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Review

Fungi took a unique evolutionary route to multicellularity: Seven key challenges for fungal multicellular life

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ABSTRACT

The evolution of multicellularity has been one of the major transitions in the history of life. In contrast to animals and plants, how multicellularity evolved in fungi and how it compares to the general principles distilled from the study of more widely studied model systems, has received little attention. This review broadly discusses multicellular functioning and evolution in fungi. We focus on how fungi solved some of the common challenges associated with the evolution of multi-celled organisms and what unique challenges follow from the peculiar, filamentous growth form of fungi. We identify and discuss seven key challenges for fungal multicellular growth: apical growth, compartmentalization, long-distance mass transport, controlling mutational load, cell-to-cell communication, differentiation and adhesion. Some of these are characteristic of all multicellular transitions, whereas others are unique to fungi. We hope this review will facilitate the interpretation of fungal multicellularity in comparison with that of other multicellular lineages and will prompt further research into how fungi solved fundamental challenges in one of the major transitions in their evolutionary history.

1. Introduction

The evolution of multicellularity is invariably considered one of the major transitions in the history of life, that allowed the evolution of complex life forms (Grosberg and Strathmann, 2007; Knoll, 2011; Szathmáry and Smith, 1995). Life started as unicellular and has given rise to multicellular forms of various complexity levels, from simple aggregates of cells to colonies, filaments and sheets and complex, 3-dimensional structures. Multicellularity arose convergently several times during evolution in almost all of the major lineages (Grosberg and Strathmann, 2007; Nagy et al., 2018; Niklas and Stuart, 2019; Ratcliff et al., 2012; Rokas, 2008; Umen, 2014). Estimates for the number of independent origins range from a dozen to >30, both among pro- and eukaryotes. Most multicellular lineages are relatively simple, comprised of clonally generated colonies, filaments or other aggregates, whereas a small number of lineages reached significantly

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higher complexity (Claessen et al., 2014; Herron et al., 2019; Knoll, 2011; Nagy et al., 2018; Taylor and Ellison, 2010; Umen, 2014). These lineages are referred to as complex multicellular and comprise animals, plants, red and brown algae, as well as fungi (Knoll, 2011; Nagy et al., 2018).

The large number of independent origins of multicellularity have even led some to question whether it is a major transition in terms of genetics (Grosberg and Strathmann, 2007), or if there are mechanisms that make this transition easier somehow (Cavalier-Smith, 2017; Knoll, 2011; Merényi et al., n.d.; Nagy et al., 2018; Ratcliff et al., 2012). Each instance of multicellularity involves somewhat different evolutionary and molecular mechanisms, which result from lineage-specific traits, environmental conditions and reflect predisposition effects of the molecular toolkits of ancestors (Niklas and Newman, 2013; Niklas and Stuart, 2019; Ruiz-Trillo et al., 2007). Among the ensemble of multicellular forms, two major modes of evolving multicellularity are distinguished (Brunet and King, 2017; Du et al., 2015; Niklas, 2014; Niklas and Newman, 2013; Sebé-Pedrós et al., 2017, 2013). Clonal multicellularity refers to groups of genetically related cells that remain stuck together or fail to separate from each other after cytokinesis. Most multicellular lineages (e.g. choanoflagellates, filamentous bacteria, green plants, red and brown algae) including the largest ones (e.g. animals, plants) evolved via this route, probably because kinship reduces genetic conflicts among individuals and thus lead to more stable associations (Fisher et al., 2013). In contrast, aggregative multicellular lineages feed as individual cells and aggregate in response to external cues into multi-celled assemblages (e.g. *Dictyostelium* (Du et al., 2015)). Cells in these assemblages are not necessarily close relatives of each other, which is a potential source of genetic conflict and evolutionary instability (Du et al., 2015; Fisher et al., 2013).

How fungi fit into this picture has been debated for a long time (Knoll, 2011; Nagy et al., 2018, 2017), partly due to the unique growth form of fungi and partly because little research has been devoted to understanding fungal multicellularity. Recently, a number of steps have been taken to remedy this profound lack of information, although several questions remain open for further research. In this article, we systematically review multicellularity-related traits of fungi with special emphasis on their evolution and comparison with other multicellular lineages (mainly plants and animals). We do not provide a detailed overview of any multicellularity-related trait or its genetic bases, rather, provide a synthesis of current knowledge and orientation towards more specific and detailed literature. In many cases, the biology of fungi has been traditionally approached from the perspective of medical or biotechnological perspectives. Here we also intend to scrutinize accumulated knowledge in these fields from the perspective of the evolution of multicellularity.

This review is intended for fungal biologists interested in how fungal multicellularity compares to that of other lineages and for biologists interested in basic principles of multicellular growth in fungi. We discuss seven challenges that had to be overcome by multicellular fungi during evolution. Some of these are challenges other multicellular linages face too (e.g. cell-to-cell communication, differentiation, long-distance transport, controlling mutational load, adhesion), but some are unique to fungi (e.g. apical growth or septation) and there are several idiosyncrasies of fungal multicellularity, which will be emphasized in more detail.

2. Multicellularity in fungi and other lineages

Multicellularity evolved convergently several times, of which fungi represent a single clade. Each of the multicellular lineages shows a unique combination of genetic and mechanistic solutions to major hurdles to multicellularity, which is dependent on the lineage's ecology, ancestry, genetic heritage and the geological conditions under which they evolved (Grosberg and Strathmann, 2007; Nagy, 2017; Niklas, 2014; Niklas and Newman, 2013; Parfrey and Lahr, 2013). However, some similarities come up in all or most lineages that reflect common barriers to the evolution of multicellularity.

A recurrent theme theorizes that the evolution of stably multicellular organisms involves a sequence of evolutionary innovations in adhesion − communication − differentiation (Abedin and King, 2010; Knoll, 2011; Niklas and Newman, 2013; Parfrey and Lahr, 2013) (Fig. 1A). Adhesion is the first step for most lineages for forming multi-celled assemblages and is usually mediated by sticky cell surface molecules (proteins, glycoproteins, polysaccharides, etc). Some of the multicelled clusters are transient, whereas others are stable throughout the life cycle (Fig. 1B). In the growing colonies conflict naturally arises between individual cells as competition for resources or for reproduction ensues (Michod and Roze, 2001; Ratcliff et al., 2012). Lineages that successfully resolved these conflicts evolved mechanisms for cell-to-cell communication (with the potential exception of volvocine algae), which enabled a division of labor between cells and higher level differentiation (e.g cell types, tissues) and eventually a genetically encoded developmental program that choreographs the emergence of species-specific morphologies.

This model scenario largely emerged from research on the animal lineage, in which choanoflagellate-like morphologies are hypothesized to represent intermediate forms (Brunet and King, 2017; Knoll, 2011; Niklas and Newman, 2013; Sebé-Pedrós et al., 2017). It seems to apply well to most lineages of clonal multicellular organisms in general (e.g. Fig. 1A, C). Fungi have been interpreted as a lineage of clonally multicellular organisms (Brunet and King, 2017) (because of the continuous multiplication of nuclei within a thallus) that grow as apically extending hyphae. However, due to their peculiar growth form, the emergence of multicellularity in fungi followed somewhat different principles. First, the evolution of multicellular fungi probably started with the gradual elongation of root-like chytrid structures (Dee et al., 2015; Harris, 2011; Kiss et al., 2019) and the evolution of an efficient cellular machinery that enables fast apical extension of hyphae (see below). Because of the coenocytic nature of hyphae, clonality in fungi need to be interpreted at the level of nuclei (in coenocytic hyphae) rather than at the level of cells (as e.g. in choanoflagellates).

