multicellular structures produced by fungi in the Pucciniomycotina (spermogonium, aecium) and Ascomycota (acervulus, pycnidium, sporodochium, coremium = synnemata). Note that these structures, like all complex multicellular structures, have a genetically determined shape and size and a tightly integrated developmental program. Plant tissue is shaded green.

various structural (veils, setae, hairs, spikes) and chemical defence systems. Insecticidal and antimicrobial armouries are particularly rich in fruiting-body-forming fungi and include secondary metabolites, pore-forming toxins (Plaza *et al.*, 2014) and lectins, many of which are encoded by genes acquired horizontally from bacteria (Kunzler, 2015).

In addition to sexual fruiting bodies, fungi produce a plethora of structures that conform to some or all aspects of complex multicellularity. Asexual fruiting bodies of Ascomycota (pycnidia, acervuli, sporodochia and coremia) are three-dimensional reproductive structures, that harbour asexual spores (conidia). They range from submacroscopic sizes to several centimeters (Fig. 2) and are composed of more or less tightly arranged hyphae. Size, shape and colouration are genetically determined, but cellular differentiation is often limited to a few cell types. Spermogonia, uredinia, aecia and telia (including macroscopic telial horns) are reproductive structures of rust fungi (belonging to the Pucciniomycotina). Although mostly sub-macroscopic (except for the telial horns of *Gymnosporangium* spp.), they have a predetermined developmental program and show cell differentiation and adhesion of almost isodiametric cells (Fig. 2). Ectomycorrhizae, rhizomorphs and sclerotia are also three-dimensional structures (Kues, 2000), however, there might be a looser genetic control over their size and shape than in fruiting bodies. Ectomycorrhizae are small cylindrical structures of mutualistic interaction formed by fungi and their plant partner. In many species, the hyphal mantle formed around the root tips of host plants comprises tightly adhering hyphae that show a complex cellular organization often resembling that seen in fruiting bodies of the same species. Rhizomorphs are shoestring-like organs of clonal dispersal and/or nutrient transport observed in some Basidiomycetes. Sclerotia are compact, three-dimensional structures that fungi develop under unfavourable conditions and that can remain dormant for long periods of time. They evolved convergently in many fruiting-body-forming lineages. Interestingly, ectomycorrhizae, rhizomorphs and sclerotia are found exclusively in fruiting-body-forming fungi, suggesting a link between their development and fruiting body development.

IV. COMPLEX MULTICELLULAR FUNCTIONING IN FUNGI

How complex multicellularity manifests during fruiting body development has been of interest among mycologists for a long time. Information based on mutant screens and classical genetic techniques (Kues, 2000; Pöggeler, Nowrousian, & Kück, 2006; Pelkmans, Lugones, & Wosten, 2016) is being increasingly complemented by high-throughput studies based on whole-genome sequencing and RNA-sequencing (RNA-Seq; Nowrousian, 2014). Studies involving whole-transcriptome comparisons (Table 1) have revealed important principles of fruiting body development in both

Table 1. High-throughput gene expression studies of fungal multicellular development

Model species	Classifica-tion	Reference	Technology used
Agaricus bisporus	Basidiomycota	Morin *et al.* (2012)	RNA-Seq (Illumina)
Antrodia cinnamomea		Lu *et al.* (2014)	RNA-Seq (Illumina)
Armillaria ostoyae		Sipos *et al.* (2017)	RNA-Seq (Illumina)
Auricularia polytricha		Zhou *et al.* (2014)	RNA-Seq (Illumina)
Coprinopsis cinerea		Cheng *et al.* (2013)	5'-SAGE
		Muraguchi *et al.* (2015)	RNA-Seq (Illumina)
		Plaza *et al.* (2014)	RNA-Seq (SOLiD)
Flammulina velutipes		Park *et al.* (2014)	RNA-Seq (Illumina)
Hypsizygus marmoreus		Zhang *et al.* (2015)	RNA-Seq (Illumina)
Lentinula edodes		Wang, Zeng, & Liu (2018)	RNA-Seq (Illumina)
Pleurotus touliensis		Fu *et al.* (2017)	RNA-Seq (Illumina)
Schizophyllum commune		Ohm *et al.* (2010)	5'-SAGE
		Ohm *et al.* (2011)	RNA-Seq (Illumina)
Termitomyces heimii		Rahmad *et al.* (2014)	MADLI TOF
Ustilago maydis		León-Ramírez *et al.* (2017)	Microarray
Fusarium graminearum	Ascomycota	Son *et al.* (2016)	RNA-Seq (Illumina)
Fusarium graminearum, *F. verticilloides*		Rani-Sikhakolli *et al.* (2012)	RNA-Seq (Illumina)
Ophiocordyceps sinensis		Xiang *et al.* (2014)	RNA-Seq (454)
Neurospora crassa		Wang *et al.* (2014)	RNA-Seq (Illumina)
Neurospora crassa, *N. tetrasperma*, *N. discreta*		Lehr *et al.* (2014)	RNA-Seq (Illumina)
3 *Neurospora*, 2 *Fusarium* spp.		Trail *et al.* (2017)	RNA-Seq (Illumina)
Pyronema confluens		Traeger *et al.* (2013)	RNA-Seq (Illumina)
Sordaria macrospora		Teichert *et al.* (2012)	Single-cell RNA-Seq (Illumina)

MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; RNA-Seq, RNA sequencing; SAGE, serial analysis of gene expression; SOLiD, sequencing by oligonucleotide ligation and detection.

model and non-model fungal species. It is becoming evident that in terms of morphogenesis and function, there are a number of similarities and differences between fungi and other complex multicellular clades, although much remains to be clarified regarding the evolutionary origins and genetic underpinnings of complex multicellularity in fungi. In the following sections we discuss known patterns of development, cell adhesion and signalling in fruiting-body-forming fungi, with a particular emphasis on general principles.