Also as a consequence of hyphal growth, the emergence of the first multi-celled fungi did not involve the adhesion of cells to each other. Adhesion, therefore, likely had little importance in the first steps of the evolution of multicellular

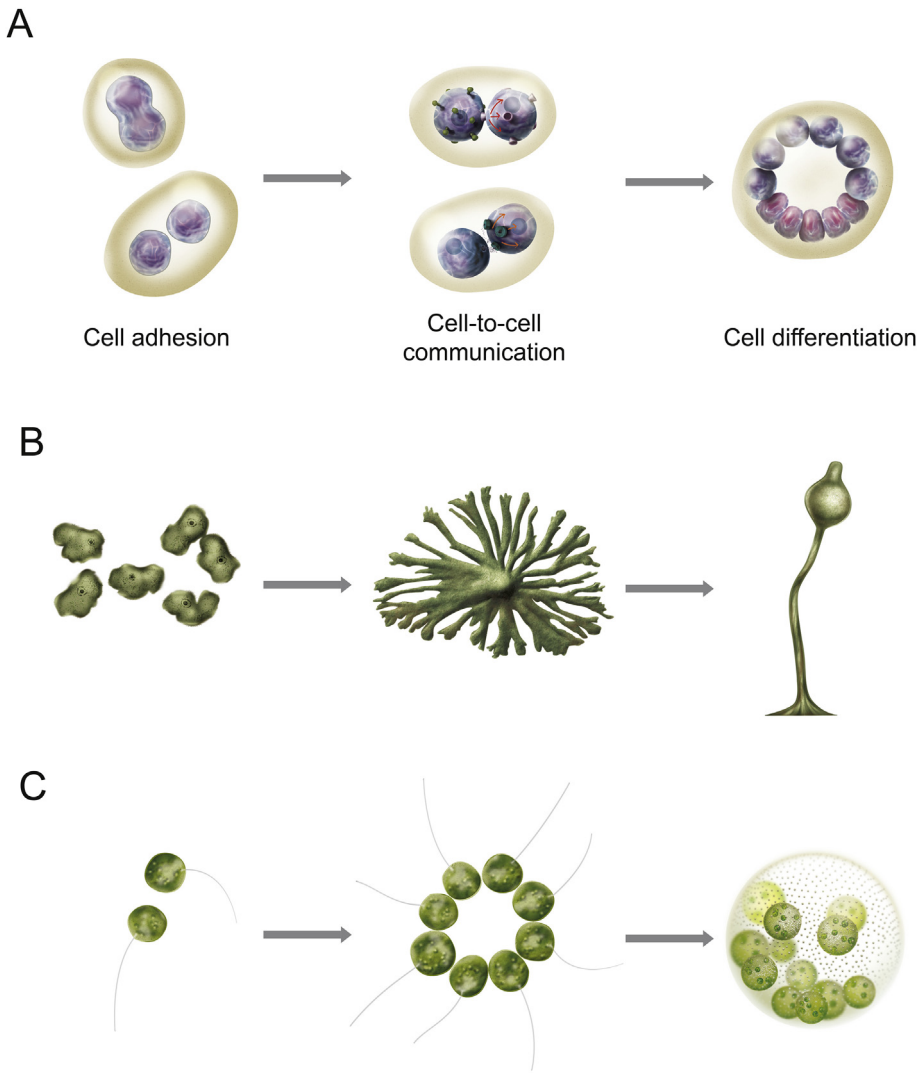

Fig. 1 – A general scheme for the evolution of multicellularity in various lineages. A, the evolution of clonal multicellularity generally requires evolutionary innovations in cell adhesion, communication (marked by arrows in neighboring cells) and differentiation. B, Transient multicellularity in aggregative multicellular lineages. C, scheme for the evolution of clonal multicellularity through the example of green algae, starting from unicellular *Chlamydomonas*-like state through undifferentiated multi-celled clusters (e.g. *Yamagishiella* spp) to *Volvox*-like complex morphologies.

fungi. Consistent with this, reconstructed ancestral repertoires of adhesion-related and kinase genes are low in fungi, with no apparent gene family expansion coincident with the evolution of multicellular hyphae (Kiss et al., 2019). The emergence of multicellular fungi, however, required mechanisms for closing cellular compartments off of each other by septa and for synchronizing cellular behaviors via communication. Cell-to-cell adhesion in fungal multicellularity became important in 3-dimensional structures, such as fruiting bodies, mycorrhizae, sclerotia, etc, which emerged much later during fungal evolution (Kiss et al., 2019; Krizsan et al., 2019; Nagy et al., 2018). Therefore, we suggest that instead of the common sequence of innovations in adhesion, communication and differentiation, the evolution of multicellular fungi may have been choreographed by key innovations in elongation, compartmentalization, communication, differentiation and adhesion. From this it follows that a number of hurdles to multicellularity affected fungi differently than they did in other lineages. In the next sections, we will discuss these and the mechanisms that fungi evolved, with comparisons to other multicellular lineages.

3. Complex or simple multicellularity: where are the limits?

Along the continuum of complexity levels of multicellular organisms, simple and complex multicellularity are often distinguished. Even though nature usually resists discretely categorizing its diversity, a distinction between simple and complex multicellularity seems practical for many reasons. Knoll (Knoll, 2011; Knoll and Hewitt, 2013) exhaustively discussed this distinction and provided a definition that is easy to apply to any organism. In simple words, he considers 3-

dimensional, compact structures as complex multicellular and everything else as simple multicellular. Technically, the key consideration of his definition is whether or not all cells of the organism are in direct contact with the environment. In simple multicellularity, each of the cells can interact with the environment and acquire nutrients or oxygen, whereas in complex multicellularity there are cells which share contact only with other cells (Fig. 2B). Cells that are separated from the environment by several cell layers can not acquire oxygen and nutrients simply by diffusion, necessitating mechanisms that circumvent this limitation.

The term complex multicellularity has been used with various meanings in fungi. For example, Nguyen et al. (Nguyen et al., 2017), Etxebeste et al. (Etxebeste et al., 2019), Goncalves et al. (Gonçalves et al., 2019) recently considered all fungi that produce a vegetative mycelium as complex multicellular. Goncalves et al. and Etxebeste et al. emphasized the genetically programmed, albeit simple, developmental program that occurs in asexually sporulating fungi as a key complex trait. We followed Knoll in considering only three-dimensional structures, such as fruiting bodies, sclerotia, ectomycorrhizae, etc, of Asco-(Merényi et al., n.d.; Nowrousian, 2018; Taylor and Ellison, 2010) and Basidiomycota (Kiss et al., 2019; Krizsan et al., 2019; Kües et al., 2018a; Nagy, 2017; Nagy et al., 2018) as complex multicellular. The seemingly conflicting application of the term roots in the unique dimensionalities and the continuum of developmental complexity levels that exist in fungi (Fig. 2). First, vegetative mycelia follow fractal-like branching patterns (Harris, 2019; Laundon et al., 2019; Obert et al., 1990) and are, therefore,