(1) Fungal development

Fungi are unique among complex multicellular organisms in that they can switch between simple and complex multicellularity during their life cycle. While the vegetative mycelium is composed of indeterminately growing hyphae that rarely adhere to each other and are thus considered to exhibit simple multicellularity herein, fruiting bodies are clearly complex multicellular structures: their development is a genetically determined process that involves adhesion, cell differentiation, growth, PCD and senescence. It starts with a transition from fractal-like growing vegetative hyphae to a three-dimensional hyphal aggregate through intense localized hyphal branching and adhesion (Kues, 2000; Pöggeler *et al.*, 2006; Lakkireddy *et al.*, 2011; Lichius *et al.*, 2012) (Fig. 3). In the Basidiomycota, this aggregate is known as the primary hyphal knot. In the Ascomycota, development has been most widely studied in perithecium-forming Sordariomycetes (e.g. *Sordaria, Neurospora*), where the earliest complex multicellular stage is the protoperithecium (Fig. 3). The development of the hyphal knot and the protoperithecium involves the reprogramming of hyphal branching patterns to form the first step of complex multicellularity. Subsequently, the differentiation of major tissue types takes place in secondary hyphal knots and perithecia in the Basidio- and Ascomycota, respectively. It has been estimated that perithecia can differentiate up to 13 cell types (Lord & Read, 2011), although the actual number of cell types, especially in the Basidiomycota, might be significantly higher.

The development of mature fruiting bodies follows genetically encoded programs that determine the species-specific morphologies (Kues, 2000; Kamada, 2002; Pöggeler *et al.*, 2006; Kamada *et al.*, 2010; Trail & Gardiner, 2014), followed by senescence through the action of various oxidative enzymes (laccases, phenol oxidases), cell-wall degrading enzymes and tyrosinases, among others (Moore, 2005; Sakamoto *et al.*, 2017). This is similar to death or organ senescence in other complex multicellular lineages and putatively serves the purpose of giving way to new reproducing generations of fruiting bodies and possibly recycling of cellular components towards reproductive cells (Moore, 2005). Growth remains apical even within fruiting bodies, but cell shape is extensively modified, ranging from hyphal to inflated and even isodiametric or polyhedral [referred to as conglutinate cells in *Sordaria* (Lord & Read, 2011)], similar to animal and plant cells. Non-terminal cells might form side branches, but regions of cell proliferation, resembling that in animals, to the best of our knowledge do not exist. Following a wave of cell-differentiation events, growth in fruiting bodies

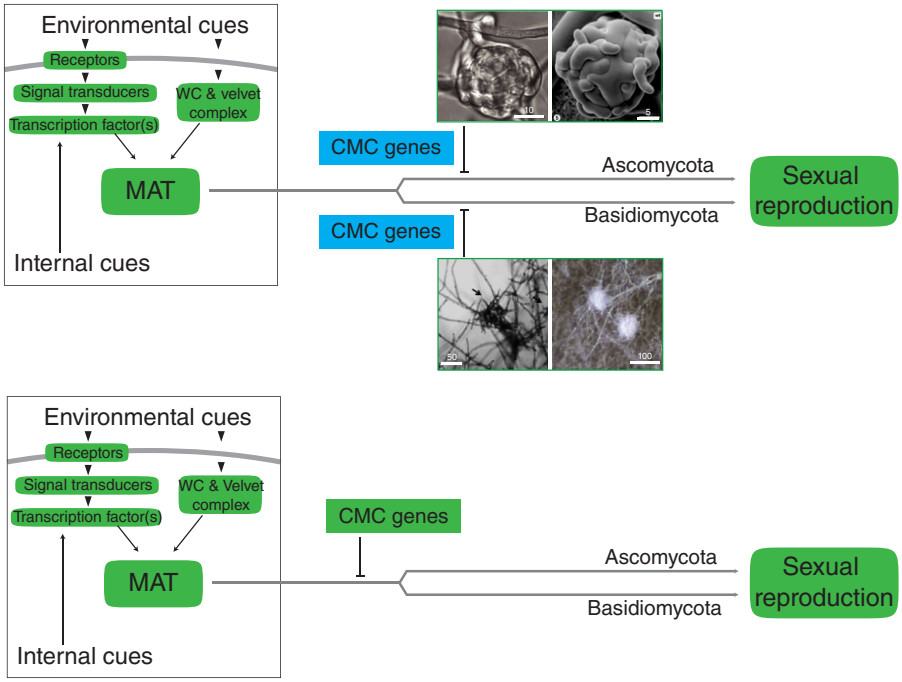


Fig. 3. Two alternative hypotheses for the evolution of complex multicellularity in fungi illustrated using a simplified case comprising Asco- and Basidiomycota. The initiation and trajectory of sexual reproduction in fungi comprises universally conserved mechanisms (highlighted in green). Genetic circuits involved in the development of fruiting bodies therefore should be linked with these conserved developmental pathways. A central question from the perspective of the evolution of fungal multicellularity is how genetic mechanisms of fruiting body development are linked to conserved circuits of sexual reproduction. The convergent origins model (top) implies that genetic mechanisms for fruiting body morphogenesis evolved independently along all lineages of complex multicellular fungi, whereas a single origin model (bottom) implies that at least part of the genetic toolkit of fruiting body development arose before the divergence of complex multicellular lineages. The presence of such genetic circuitries may predispose fungi to the recurrent evolution of complex multicellularity. The earliest complex multicellular stages, protoperithecia and primary hyphal knots for the Asco- and Basidiomycota are shown in the upper and lower images in A, respectively. Images are from Lord & Read (2011), Mayrhofer, Weber, & Pöggeler (2006) and Lakkireddy *et al.* (2011). CMC, complex multicellularity; MAT, mating genes; WC, whilte collar.

is achieved by manipulating cell size through turgor and cell wall expansion.