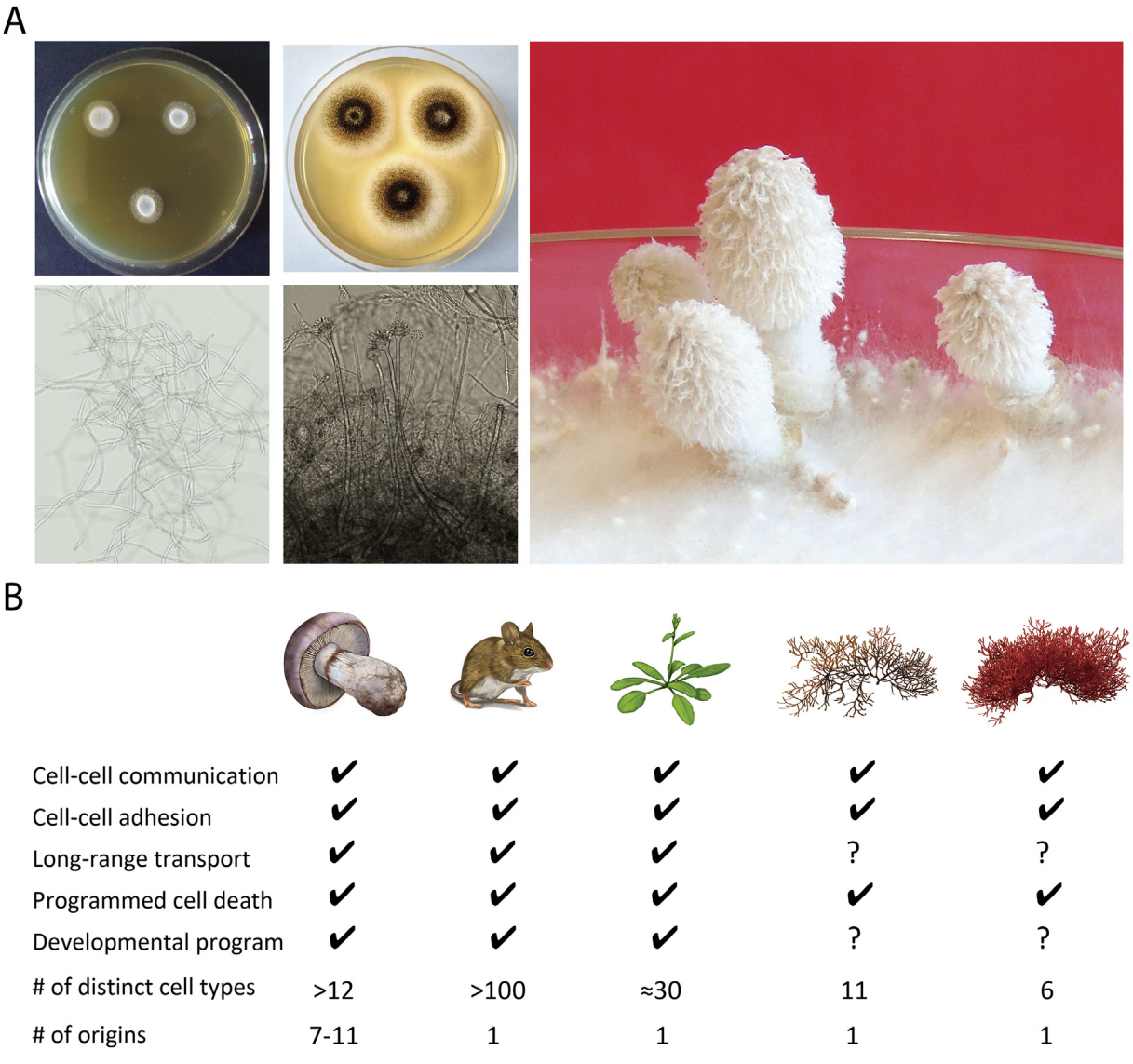

Fig. 2 – Complex vs. simple multicellularity in fungi. A, Complexity levels of multicellular fungi, ranging from vegetative mycelium (*Aspergillus niger*, left), simple developmental processes (conidiation of *A. niger*, middle) and complex multicellular development (fruiting bodies, *Coprinopsis cinerea*, right) B, examples of the five main complex multicellular lineages (fungi, animals, green plants, red and brown algae), with key traits of complex multicellularity shown beneath for each.

not clearly 3-dimensional. Analyses of hyphal growth using fractal geometry reported fractal dimensionalities ranging from 1.2 to 2.0 (Papagianni, 2006; Prosser, 1995). Second, genetically encoded developmental programs exist in most fungi (even unicellular chytrids or yeasts) and their products range from simple sporangia and conidiophores to the most complex mushroom morphologies of the Agaricomycotina.

However, only in some fungal developmental structures harbor cells that are not in direct contact with the environment. Therefore, it appears that fungi comprise a case that is hard to unambiguously squeeze into discrete categories created for other lineages. However, if we interpret complexity at the level of different developmental stages, then dimensionalities and cell-to-cell or cell-to-environment contacts can provide a clear picture for guiding classifications. Vegetative mycelia, reproductive structures made up of individual hyphae (e.g. conidiophores, scattered basidia) as well as yeast colonies and biofilms seem to be closer to simple multicellular than complex, whereas fruiting bodies (both asexual and sexual), rhizomorphs, ectomycorrhizae, sclerotia, and other 3-dimensional structures are complex multicellular. Given that organisms rarely evolve to be fit into 'boxes', yet other, hard to classify, intermediate forms may still exist.

4. Apical growth and terrestrialization

Much of the uniqueness of fungal multicellularity derives from the fractal-like growth patterns of hyphae. Hyphal growth via polarized apical extension is undoubtedly a key innovation for fungi, with similar solutions being limited across the tree of life (e.g. Oomycota, pollen tubes or axons). It is important to note that hyphae are not analogous to other forms of filamentous multicellularity that evolved in several prokaryotic (cyanobacteria) or algal lineages: these emerge by the failure of cell division to complete with cell separation (Claessen et al., 2014; Niklas, 2014; Rossetti et al., 2011), whereas hyphae grow at the tip and cellular compartments get closed off by cross-walls in a sequential manner.

What could have driven the evolution of hyphae?

Apical growth is achieved by the coordinated secretion and incorporation of cell wall materials in the tip region. The cellular mechanisms of hyphal growth have been reviewed in detail recently (Harris, 2011; Riquelme et al., 2018; Steinberg, 2007; Sudbery, 2011), here we focus on the putative driving forces of the evolution of hyphae. The first question to examine is why a hyphal thallus represents the optimal solution for fungal multicellularity? Three important factors driving the evolution of hyphae might have been the evolution of the cell wall, that of osmotrophy (feeding by the uptake of the products from the extracellular decomposition of macromolecules, more precisely, lysotrophy (Berbee et al., 2017; Kiss et al., 2019; Leonard et al., 2018; Nagy et al., 2018; Richards and Talbot, 2018)) and the invasion of land (Dee et al., 2015; Harris, 2011; Kiss et al., 2019; Sekimoto et al., 2011). Mutualistic interactions between fungi and plants are widely accepted as a prerequisite for plant terrestrialization, which, in turn, might have also required that the fungal partner evolves efficient mechanisms for nutrient acquisition across patchy habitats. Evolutionary precursors of fungi were probably wall-less, phagotrophic opisthokonts. The evolution of osmotrophy and that of a rigid cell wall first emerged in a part of the life cycle (Richards et al., 2017) (e.g. sporangia of chytrid or Blastocladiomycota fungi), probably way before hyphae. The combination of a sessile, walled thallus and osmotrophic feeding habit constrains how nutrients can be obtained, especially in terrestrial habitats. It follows that the acquisition of patchy terrestrial nutrients required mechanisms for traversing larger distances, for which fast apical growth of tubular hyphae might have offered a solution. Heaton et al. recently showed by computational modeling that in the presence of patchy and recalcitrant resources a hyphal advantage emerges, which explains mechanistically why filamentous fungi, not unicellular microbes dominate hard-to-digest food sources (Heaton et al., 2020). A complementary consideration is that the cell wall and osmotrophy have passively driven the emergence of hyphae by constraining the potential evolutionary trajectories fungi might have taken on their route to invading land (Kiss et al., 2019).

Avoiding conflicts and maximizing the efficiency of acquiring soluble goods might have also favored the evolution of a filamentous growth form in evolution. The production of extracellular enzymes is costly, and it is the interest of the organism to optimize investment/nutrient uptake ratios. Hyphal filaments fill available space in a fractal-like manner, ensuring that soluble nutrient compounds generated by secreted lytic enzymes can be most efficiently absorbed. Related to this concept, competition for food by neighboring cells can lead to intra-group conflicts (Rokas, 2008). By fractal-like growth, neighboring hyphae minimize the amount of conflict that might arise within the colony. Whether maximizing nutrient acquisition efficiency, minimizing conflicts or both, drove the evolution of fractal-like growth patterns of hyphae is hard to speculate, nevertheless, hyphal multicellular systems display both properties.

In which groups did hyphae evolve?

Looking across the fungal tree of life, the earliest diverging clades in which we find hyphae-like structures are the Blastocladio- and Chytridiomycota, suggesting that hyphae evolved already in these groups (Harris, 2011; Stajich et al., 2009). In these clades we find a remarkable diversity of hyphae-like forms, from thin aseptate ones resembling Mucoromycota hyphae to wide, sausage-like, zoospore-bearing structures of *Allomyces*. However, the phylogenetic distribution of hypha-like structures in these clades is patchy, indicating an intricate evolutionary history of hyphae. For example, phylogenetic analyses indicated that unicellular and hyphal Monoblepharidomycetes (Chytridiomycota) are sister taxa, which implies an independent origin of hyphae in this class (Dee et al., 2015). The Monoblepharidomycetes and other taxa with hypha-like structures provide evidence for convergence of hyphae in early-diverging clades (Dee et al., 2015; Kiss et al., 2019), which probably evolved in response to common driving forces mentioned above. Hyphae become dominant in the Mucoromycota (Stajich et al., 2009), which contain some of the common molds that grow on dung or damaged fruits. Most early

hyphae were aseptate, although it's worth noting that septa can develop between reproductive and vegetative structures already in the earliest fungal clades. Hyphae remained conserved across most fungi, with a few apparent losses in yeast-like lineages, although it appears that hyphal growth is rather suppressed in these lineages instead of being irreversibly lost (Kiss et al., 2019; Nagy et al., 2014).

What could have been the precursor to hyphae?

What morphological structure hyphae derive from has interested mycologists for a long time. Both hypotheses that have been put forth on the morphogenetic origins of hyphae designate chytrid fungi as precursors (Dee et al., 2015; Harris, 2011) (Fig. 3). Harris posited that substrate-anchoring rhizoids of chytrids were the morphological precursors of hyphae (Harris, 2011, 2008). Rhizoids are thin, apically growing structures that anchor chytrid sporangia to various substrates (Fig. 3). The fractal-like branching typical of hyphae emerged at least as early as substrate-anchoring rhizoids of chytrid fungi and it was recently shown that the chytrid *Rhizoclosmatidum globosum* can control rhizoid morphogenesis in response to starvation (Laundon et al., 2019), just like hyphae do. These collectively suggest that a morphogenetic link between nutrient uptake and fractal-like growth emerged already in chytrids that did not yet have real hyphae. These observations on chytrid rhizoid biology combined with other similarities (e.g. actin cytoskeleton structure (Dee et al., 2019)) between rhizoids and hyphae suggest that rhizoid-like structures were likely precursors to fungal hyphae (Dee et al., 2019, 2015; Harris, 2011, 2008; Kiss et al., 2019; Nagy et al., 2018, 2017; Stajich et al., 2009). In marginal support of this hypothesis, rhizoidal hyphae also exist in some Mucoromycota (e.g. *Rhizopus stolonifer*, see Fig. 3), which anchor the coenocytic filamentous thallus to the substrate (Hernandez-Lauzardo et al., 2006). The other hypothesis posits that hyphae-like connections between thalli of polycentric chytrids (e.g. *Physocladia* spp.) were intermediates to true hyphae (Dee et al., 2015).

When did hyphae evolve?

Fossils, unfortunately, provide little information, despite recent discoveries of very old fossils that convincingly appear fungal (Bonneville et al., 2020; Loron et al., 2019) and push the fossil record of fungi back by 250–500 myr. One of the recently discovered fossils (Bonneville et al., 2020) from the Neoproterozoic (810–715 million year old) is a filamentous network containing traces of chitin and partial septa. Based on morphology, a similarity of this fossil to extant Blastocladiomycota has been noted. This fossil, if truly was a hyphal fungus, represents a step towards closing the gap between the fossil record and molecular clock estimates. Recent molecular clock studies provided age estimates ~700 million years for the last common ancestor of the Dikarya and the Mucoromycota (Chang et al., 2015), which conceivably had true hyphae (also supported by phylogenetic analyses, see (Berbee et al., 2017; Dee et al., 2015; Kiss et al., 2019)). Other hyphal remains of fungi, resembling Blastocladio- and Glomeromycota, have been reported from the Devonian Rhynie chert, ~407 million years ago (Berbee et al., 2017; Strullu-Derrien et al., 2018).

What genetic innovations underlie the evolution of hyphae?

Comparisons of the genomes of filamentous and unicellular fungi revealed surprising conservation of genes related to hyphal growth in plesiomorphically unicellular fungi and related eukaryotes (Kiss et al., 2019). Hyphal growth involves various molecular processes and components, such as polarity establishment and maintenance, septum formation, microtubules, the actin cytoskeleton, vesicle transport, cell wall biogenesis and modification as well as corresponding transcriptional

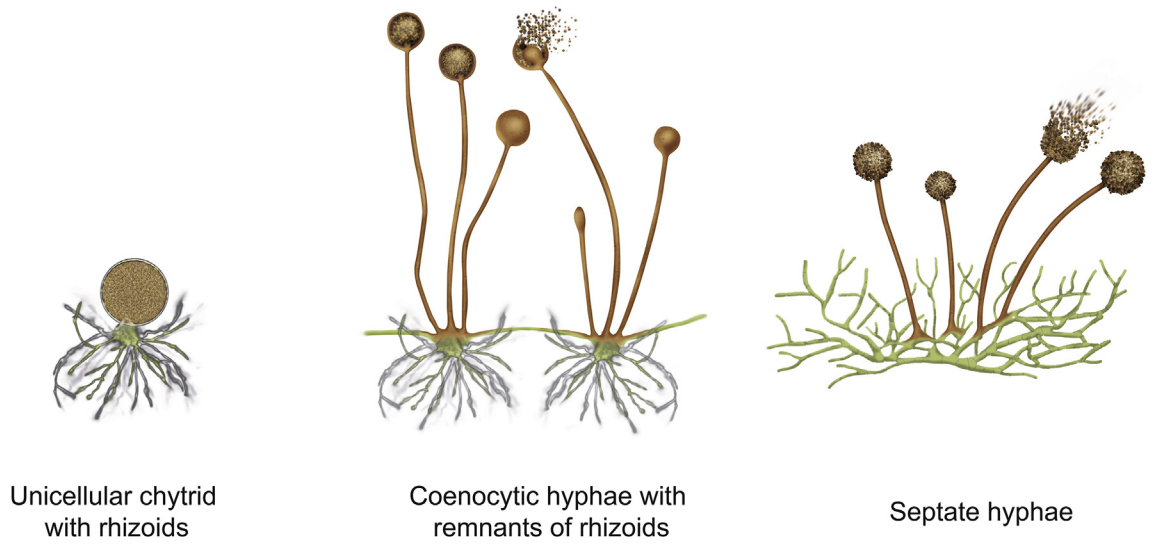

Fig. 3 – Key stages of the evolution of multicellularity in fungi. Hyphae presumably evolved from substrate-anchoring rhizoids of unicellular chytid fungi (left). Rhizoids exist in some Mucoromycota species, (e.g. *Rhizopus* spp, middle) providing evidence for probable morphological precursors towards filamentous fungi (e.g. *Aspergillus* spp., right).

regulators and signaling pathways. By systematically reconstructing the evolution of genes related to these functions, we showed that most genes used by extant fungi for hyphal growth are deeply conserved among eukaryotes (Kiss et al., 2019). Most of these gene families showed no or little duplication in correlation with the emergence of hyphae, indicating that fungi likely co-opted conserved eukaryotic genes for hyphal growth during evolution. While very few duplications were observed, the emergence of hyphae coincided with some changes to the sequence properties (gene lengths, domain composition, and intron properties) of hyphal morphogenesis genes. Only a small number of hypha-related families (17) were found to have originated in correlation with the emergence of hyphae.

These observations mirror patterns seen in the evolution of multicellular animals (Sebé-Pedrós et al., 2017): most multicellularity-related genes seem to have been co-opted from ancestral, unicellular precursors with limited innovation through duplication. A surprising pattern that seems to be characteristic of fungi is the delayed expansion of kinases, cell surface receptors and adhesion-related genes. These genes are expanded in most multicellular lineages, ranging from animals (Grau-Bové et al., 2017; Miller, 2012), plants (Umen, 2014) and brown algae (Cock et al., 2010), however, in filamentous fungi we did not observe any diversification coincident with the emergence of hyphae. Kinases and adhesion-related genes expanded in the Agarico- and Pezizomycotina, presumably reflecting the origins of complex multicellular fruiting bodies in these clades, which require more sophisticated signaling and adhesion mechanisms (Kiss et al., 2019).