There is evidence for autophagic cell death playing a role in sculpting fruiting bodies of both the Asco- and Basidiomycota. It should be noted that PCD of non-terminal cells may be counter-selected in fruiting body development, because it disrupts nutrient transport along the hyphae. Nevertheless, PCD has been reported to play a role in forming the gill cavity of *Agaricus bisporus* (Umar & van Griensven, 1998; Lu, 2006) (although this has been disputed) and in removing paraphyses from within ascomycete perithecia, presumably to give way to asci and spore release (Trail & Gardiner, 2014). Further, autophagy genes are required for fruiting body development in *Sordaria macrospora* (Voigt *et al.*, 2013; Voigt & Pöggeler, 2013), although how their defects disrupt development is not yet known.

(2) Cell adhesion in fungi

Most of our knowledge on adhesive proteins of fungi pertains to adhesion to animal and plant hosts and various surfaces (e.g. medical devices) and comes primarily from simple multicellular and secondarily unicellular (i.e. yeast) species. Adhesion is mediated by a combination of sticky cell wall proteins and secreted carbohydrates, although the precise composition of fungal adhesives is highly heterogeneous (Tucker & Talbot, 2001; de Groot *et al.*, 2013; Epstein & Nicholson, 2016). Most cell wall proteins with glycosylphosphatidylinositol (GPI) anchoring (Sundstrom, 2002; de Groot *et al.*, 2013) to the cell wall have adhesive properties (Weig *et al.*, 2004; Dranginis *et al.*, 2007) and include adhesins (Sundstrom, 1999, 2002; Weig *et al.*, 2004), flocculins (Dranginis *et al.*, 2007) and sexual agglutinins (Lipke & Kurjan, 1992) that mediate in the adhesion of yeast cells. Other adhesive molecules include glycoproteins (Newey, Caten, & Green, 2007) that are linked to cell wall sugars through N- or O-linked oligosaccharides (Bowman & Free, 2006) (mostly mannose or galactomannan) and secreted mannosyl and glucosyl residues. Although not much is known about the role and composition of the extracellular matrix in complex multicellular fungi, its deposition has been observed even in the earliest stages of fruiting body development (Lichius *et al.*, 2012).

Our understanding of cell adhesion within fruiting bodies is far from complete (Lord & Read, 2011; Trail & Gardiner,

2014), nevertheless, many of the adhesive proteins described from simple multicellular fungi have been detected in fruiting bodies. GPI-anchored and fasciclin-like proteins (Miyazaki *et al.*, 2007; Liu *et al.*, 2009) have been implicated in cell adhesion within fruiting bodies (Trail, 2013), whereas hydrophobins have been suggested to form air channels that circumvent the limits of diffusion in three-dimensional structures (Lugones *et al.*, 1999). Similarly, lectins have been detected in fruiting bodies of Asco- and Basidiomycota, and might be involved in cell adhesion (Hassan *et al.*, 2015) but also in defence against predators (Hassan *et al.*, 2015). These proteins are conserved across the Asco- and Basidiomycota at the family level which is consistent with both vertical inheritance of function and their parallel co-option for hypha–hypha adhesion in complex multicellular lineages. This would parallel adhesive molecules of complex animals having presumably evolved early in protist evolution for prey capture, later co-opted for cell–cell adhesion (King *et al.*, 2003; Abedin & King, 2008, 2010; Rokas, 2008; Richter & King, 2013).

(3) Cell–cell communication and signalling

Multicellular organisms mediate transcriptional responses to external stimuli and synchronize cell functioning by various signal transduction pathways both within and between cells (King *et al.*, 2003; King, 2004; Miller, 2012; de Mendoza, Sebé-Pedrós, & Ruiz-Trillo, 2014). Because of how cells arise in fungi, communication along and between hyphae necessarily follows different principles. There are known mechanisms for information processing along hyphae in vegetative mycelia: cytoplasmic connections through septal pores are channels for cell–cell communication (Extebeste & Espeso, 2016; Kues *et al.*, 2018). However, there is no functional analogue of plasmodesmata or gap junctions that would mediate crosstalk between neighbouring (Bloemendal & Kuck, 2013) hyphae in fruiting bodies. Intercellular communication in fungi relies on the diffusion of chemical signals through the extracellular space, such as pheromones, volatile compounds, and quorum-sensing molecules (Albuquerque & Casadevall, 2012; Cottier & Mühlschlegel, 2012; Wongsuk, Pumeesat, & Luplertlop, 2016; Kues *et al.*, 2018), including small proteins (Wang *et al.*, 2013; Gyawali *et al.*, 2017). It has evolved to signal through a loosely occupied space or among unicells and primarily suits the needs of vegetative mycelium or yeast cells. Nonetheless, such systems could be easily co-opted to communicate across tightly arranged hyphae in fruiting bodies, as suggested by a higher diversity (Pöggeler *et al.*, 2006; Busch & Braus, 2007; Stajich *et al.*, 2010; Frey, Reschka, & Poggeler, 2015; Kuck, Beier, & Teichert, 2016) of certain kinase gene families in fruiting-body-forming fungi, the expression of several kinases in fungal fruiting bodies and defects in fruiting body development in many kinase mutants (Pöggeler *et al.*, 2006). Remarkably, defects in either of the three mitogen-activated protein kinase (MAPK) pathways of fungi impact fruiting body initiation (Kicka & Silar, 2004; Lichius *et al.*, 2012). Self-signalling, hypha-to-hypha dialog and fusion play fundamental roles in sclerotium and rhizomorph development (Erental, Dickman, & Yarden, 2008). Although the precise mechanisms of interhyphal communication within fruiting bodies remain unknown so far, the lack of intercellular channels between neighbouring hyphae suggests that fungi use different strategies to orchestrate the functioning of complex multicellular structures compared to plants and animals.

V. CONVERGENT ORIGINS OF COMPLEX MULTICELLULARITY IN FUNGI

Complex multicellularity evolved in only five eukaryotic groups (Cock *et al.*, 2010; Parfrey *et al.*, 2011; Niklas, 2014; Umen, 2014; Brawley *et al.*, 2017). Within fungi, it occurs in most major clades and shows signs of convergent evolution (Sugiyama, Hosaka, & Suh, 2006; Schoch *et al.*, 2009; Taylor & Ellison, 2010; Knoll, 2011) (Fig. 1). The best known complex multicellular clades are Pezizomycotina and Agaricomycotina in the Asco- and Basidiomycota, respectively, where the majority of fruiting-body-forming fungi are found (Fig. 1). Although two origins of complex multicellularity are generally proposed for fungi (Schoch *et al.*, 2009; Stajich *et al.*, 2009; Knoll & Lahr, 2016), complex multicellular structures also occur in the early diverging Mucoromycota, the primarily yeast-like Taphrinomycotina as well as the Puccinio- and Ustilaginomycotina. Of these, the earliest diverging is the Mucoromycota, which primarily contains simple multicellular moulds but also three small groups of fruiting-body-forming fungi. Members of the Endogonales (Mucoromycotina) form globose, truffle-like sporocarps filled with zygospores (Fig. 1), while *Modicella* (Mortierellomycotina) forms small stalked fruiting bodies that contain sporangia and sporangiospores (Smith *et al.*, 2013) (Fig. 1). Similarly, some Glomeromycotina species produce small, underground sporocarps (Blaszkowski, 2012; Smith *et al.*, 2013). The Taphrinomycotina (Ascomycota) contains a single known fruiting-body-forming genus, *Neolecta* that forms brightly coloured irregular or tongue-like fruiting bodies on soil (Nguyen *et al.*, 2017). This genus is particularly interesting from a developmental perspective as it is nested in a clade of primarily unicellular yeasts, and has a yeast-like genome architecture (Nagy, 2017; Nguyen *et al.*, 2017).

In the Basidiomycota, the largest fruiting-body-producing lineage is the Agaricomycotina, where multicellularity reached its highest complexity in fungi. Nearly all of the species produce fruiting bodies, with the exception of some secondarily reduced yeast lineages in the Tremellomycetes (Nagy *et al.*, 2014), the Serendipitaceae (Weiss *et al.*, 2016), and ant-associated Pterulaceae that might have lost the ability to form fruiting bodies (Mueller, 2002). This group also includes the most typical manifestations of agaricoid 'mushroom' morphologies as well as an array of morphologically diverse forms (Hibbett & Binder, 2002; Hibbett, 2007). Aside from the Agaricomycotina, complex multicellular species are found in the Puccinio- and Ustilaginomycotina (generally

known as rust and smut fungi) as well, although they represent the minority of species in their clades compared to simple or yeast-like forms. Fruiting bodies are known in at least four classes of the Pucciniomycotina (Aime *et al.*, 2006) (Atractelliomycetes, Agaricostilbomycetes, Pucciniomycetes, Microbotryomycetes) and include simple capitate (*Phleogena*) or cup-shaped (*Platygloea*, *Kriegeria*; Fig. 1) morphologies, but also crust-like (e.g. *Septobasidium*) and gelatinous (e.g. *Helicogloea*) forms resembling those found in early-diverging Agaricomycotina. As the relationships of fruiting-body-forming classes of Pucciniomycotina are still unresolved (Aime *et al.*, 2006; Bauer *et al.*, 2006; Wang *et al.*, 2015), there is uncertainty as to the number of independent origins of fruiting body development in this subphylum. The occurrence of true fruiting bodies in the Ustilaginomycotina may be controversial. *Ustilago maydis* was recently reported to produce fruiting-body-like structures *in vitro* (Cabrera-Ponce *et al.*, 2012) whereas other species (e.g. *Testicularia* spp., *Exobasidium* spp.) produce gall-like swellings on parasitized plants that are mostly made up of fungal hyphae but incorporate some of the plant tissue too. Although these show some features of complex multicellularity (e.g. tight arrangement of hyphae, adhesion), whether their development follows a genetically pre-determined program or their growth is determinate, remains to be understood (Nagy, 2017).

The phylogenetic distribution of complex multicellular fungi is patchy and the above-mentioned lineages outline at least eight complex multicellular clades. However, the Pucciniomycotina, Glomeromycotina and potentially the Ustilaginomycotina may represent more than a single origin of fruiting-body-producing species, yielding 11 as a conservative upper estimate for the number of independent complex multicellular clades in fungi, although this number may be refined as better resolved phylogenies become available.