5. Compartmentalization and network formation

As explained above, the first challenge for fungi to overcome may have been the patchy distribution of nutrients on land, for which apically growing hyphae might have been an optimal solution. However, in parallel with the emergence of a hyphal network, new challenges also arose. Early fungal thalli were probably filamentous cellular syncytia, in which the flow of cytoplasm and that of organelles were hardly regulated (Riquelme et al., 2018; Roper et al., 2015). While a syncytial continuity of the cytoplasm facilitates growth and communication (see below), it potentially also allows the fast spread of pathogens through the network or the 'bleeding' of cytoplasmic contents upon an injury. Fungi evolved various mechanisms to compartmentalize hyphae and to regulate the flow of organelles and cytoplasm.

Fungi form individual cells by septation, the process of compartmentalizing hyphae by various septal structures and occlusions. The mechanisms of septum formation have recently been reviewed in detail (Riquelme et al., 2018; Steinberg et al., 2017). Briefly, septum formation is analogous to cytokinesis in animal and yeast cells and involves the formation of a contractile actomyosin ring that leaves a central pore on the cross-wall between adjacent cells. From an evolutionary perspective, a continuum of septum morphologies and diameters exist; from narrow invaginations that hardly interfere with flow, to complete closure of neighboring hyphal

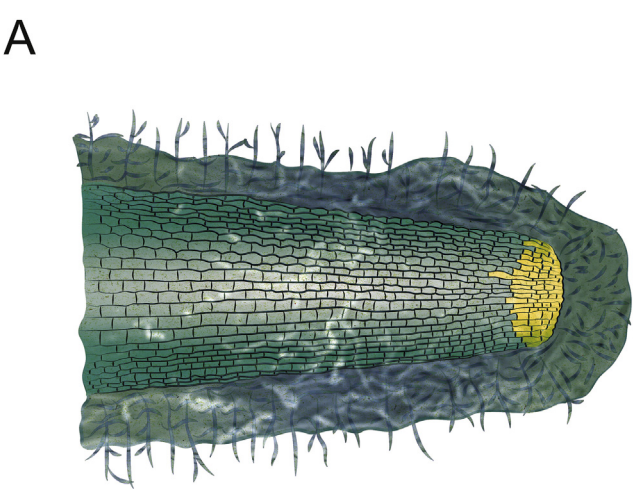

Fig. 4 – The tip of an *Armillaria* rhizomorph. The yellow subapical region represents a slowly dividing group of cells.

cells. Mechanisms for completely plugging the septa evolved in the Dikarya (Berbee and Taylor, 2010; Jedd, 2011; Stajich et al., 2009), whereas earlier diverging fungi in the Mucoromycota, Zoopagomycota, Chytridiomycota and Blastocladiomycota mostly form incomplete septa. Both main phyla in the Dikarya, the Asco- and Basidiomycota, evolved complex mechanisms for septal pore gating. Although these evolved for the same general function, the two phyla evolved different solutions: while Ascomycota (Pezizomycotina) have Woronin-bodies, the Basidiomycota evolved various septal pore cap structures, which presumably differentiate from the endoplasmic reticulum (Jedd, 2011).

Besides apical growth and branching, the third fundamental process that contributes to the topology of the mycelium is fusion (Fleißner and Glass, 2007; Read et al., 2009). Hyphal fusion increases the connectivity of the network, which contributes to network robustness and has a number of positive consequences, for example, in how fast mass flow can be rewired across the network. Interconnections between hyphae appear key for long-distance nutrient translocation by providing pathways along which reallocation of soluble good can be organized (Fricker et al., 2017). Hyphal fusion starts with a communication process that is relatively well known in filamentous fungi. The process is best studied in *Neurospora crassa* which provides a model system for understanding fungal communication (Daskalov et al., 2017; Fischer and Glass, 2019; Fleißner et al., 2008; Glass and Dementhon, 2006). Fusion of genetically identical cells in *Neurospora* and *Sordaria* includes >70 genes (e.g. the STRIPAK complex (Elramli et al., 2019; Reschka et al., 2018; Kück et al., 2016)) and is tightly integrated with the recognition of non-compatible partners, although the signal molecule is not known. Interactions between genetically incompatible cells, on the other hand, can trigger cell death (Glass and Dementhon, 2006; Gonçalves et al., 2017), an important trait of multicellular organisms for removing unnecessary cell (populations), sculpting morphological structures, or containing the spread of infections or cheater genotypes.

6. Controlling mutational load

Each multicellular organism faces the challenge of preventing the accumulation of deleterious mutations resulting from DNA copy errors during cell division. Animals achieve this by segregating a quiescent germ-line from somatic cells early in their development (Weismann, 1893). Plants also segregate a slowly dividing cell population, called meristem, that effectively decreases the number of mutations passed on to the next generation (Lanfear, 2018; Walbot, 1985).

Fungal mycelia continuously accumulate mutations as nuclei divide, requiring mechanisms for avoiding mutational meltdown. Although measuring the mutation rate of filamentous fungi is difficult, estimates suggest that they have remarkably robust genomes: the mutation rate per site per mitosis was estimated between $1.1–4.2 \times 10^{-11}$ for *Aspergillus* (Álvarez-Escribano et al., 2019), 7.2×10^{-11} for *Neurospora crassa* (Drake, 1991), with significantly lower estimates, 3×10^{-14} for *Armillaria gallica* (Aanen, 2014; Anderson and Catona, 2014) and 3.8×10^{-12} for *Marasmius oreades* (Hiltunen et al., 2019). For comparison, the mutation rate of human somatic tissues varies between 1.6×10^{-7} and 3.5×10^{-9} per site per mitosis (Werner and Sottoriva, 2018), it is 7×10^{-9} for *Arabidopsis thaliana* somatic cells (Ossowski et al., 2010), and 3.3×10^{-11} for human germline cells (Milholland et al., 2017)

Due to the apical growth of hyphae, fungi are not able to segregate slowly dividing cell populations, necessitating different mechanisms for keeping mutations in check. Several hypotheses have been put forth in fungi (Aanen, 2014; Hiltunen et al., 2019). One possibility is that DNA repair mechanisms evolved higher fidelity, for example through the increase of DNA repair gene copy numbers, as it was reported for long lived animals (Abegglen et al., 2015), though recent data does not seem to support this (Hiltunen et al., 2019). *Armillaria* rhizomorphs (shoestring-like underground organs for clonal dispersal), were hypothesized to harbor a slowly dividing apical cell population and that cell divisions take place in the subapical zone, minimizing the number of cell divisions, similarly to plant shoots (Anderson and Catona, 2014) (Fig. 4). However, most fungi lack rhizomorphs, suggesting the existence of other, more universal mechanisms in fungi.

Another possibility to explain the low number of mutations in fungi is the non-random segregation of sister-chromatids during cell division. DNA-replication is a semi-conservative process and results two DNA duplexes, each containing a template and a daughter strand. After these newly formed DNA duplexes are replicated once more, one of the sister chromatids will contain the ''old'' template strand, therefore sister chromatids will differ in age. In case of non-random segregation of sister chromatids during mitosis, the template containing sister chromatids co-segregate. To investigate the behavior of sister chromatids in fungi, Rosenberger and Kessel