VI. EVOLUTIONARY TIMESCALE FOR COMPLEX MULTICELLULAR FUNGI

Complex multicellular arose over a vast timespan, yet their origins and diversification might have required some basic similarities such as eukaryotic prehistory and geological and abiotic conditions [e.g. O_2 or sulfide concentrations (Canfield & Teske, 1996; Canfield, Poulton, & Narbonne, 2007; Johnston *et al.*, 2010; Richter & King, 2013)]. Whereas simple multicellular lineages may be as old as 3.5 Ga (Aguilar *et al.*, 2015), complex multicellular organisms originated much later. Recent studies (Parfrey *et al.*, 2011; dos Reis *et al.*, 2015; Sharpe *et al.*, 2015) date complex multicellular clades to between 175 and 800 million years ago (Mya), with the Metazoa being the oldest (700–800 Mya), followed by Florideophyceae red algae (Xiao *et al.*, 2004; Parfrey *et al.*, 2011) (550–720 Mya), the Embryophyta (430–450 Mya) and macroscopic brown algae (175 Mya) (Silberfeld *et al.*, 2010). Due to the soft texture of fungal fruiting bodies, the fossil record is very patchy and available fossilized fruiting bodies are far too recent to provide reasonable estimates for the age of complex multicellular clades (Poinar & Singer, 1990; Hibbett, Grimaldi, & Donoghue, 1995, 1997; Hibbett *et al.*, 2003; Berbee & Taylor, 2010; Cai *et al.*, 2017). Yet, the oldest known fruiting body fossil, a perithecium known as *Paleopyrenomycites devonicus* (Taylor *et al.*, 2005) is from the early Devonian (*ca.* 400 Mya) placing the earliest physical evidence for complex multicellular Ascomycota as roughly the same age as the origin of embryophytes or red algae. Based on this and other Ascomycota fossils, molecular clock analyses inferred the origins of the Pezizomycotina, the largest complex multicellular clade in fungi, at 537 (443–695) Mya (Prieto & Wedin, 2013; Beimforde *et al.*, 2014). The age of the Agaricomycotina has been inferred at 429–436 Mya based on multiple calibration points and phylogenomic data sets (Floudas *et al.*, 2012; Chang *et al.*, 2015; Kohler *et al.*, 2015). To the best of our knowledge, no molecular age estimates are available for *Endogone* and *Modicella*, nevertheless, their limited diversity and recent divergence from simple multicellular fungi suggest they are much younger than either the Agarico- or Pezizomycotina. Similarly, although chronological information is lacking for complex multicellular Puccinio-, Ustilagino- and Taphrinomycotina, the patchy distribution of complex multicellular taxa in these clades suggests relatively recent origins. Taken together, the origins of the Pezizomycotina and Agaricomycotina seem to coincide with the origins of complex multicellular plants and algae in the Paleozoic, although significantly older estimates have also been published (Heckman *et al.*, 2001; Berbee & Taylor, 2010). The much younger ages for smaller complex multicellular clades suggests that the evolution of complex multicellularity in fungi is not tied to specific geologic events, as suggested for animals (Rokas, 2008), but was probably dependent on internal contingencies.

VII. IS THERE A LARGE GENOMIC HURDLE TO COMPLEX MULTICELLULARITY?

Although a complete understanding of multicellularity-related genetic elements is lacking for any lineage, the significant increases in phenotypic complexity associated with the evolution of complex multicellularity suggests that a comparably large set of genetic novelties was required (Cock *et al.*, 2010; Knoll, 2011). This would be concordant with it being a rare event in evolution. Genetic innovations underpinning the evolution of multicellularity have mostly been discussed in the context of gene duplications (Rokas, 2008; Cock *et al.*, 2010; Stajich *et al.*, 2010; Miller, 2012; Richter & King, 2013; Brawley *et al.*, 2017; Brunet & King, 2017; Sebé-Pedrós *et al.*, 2017), co-option (King *et al.*, 2003; Richter & King, 2013; Brunet & King, 2017; Sebé-Pedrós *et al.*, 2017) and to a lesser extent other sources of genetic novelty. In fungi, a number of transitions to complex multicellularity are coupled with surprisingly limited gene family diversification. The genus *Neolecta* (Taphrinomycotina)

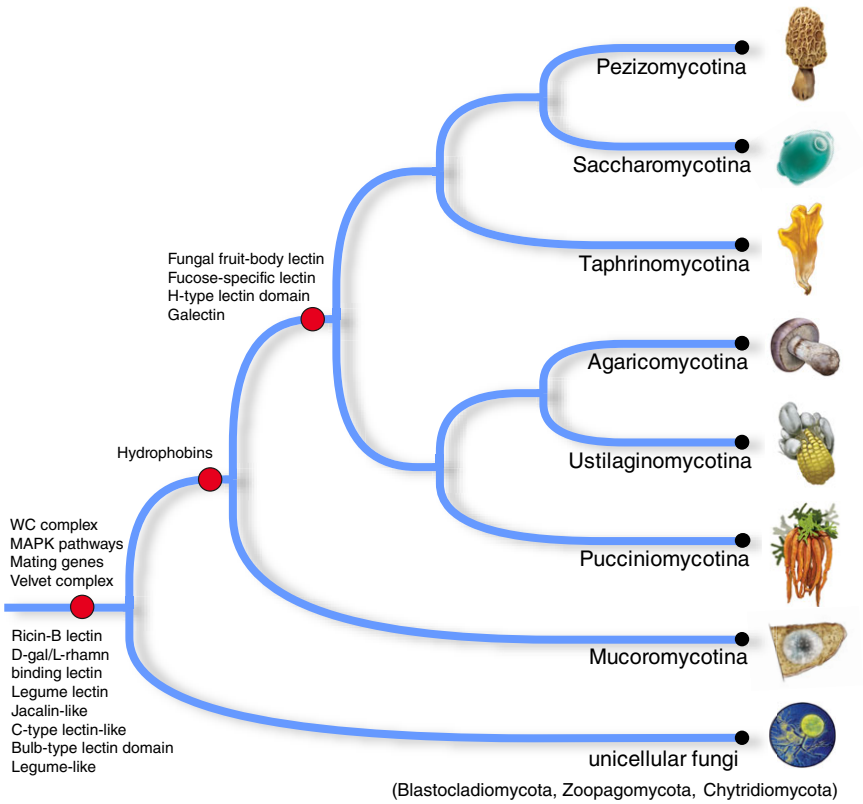


Fig. 4. The conservation of characteristic gene families related to complex multicellularity in fungi. Several gene families involved in cell adhesion, defence, fruiting body initiation and morphogenesis are conserved across fungi, suggesting that the genetic prerequisites for multicellular functioning are widely available in uni- and simple-multicellular fungi. Note that the emergence of most families pre-dates the divergence of major clades of complex multicellular fungi, including the largest clades Pezizomycotina and Agaricomycotina. MAPK, mitogen-activated protein kinase, WC, white collar.

and fruiting-body-forming members of the Tremellomycetes and Pucciniomycotina, possess small genomes with a secondarily reduced protein-coding capacity, similar to that of secondarily unicellular yeasts (Stajich *et al.*, 2010; Nagy, 2017). Consistent with an independent origin of complex multicellularity, *Neolecta* is nested in a clade of yeast-like and simple multicellular fungi (Taphrinomycotina) (Fig. 4), which, we estimate, split from its closest extant complex multicellular relative >500 million years ago (based on Kohler *et al.*, 2015). Yet, its genome encodes as few as 5500 protein-coding genes (fewer than that of *Saccharomyces cerevisiae*) and very limited gene family diversification has been inferred along the evolutionary route to *Neolecta* (Nguyen *et al.*, 2017). This is consistent with three hypotheses. First, the genetic hurdle to complex multicellularity may not be great and it may be relatively 'easy' for fungi to evolve complex multicellular structures. Second, gene duplications might not be the key changes underlying the evolution of complex multicellularity. Rather, building on a conserved gene repertoire shared with other Ascomycota, other sources of genetic innovations (e.g. gene regulatory network rewiring, alternative splicing patterns, non-coding RNA species) could underlie the independent origin of fruiting bodies in *Neolecta*, similarly to the picture emerging from studies of animal multicellularity (Richter & King, 2013; Grau-Bove *et al.*, 2017; Sebé-Pedrós *et al.*, 2017). Third, a single origin of fruiting bodies in the Ascomycota could explain limited gene family diversification on the evolutionary path leading to *Neolecta*, but would not explain the lack of known fruiting body genes of the Pezizomycotina from its genome (Nguyen *et al.*, 2017). This would also be a relatively unparsimonious scenario, requiring several losses of fruiting body production in the Taphrinomycotina and the Saccharomycotina, among others. Which of these, or their combination best explains the evolution of complex multicellularity in *Neolecta* and other fungi remains to be understood.

VIII. HOW MANY ORIGINS OF COMPLEX MULTICELLULARITY IN FUNGI?

Complex multicellularity in fungi is a patchy character (Telford *et al.*, 2015) that appears in many phylogenetically distant clades. The prevailing view is that fungal fruiting bodies arose through convergent evolution (Schoch *et al.*, 2009; Stajich *et al.*, 2009; Taylor & Ellison, 2010; Knoll, 2011; Sebé-Pedrós *et al.*, 2017), which is supported by the

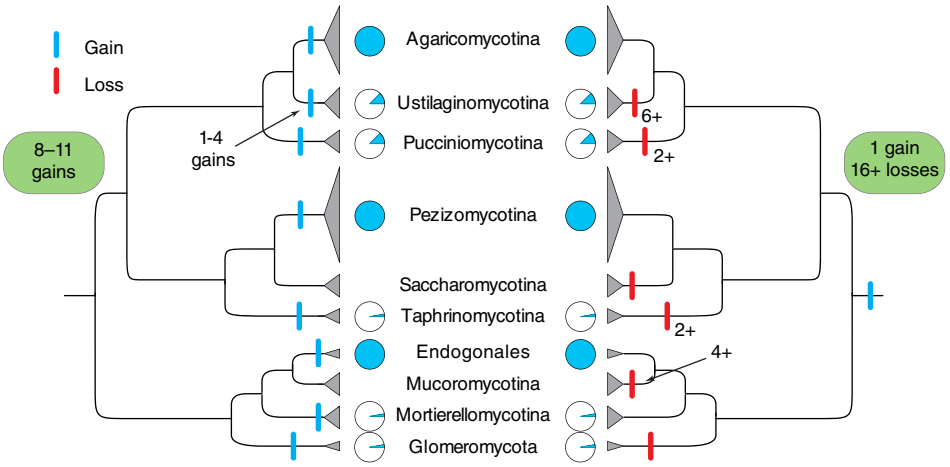


Fig. 5. Alternative phylogenetic models for the recurrent origins of complex multicellularity in fungi. Gains and losses of complex multicellularity across fungi under two contrasting models are shown by vertical blue and red bars, respectively. The model implying convergence requires 8–11 independent origins to explain the phylogenetic distribution of complex multicellular fungi, whereas a model implying a single origin requires one gain and >16 losses. Clades containing complex multicellular species are marked by pie charts with the blue section corresponding to the estimated fraction of complex multicellular species.

apparent lack of homologies between fruiting bodies in different clades. In Section V we discussed eight major clades of complex multicellular fungi (Fig. 1), although there might be as many as 11, depending on the number of independent fruiting-body-forming clades in the Pucciniomycotina. If all of these clades evolved complex multicellularity independently, there are 8–11 origins of this trait within fungi, compared to only four outside fungi. This large number and density of fungal complex multicellular clades prompts us to examine alternative views for the origin of complex multicellularity in fungi. How would models implying a single origin of complex multicellularity compare to those implying multiple origins? Phylogenetically, a multiple-origins model is more parsimonious than a single-origin model, requiring 8–11 origins compared to one origin and >16 losses to explain the distribution of complex multicellularity across fungi (Fig. 5). However, the likelihood of the recurrent evolution of multigenic traits might be orders of magnitudes lower than that of a single origin followed by multiple losses. Below we discuss how the phylogenetic conservation of developmental modules, genes and pathways underlying fruiting body development fits alternative scenarios of the evolution of complex multicellularity in fungi.

(1) Homologies between independently evolved complex multicellular fungi?