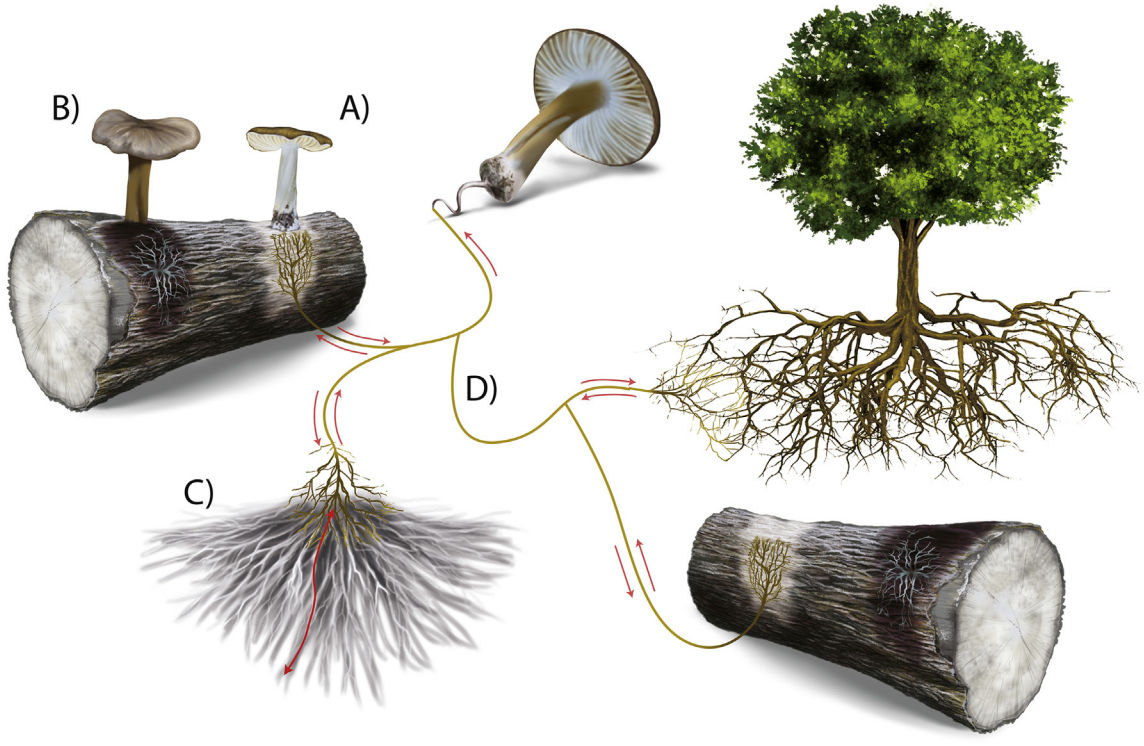

Fig. 5 – Long-distance mass transport in fungi. Non-resource-unit-restricted species (A) can exploit new food sources by overgrowth nutrient pour areas, while other species (B) can exploit new resources exclusively via spore propagation. Long-distance mass transport can be achieved at the level of mycelium (C) with e.g. trunk hyphae or between different food sources with long-distance transport structures (LTS) (D). LTS can connect different food sources, mycorrhizal partners, the developing fruiting bodies or the growing mycelium. Red arrows show the presumed direction of mass flow, which can be driven by several mechanisms, but regulation at the whole organism level is barely known.

labeled the nuclei of *Aspegillus nidulans* by radioactive adenine. Inoculating uninucleate conidia – that contain labeled DNA – on non-radioactive media they were able to follow the original chromosome set during 4 and 8 rounds of mitosis. They have found that the labeled DNA is not dispersed randomly along the hyphae, instead, it can be found near the growing tip. This process can be explained by the non-random segregation of sister chromatids during mitosis and suggests the existence of a mechanism that can differentiate and position "old" nuclei to the tip of the hyphae (Rosenberger and Kessel, 1968). Later, Cairns proposed the immortal strand hypothesis, which posits that, via the co-segregation of sister chromatids containing "old" DNA, stem cells retain the original genome to prevent the accumulation of mutations passed on to next generations (Cairns, 1975). This hypothesis was invoked recently to explain the extremely low mutation rates in *M. oreades* (Hiltunen et al., 2019) and *A. gallica* (Anderson and Catona, 2014).

In the context of fungal hyphae, the immortal strand hypothesis would imply that the old DNA strands are retained at the growing tip. However, in a mycelial colony hyphae branch extensively. During branching, one of the tips inevitably receives the copy strands, resulting in a mutational load in each hyphal tip proportional to the branching events, which still results in the accumulation of mutations over time. To resolve this contradiction, Aanen propounded that in a radially growing colony, after reaching an optimal hyphal density, the number of branching events needed to maintain it, decreases (Aanen, 2019). Together, non-random segregation of sister chromatids and the decrease in the number of branching events as the mycelial colony expands might keep the number of accumulated mutations low at the growth front.

In a mycelial colony, the growth front continuously renews and expands the colony that can reach extreme sizes and age (Anderson and Catona, 2014). Therefore, it is crucial to keep relatively intact genetic material at that region. The non-random segregation of sister chromatids and the positioning of the "old" DNA to hyphal tips can be seen as a nucleus level mechanism for "germline-soma segregation" in fungi, that might spatially control the accumulation of new mutations resulting from the increasing number of cell divisions. However, more experimental support is needed to understand how conserved this mechanism is among fungi.

7. Long-distance transport

While increased body size might have been the main driver of the evolution of long-distance transport in most multicellular organisms, the patchiness of food sources probably played a major role in fungi. Feeding over patchy nutrients requires the ability of efficient long-distance mass transport to bridge over nutrient rich areas (even meters away). Species capable of that (referred to as non-resource-unit-restricted (Anderson et al., 2018; Boddy, 1999; Heaton et al., 2010; Olsson, 2001), can flexibly react to spatial changes in food availability and could gain advantages over others in spatially diverse environments (Heaton et al., 2020). Fungi, just like animals and plants, evolved a range of long-distance mass transport mechanisms (Fig. 5) (Fricker et al., 2017; Monahan-Earley et al., 2013; Raven, 2018). Motor-driven transport can outperform diffusion, but it has a limited capacity (up to 1.44 cm h^{-1}). Mass-flow is the most effective solution in plants and animals, but the driving force can vary a lot. In animals mass transport can be driven by pressure generated by contractile tissues or organs (Monahan-Earley et al., 2013; Sherman, 1981), while in plants various models of transport based on actively/passively generated pressure differences, evaporation or cohesion were proposed (for detailed description see e.g.(De Schepper et al., 2013; Raven, 2018). In fungi, mass-flow can also be driven by several mechanisms: evaporation or guttation at specific sites of the mycelium (Brownlee and Jennings, 1981; Coggins et al., 1980), or in the fruiting body (Cowan et al., 1972; Geer et al., 1990), low pressure generated by a mycorrhizal partner (Cairney, 1992; Whiteside et al., 2019) or a negative pressure created by the expanding hyphal tip (referred to as growth-induced mass flow (Heaton et al., 2010). These mechanisms can potentially work simultaneously in one fungal thallus, but their regulation at the whole organism level remains unknown. Some mechanisms have been described which can regulate mass flow locally (Fricker et al., 2007), like the insulation of hyphae by hydrophobins (Bayry et al., 2012) or pigments (Cordero and Casadevall, 2017); forming septa and changing their permeability (Daly et al., 2020; van Peer et al., 2009); creating anastomoses (Simonin et al., 2012); moving water against the free energy gradient via the activity of chloride-cation cotransporters (Hartmann et al., 2014) or non-circadian oscillatory control of the direction of transport (Schmieder et al., 2019; Tlalka et al., 2003).

Besides the above mentioned mechanisms, mass transport can also be facilitated by specialized structures (Fig. 5). These include transporting hyphae, hyphal aggregates, strands, cords or rhizomorphs. Transport-specialized trunk hyphae, in which the direction of transport altered in every 3 h, were characterized in *Coprinopsis cinerea* (Schmieder et al., 2019). More complicated structures differ in morphological complexity or the mode of development, but we here discuss them under the term long-distance transport structures (LTS). The simplest LTS are homogeneous aggregates of hyphae, whereas more complex LTS contain vessel, tendril or fiber hyphae (Clémencon et al., 2012; Fricker et al., 2017) and may be differentiated into rind, cortex and medulla (e.g. *Armillaria* rhizomorphs) (Yafetto, 2018). These structures could be key for reaching a high velocity (from 2 to 148 cm h^{-1}) of mass transport in fungi (Fricker et al., 2017).

To our best knowledge no study on the evolutionary origins of long-distance transport in fungi exists. LTS could have existed in the Silurian–Devonian periods based on fossil evidences (*Tortotubus protuberans* (443–359 mya) (Auxier et al., 2016; Smith, 2016), or *Glomites rhyniensis* (419–393 mya) (Taylor et al., 1995)), however how long-distance transport evolved in hyphae or in LTS is still unknown. It is conceivable that long-distance transport could have evolved in parallel with other fungal traits (septum formation, hyphal fusion, etc.), which made exploration of food resources and living in heterogeneous environments possible by circumventing the limitation of diffusion and connecting resource rich and poor sites within the fungal body.

8. Communication

Cell-to-cell communication is key in multicellular organisms, for organizing group behaviors, the division of labor, differentiation or defense (Du et al., 2015; Knoll, 2011). Cells need to be able to send and sense signals that inform them and their neighbors on spatial coordinates, nutritional status, morphogenetic states and a range of other attributes that multicellular organisms need to coordinate. Cell-to-cell communication and associated signal transduction pathways have been extensively studied in multicellular animals and plants (Bich et al., 2019; Bloemendal and Kück, 2013; Brunet and King, 2017; Sebé-Pedrós et al., 2017; Tong et al., 2017), whereas comparatively less knowledge is available in fungi. Fungi engage in a wide range of communication processes to relay information between their own cells as well as towards other microbes they share their habitat with (Cottier and Mühlschlegel, 2012; Kües et al., 2018b). Here we focus on cell-to-cell communication that happens among cells of the same fungal individual. Inter-species communication and warfare (e.g. via secondary metabolites) is an intensely researched field, but is not strictly a multicellular function, so we only refer to recent reviews on this topic (Bahn et al., 2007; Biswas et al., 2007; Brown et al., 2018) and not discuss it further here.

In animals and plants the main channels for communication are gap junctions and plasmodesmata, respectively, besides secreted molecules in both kingdoms. The best known intercellular channel for cell-to-cell communication in fungi are pores at septa and associated septal pore structures that separate hyphal compartments from each other (Bloemendal and Kück, 2013). Septal pores allow the regulated flow of molecular signals, cytoplasm and even organelles (Jedd, 2011). However, communication through septal pores enables the streaming of information only along the hyphal axis. Fungal hyphae fill a 3-dimensional space, necessitating mechanisms for communication between neighboring or even more distantly spaced hyphae, similarly to what occurs in plants and animals through hormones and other secreted molecules. However, this mode of communication in fungi is much less known.

Signal transduction pathways became significantly more diverse in multicellular species compared to unicellular ones, and comparative genomic analyses have shown that this diversity stems from an expansion of corresponding gene families coincident with the emergence of multicellularity. Cell surface receptors (e.g. G-protein coupled receptors, GPCRs), receptor kinases as well as tyrosine and serine–threonine kinases have expanded in multicellular animals (Brunet and King, 2017; de Mendoza et al., 2014; King and Carroll, 2001; Miller, 2012; Pincus et al., 2008; Sebé-Pedrós et al., 2010; Suga et al., 2012; Tong et al., 2017), plants (Bowles et al., 2020; Coates et al., 2015; De Clerck et al., 2018; Umen, 2014) and even in brown algae (Cock et al., 2010). While receptor kinases (proteins that contain both a receptor and an intracellular kinase domain) are central to plant and animal signal transduction (De Clerck et al., 2018; Miller, 2012; Pincus et al., 2008; Shiu and Bleecker, 2001), we recently did not find evidence for the presence of receptor kinases (except the low diversity histidine kinases) in fungi (Kiss et al., 2019; Krizsan et al., 2019). Orthologs of animal tyrosine kinases seem to be missing in fungi (Krizsan et al., 2019; Miller, 2012; Zhao et al., 2014), however, tyrosine kinase-like kinases (TKLs) show characteristic expansions in complex multicellular fungi. Fungi also show expansions in unique kinase families, including the FunK1 family, which seems to be specific to fungi and is greatly expanded in some lineages (Stajich et al., 2010).

While recent comparative genomic analyses uncovered significant expansions of intracellular kinases in complex multicellular fungi (e.g. (Krizsan et al., 2019)), whether these kinases are functionally linked to the higher complexity level of these fungi remain to be established. Further, while the observed kinase expansions conform to the expectations of multicellular evolution we derived from animal and plant lineages (e.g. (Miller, 2012)), what proteins transmit extracellular signals remain completely unknown. It is a challenge for the coming years to identify the receptors involved in multicellular functioning and test whether they have undergone corresponding evolutionary expansions. GPCRs are one of the main cell surface receptor families. While animals and plants harbor diverse GPCR repertoires (>500 genes/species), described GPCR diversity of fungi (see recent reviews (Brown et al., 2018; Dilks et al., 2019; Krishnan et al., 2012; Xue et al., 2008)) is simply too limited (3–30 genes/species) to explain their complexity. A comparative analysis of GPCRs across 71 fungal and animal genomes recently revealed a surprising contrast between GPCR expansions in multicellular fungi and animals: whereas significant GPCR expansion coincides with the emergence of multicellular animals, a similar expansion was not observed in fungi (Kiss et al., 2019), suggesting that further GPCRs or other receptor classes should be involved in organizing multicellular complexity in fungi.

A large number of signal molecules have been described that are produced by or that mediate various processes in fungi (see e.g. (Hogan, 2006; Ugalde, 2006)), although their exact mode of action or the corresponding receptors are poorly known. These are chemically diverse compounds (Cottier and Mühlschlegel, 2012; Gessler et al., 2017; Kües et al., 2018b; Tsitsigiannis and Keller, 2007), including lipids (e.g. oxylipin), short peptides (e.g. mating pheromones), alcohols (e.g. farnesol), volatiles and gases (CO_2, nitrogen oxides). Much of our knowledge on fungal communication comes from yeast-like fungi, which, although spend most of their life cycle as unicellular yeasts, are capable of multicellular hyphal growth and/or behaving as communities with synchronized developmental transitions (e.g. in biofilm formation or the white-to-opaque transition in *Candida* (Gulati and Nobile, 2016; Váchová and Palková, 2018)). A large body of knowledge has also been accumulated on regulatory compounds of spore germination and colony morphogenesis in filamentous fungi (Hogan, 2006). Quorum sensing appears to be a main mechanism of communication in yeast-like fungi like *Candida albicans* (Albuquerque and Casadevall, 2012; Kruppa, 2009; Padder et al., 2018) or *Cryptococcus neoformans* (Albuquerque et al., 2013; Tian et al., 2018).

Taken together, although a large number of fungal communication strategies, signal transduction pathways have been described and several signal molecules have been

characterized, our knowledge on fungal cell-to-cell communication is far from complete. A very exciting but particularly poorly known aspect is how communication and signal transduction mechanisms correlate with multicellular complexity and what receptors and ligands might underlie the development of complex structures.

9. Differentiation and development

One of the fundamental advantages that multicellularity confers to organisms is the possibility for labor division between cells and therefore higher functional versatility at the organism level (Brunet and King, 2017; Ispolatov et al., 2012; Knoll, 2011). The division of labor requires that cells specialize for specific functions by differentiating their phenotypic outputs. Differentiation results in the generation of a genetically encoded diversity of cell types, the emergence of which is under tight spatial and temporal regulation.

The evolutionary origins of cell-type determination are among the hardest questions in biology, which has been puzzling scholars for decades even in multicellular groups that received the most attention historically (Brunet and King, 2017; Sebé-Pedrós et al., 2018, 2016; Sogabe et al., 2019). Like other multicellular organisms, fungi differentiate a range of different cell types, which include both vegetative (e.g. hyphae, yeasts), resting (e.g. sclerotia) and reproductive cell types. Current estimates of cell type diversity in fungi are based on counting of morphologically distinct cellular morphologies in fungal colonies. Precise estimates of cell type diversity, especially in complex multicellular fruiting bodies, are, at the moment missing. Nevertheless, even using simple approaches, 13, 28 and 30 morphologically distinct cell types could be recognized in the ascomycetes *Sordaria* (Lord and Read, 2011), *Neurospora* (Bistis et al., 2003) and in the basidiomycete *Coprinopsis* (Kües and Navarro-González, 2015), respectively. The rapid development of ingle-cell technologies will facilitate the understanding of cell type diversity in fungi, similarly to plants and animals. A remarkable feature of fungal cell type specification is that it does not result in terminally differentiated cells, rather, most fungal cells retain their totipotency and are able to revert into vegetative cell types (Money, 2002).