If complex multicellular structures in disparate clades share homology, it should be detectable among genes involved in fruiting body development in the Asco- and Basidiomycota. Fruiting bodies in these clades show no evident homology at the phenotype level, however, this comes at no surprise as phenotypes can diverge quickly; a more appropriate question therefore is whether homologies exist at the level of the underlying genetic background. Some Asco- and Basidiomycota fruiting bodies represent the best researched complex multicellular structures of fungi and these model species belong to some of the most phylogenetically distant complex multicellular clades found in fungi, providing a suitable framework for addressing similarities between convergently evolved complex lineages. In particular, are there homologies at the level of interactions high in the gene regulatory networks (including the initiation of the complex multicellular phase) and the key cellular functions of complex multicellularity (adhesion, communication, development)?

The development of complex multicellular structures is part of the sexual reproductive program in fungi. In the most general sense, sexual reproduction, including mate detection, cell fusion and the formation of sexual propagules, and many of the associated genetic pathways are conserved across fungi. Fruiting bodies evolved to protect the developing sexual progeny and thus any gene regulatory network orchestrating their development should be involved in the pathways governing sexual development. Indeed, mating genes regulate several aspects of fruiting body development: the formation of fruiting body initials (protoperithecia) of the Ascomycota, and of primary and secondary hyphal knots of *Coprinopsis cinerea* are regulated by the *A* and *B* mating-type genes (Kues *et al.*, 1998, 2002). On the other hand, protoperithecia of *Neurospora crassa* appear in a mating-independent manner, before fertilization by a conidium of the opposite mating type. Both protoperithecium and primary hyphal knot formation are induced by nutrient (mostly N_2) starvation (Pöggeler *et al.*, 2006) through mechanisms that are widely conserved across fungi (D'Souza & Heitman, 2001; López-Berges *et al.*, 2010; Shertz *et al.*, 2010) (Fig. 3) and even deeper in the eukaryotes [e.g. *Dictyostelium* (Dubravcic, van Baalen, & Nizak, 2014)]. More generally, nutrient availability is an important signal for sex in fungi: nutrient-sensing pathways regulate sexual development through the mating-type genes (Lengeler *et al.*,

2000), similar to many other processes that impact fruiting body development.

The initiation of the complex multicellular phase is dependent on a number of additional factors, such as changing environmental conditions (e.g. temperature, CO_2 concentration) and the perception of external signals, such as light, by the vegetative mycelium. Light sensing relays several important processes of fruiting body development, including its initiation, stimulating growth in the right direction and sensing seasonal light/dark periodicity that triggers fruiting (Pöggeler *et al.*, 2006; Kamada *et al.*, 2010). Many of these responses are mediated by the blue light receptor white collar complex (WCC) which, including its regulatory role in fruiting body development, is conserved widely (Idnurm & Heitman, 2005; Rodriguez-Romero *et al.*, 2010; Verma & Idnurm, 2013), although the specific interaction may differ even among closely related species (Purschwitz *et al.*, 2008; Kim *et al.*, 2015). The WCC regulates sexual reproduction through mating genes (Idnurm & Heitman, 2005) in all fungal species examined so far (Idnurm & Heitman, 2010), except for budding and fission yeasts in which the WCC has been lost (Nguyen *et al.*, 2017). Similarly, the gross structure of mating pathways, that of mating loci, and the regulation of sexual reproduction by mating genes is conserved across the Dikarya (Asco- and Basidiomycota) and possibly in even earlier fungi (Casselton, 2002; Raudaskoski & Kothe, 2010; Jones & Bennett, 2011; Kim *et al.*, 2012). G-proteins and the MAPK cascade that transduces the signal of compatible mate partner to the nucleus are also highly conserved across fungi (Jones & Bennett, 2011; Kruzel, Giles, & Hull, 2012; Ait Benkhali *et al.*, 2013), although differences between species exist at the level of terminal transcription factor identity (Kruzel *et al.*, 2012). Two MAPK pathways (cell wall integrity and osmoregulatory) are also highly conserved across fungi and are required for fruiting body development (Lichius *et al.*, 2012). The velvet complex coordinates differentiation processes and influences (a)sexual fruiting body development. Velvet complex proteins originated before the last common ancestor of complex multicellular lineages and are conserved across most fungi (Bayram & Braus, 2012) (Fig. 4).

On the other hand, little is known about the conservation of effector genes and cellular differentiation pathways (e.g. genes involved in morphogenesis, differentiation, etc.) that implement the complex multicellular phase. Adhesion-related GPI-anchored proteins as well as hydrophobins are conserved across all fungi and are involved in fruiting body development in both the Asco- and Basidiomycota (Bruneau *et al.*, 2001; Costachel *et al.*, 2005; Szeto, Leung, & Kwan, 2007; Robledo-Briones & Ruiz-Herrera, 2013). However, given their different roles in simple multicellular and yeast species, whether their widespread role in fruiting body development evolved through parallel co-option or reflects a plesiomorphic condition is difficult to decide. Similarly, lectins have been implicated in adhesion and defence in both the Asco- and Basidiomycota fruiting bodies (Hassan *et al.*, 2015), although different clades (and often different species) made use of different lectin families.

The lack of discernible homology among known genetic aspects downstream of fruiting body initiation implies extensive convergence. This is underpinned by the fact that most transcription factors known to be involved in fruiting body morphogenesis are specific to either the Asco- or Basidiomycota, although conservation of function in sexual reproduction has been reported at the family level (e.g. high mobility group-box transcription factors) (Ait Benkhali *et al.*, 2013), which might suggest a plesiomorphic role in cell differentiation or that certain functions tend to be recruited repeatedly for fruiting body development.

Taken together, the genetic toolkit of fruiting body development includes both universally conserved and lineage-specific elements, suggesting that it has been assembled gradually during evolution. Whereas many aspects of fruiting body development show convergence, homology exists among regulatory gene circuits underlying the initiation of fruiting body development and might exist at the level of certain multicellular functionalities. This points to a single origin of some of the foundations of complex multicellularity in fungi, which is remarkable from the perspective of independent origins and raises the question of how conservation can be reconciled with genetic theories of convergent evolution.

Explaining phenotypic convergence is a major challenge in evolutionary biology. Convergence in the classic sense implies lack of homology, although recent advances revealed that this concept does not hold for several convergently evolved traits and suggests that a reassessment of evolutionary convergence is necessary (Prud'homme, Gompel, & Carroll, 2007; Gompel & Prud'homme, 2009; Stern, 2013; Nagy *et al.*, 2014). Phenotypic convergence can arise as a result of a range of genetic processes that include contributions of both homology and homoplasy (convergence/parallelism) (Panganiban *et al.*, 1997; Shubin, Tabin, & Carroll, 2009; Wake, Wake, & Specht, 2011; Nagy *et al.*, 2014). Two related concepts, deep homology (Shubin *et al.*, 2009) and latent homology (Nagy *et al.*, 2014) refer to the deep conservation of regulatory circuitries underlying different phenotypes and to developmental modules that predispose lineages for convergent evolution, respectively. Latent homologies can facilitate the recurrent evolution of similarity if minor tweaks to the spatial or temporal regulation of conserved developmental modules can result in parallel co-option for the same functionality. Essentially, this reduces the mutational target size for evolution, which leads to a significantly higher probability of convergence than if mutations were required to all genes independently. In the context of complex multicellularity in fungi, latent homologies assume the existence of universally conserved genes or modules that were convergently co-opted for complex multicellularity while either retaining or losing their ancestral functions. One could speculate that genes encoding proteins related to adhesion on host surfaces by hyphae or spores have been rewired for hypha–hypha adhesion in complex multicellular lineages. This would parallel

the long prehistory of multicellularity-related genes in unicellular and colonial protists and their extensive co-option at the origin of multicellular animals (King, 2004; Sebé-Pedrós *et al.*, 2017, 2013). Deep homology refers to homologous regulatory wiring high in the hierarchy of gene expression regulation that underlies phylogenetically and phenotypically independent traits. A classic example of deep homology is provided by arthropod and vertebrate appendages that evolved independently, yet, their development relies on the same deeply conserved gene regulatory networks. The disparate origins of many aspects of fruiting body development in the Asco- and Basidiomycota combined with shared mechanisms of their initiation raise the possibility of deep or latent homologies underlying their evolutionary history.

Fruiting body formation is a complex developmental process, and its current manifestations in both the Asco- and Basidiomycota probably evolved in a gradual manner. Similarly, gene regulatory circuits that orchestrate fruiting body development certainly also evolved in a stepwise manner, building on ancient regulatory modules, but also on co-option of conserved genes and the evolution of new ones (Fig. 4). At the moment relevant information to reconstruct precisely the evolution of the genetic toolkits underlying Asco- and Basidiomycota fruiting bodies and to answer the question whether their ancestor (or ancestors of earlier groups) was capable of forming simple fruiting bodies is lacking. Nevertheless, the high phylogenetic density of complex multicellular clades and the conservation of some mechanisms of fruiting body development suggests that convergence in the strict sense may not adequately explain the evolution of complex multicellularity in fungi. Understanding the components and conservation of early developmental modules that physically implement complex multicellularity, downstream of the initiation of fruiting body development, thus represents a key question for understanding the number of origins of complex multicellularity in fungi.

IX. CONCLUSIONS

(1) Fungi are one of the most enigmatic lineages of complex multicellular organisms. Although functional and mechanistic similarities with plant and animal multicellularity exist, there are fundamental differences in the driving forces, and in the timing and mechanisms of the evolution of simple and complex multicellularity in fungi, suggesting that there might not be a unifying framework for the evolution of multicellularity across the tree of life. Is it possible then to establish general principles of the evolution of multicellularity? In terms of complex multicellularity, there is certainly a common syndrome of traits that distinguish complex from simple multicellularity. This includes three-dimensional organization, cell adhesion and an integrated developmental program that results in a multicellular structure or individual with genetically determined size and shape. For most lineages, complex multicellularity comprises the reproducing individual, whereas it mostly serves a reproductive role in fungi. This is a fundamental difference between fungi and other lineages and provides an adaptive explanation for the patchy phylogenetic distribution of complex multicellularity in fungi.

(2) Complex multicellular fungi fall into 8–11 clades. This pattern is currently considered to have happened through convergent evolution. While the genetic bases of several key aspects (e.g. morphological) of complex multicellularity are lineage-specific and thus likely evolved convergently, most mechanisms of fruiting body initiation are universally conserved and thus likely have a single origin in fungi. The morphogenetic processes that link the conserved and lineage-specific developmental modules are among the least known aspects of fruiting body development currently, yet these might represent the crux of the matter for understanding the origins of complex multicellularity in fungi. Whether a single or multiple origins can explain the patchy phylogenetic distribution of complex multicellularity in fungi will need further research and we conjecture that focusing on the earliest cell-differentiation events in the development of complex multicellular structures holds the key to answering this question.

(3) Complex multicellular structures can be encoded by very small, yeast-like genomes, suggesting that complex multicellularity does not require many more genes than are required for the development of simple multicellular fungi or yeasts. Protein-coding repertoires of fungal genomes fail to explain differences in complexity level adequately, and there is a need for assays of other sources of genetic innovations (Nagy, 2017), including gene regulatory network rewiring, alternative splicing, various non-coding RNA species or RNA-editing pathways (Teichert *et al.*, 2017). Uncovering the genetic underpinnings of the evolution of complex multicellularity in fungi is key to understanding the general principles of evolution towards increasingly more complex organisms. Our views on evolutionary trends towards complex multicellularity in the tree of life and whether it represents a major transition in terms of genetic novelty hinges to a large extent on what we can learn through fungi. We expect that the unique ways in which fungi developed multicellularity could change paradigms in one of the central questions in biology.

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