Cell differentiation instructions are clearly genetically encoded in the organisms' genomes. They collectively comprise a developmental program that determines species-specific morphologies. Knoll (2011) considers this as one of the defining features of complex multicellular organisms. What genes and genetic interactions comprise the organism's developmental program is among the classic questions for any multicellular lineage. Development is usually orchestrated by highly interconnected networks of regulatory genes (e.g. kinases, phosphatases, transcription factors), which generate specific transcriptional outputs for precisely regulating the abundance and localization of structural proteins (Levine and Davidson, 2005). Some of the best known developmental gene networks in fungi are from simple model systems, animal or plant pathogens. For instance, detailed studies have been performed on transitions between yeast and (pseudo)hyphae in *Saccharomyces cerevisiae*, *Candida albicans*, *Histoplasma capsulatum* (Sil, 2019), *Cryptococcus neoformans*, the development of specialized plant-penetrating cells, appressoria, of the rice blast fungus *Magnaporthe oryzae* (now *Pyricularia oryzae*), or asexual sporulation of Ascomycota. Probably the best understood developmental process of fungi is asexual sporulation in the Ascomycota, which involves the sequential activation of three transcription factors of the central regulatory pathway (BrlA, AbaA and WetA) and hundreds of downstream regulators or structural genes (Adams et al., 1998; Etxebeste et al., 2019; Ojeda-López et al., 2018).

These studies provided key insights into the general principles of morphogenesis in fungi, providing foundations for studying more complex developmental programs. In comparison, less information is available on the development of complex multicellularity in fungi. Most of what we know is coming from studies of Asco-(Nowrousian, 2018; Pöggeler et al., 2018, 2006) and Basidiomycete fruiting bodies (Krizsan et al., 2019; Kües, 2000; Kües and Liu, 2000; Kües and Navarro-González, 2015; Merényi et al., n.d.; Nagy et al., 2018; Ohm et al., 2010). Profiling transcriptomic changes, the genes and gene regulatory networks underlying the development of sexual fruiting bodies of Ascomycota, including cleistothecia (Krijgsheld et al., 2013; Lord and Read, 2011), perithecia (Dyer and O'Gorman, 2012; Nowrousian, 2018; Nowrousian et al., 2012; Pöggeler et al., 2006; Teichert et al., 2014; Trail et al., 2017; Wang et al., 2018) and apothecia (Rodenburg et al., 2018; Traeger et al., 2013) is providing an ever more precise picture on sexual reproduction in the most taxonomically diverse fungal clade. Similarly, developmental processes in the Basidiomycota are being illuminated by a growing amount of information on basidiome development (Krizsan et al., 2019; Kües, 2000; Kües and Liu, 2000; Morin et al., 2012; Ohm et al., 2011, 2010; Sipos et al., 2017), basidium formation (Liu et al., 2018) or ectomycorrhiza morphogenesis (Martin et al., 2016). Despite these advances, understanding the development of Basidiomycota fruiting bodies lags behind that of Ascomycota fruiting bodies, which, in turn lags behind that of asexual development. The development of sexual fruiting bodies in both Asco- and Basidiomycota are orders of magnitudes more complex than that of asexual conidiophores. This, on the one hand, explains why the instruction set of sexual fruiting bodies is harder to decode, but, on the other, also highlights the pressing need for more emphasis on sexual development. As high-throughput tools are becoming available for interrogating several types of genomic data, including cis- (e.g. (Bartlett et al., 2017; Buenrostro et al., 2015)) and trans (Schuster and Kahmann, 2019)- elements of gene expression regulation, we can expect an acceleration in discovery in this field in the next few years.

At the moment, little information is available for comparative discussions of development among multicellular lineages, especially on the molecular level. Open questions remain as to the (multiple) origins of fruiting body formation in fungi (Nagy et al., 2018). For example, although the traditional view is that Agarico- and Pezizomycotina fruiting bodies evolved completely independent of each other, recent comparative transcriptomic analyses suggest that many of the developmentally regulated genes are shared between the two phyla, despite ~650 million years of divergence (Merényi et al., n.d.). This level of similarity among the transcriptomes

suggested that intricate evolutionary mechanisms, potential predisposition and latent homologies might underlie the repeated evolution of fruiting bodies. How fruiting bodies emerged – not only in the two mentioned clades, but others as well - will require further research.

10. Adhesion

Cell adhesion is one of the key processes in the evolution of early multi-celled clusters in clonally multicellular lineages (Abedin and King, 2010; Grosberg and Strathmann, 2007; Knoll, 2011; Nagy et al., 2018; Sebé-Pedrós et al., 2017) (e.g. choanoflagellates, animals). We discussed above that fungal multicellularity evolved via a mechanism that did not involve the adhesion of related cells to each other. This entails that cell–cell adhesion might have been of limited importance in the early evolution of multicellular fungi, which is supported by recent genomic analyses of the evolution of hyphae (Kiss et al., 2019). This is a fundamental difference from other multicellular lineages: while for most multicellular lineages the first step to group formation is adhesion, the ancestral role of adhesion in fungi is probably attachment to various non-fungal surfaces rather than to cells of the same fungal individual. Adhesion is of great importance in the attachment of hyphae, reproductive (e.g. conidia) and infectious structures (e.g. appressoria) to host surfaces and the substrate (Braun and Howard, 1994; Nicholson, 1996; Tronchin et al., 2008) and in complex multicellular structures (Nagy et al., 2018). While the former is beyond the scope of this review (and has been reviewed elsewhere (de Groot et al., 2013; Jones, 1994; Lipke, 2018)), adhesion in fruiting bodies and other 3D structures is a poorly known but interesting aspect of multicellular development.

In 3-dimensional structures, genetically encoded mechanisms for hypha-to-hypha adhesion are crucial to sculpting species-specific morphologies. Whereas adhesion in animals is traditionally discussed in the context of an extracellular matrix, little information on the existence and composition of extracellular matrix exists in fungi. Clear-cut evidence on extracellular matrix in biofilms formed by diverse filamentous and yeast-like pathogens (Mitchell et al., 2016a, 2016b; Riquelme et al., 2018) indicates that fungi are clearly able to produce extracellular matrix, but it's quite poorly understood in the context of complex development. There is some evidence for extracellular matrix deposition in both perithecial Ascomycota (Lichius et al., 2012) and mushroom-forming Basidiomycota (Boulianne et al., 2000), however, their composition and role in development are hardly known.

There are several gene families with reported sticky properties that are abundantly expressed in fruiting bodies, making them potential mediators of adhesion. Lectins' adhesivity to diverse carbohydrates might, for example, make them important families in hyphal adhesion (Hassan et al., 2015; Nagy et al., 2018) although they might also mediate signal transduction events (Walser et al., 2005). Hydrophobins, unique fungal cell surface proteins have been hypothesized to be involved in adhesion in fruiting bodies, although they more likely function in forming air channels or providing hydrophobicity to fruiting bodies (Bayry et al., 2012; Lugones et al., 1999). Several fasciclin genes are also specifically expressed in fruiting bodies, possibly reflecting adhesion-related functions (Merényi et al., n.d.; Miyazaki et al., 2007; Trail, 2013). The wide range of fruiting body-expressed laccases and glycoside hydrolases might also be involved in adhesion (e.g. by creating fruiting body-specific cell wall architectures or crosslinking walls of neighboring hyphae (Krizsan et al., 2019; Thurston, 1994), although mechanistic evidence for that is currently lacking.

Compared to adhesion within fruiting bodies, a much larger body of literature deals with attachment of conidia, appressoria and other infection structures to host surfaces. Based on the widespread ability of adhesion of conidia and other propagules to various surfaces, it is conceivable that adhesion mechanisms were ancestral in fungi. We hypothesize that these adhesion mechanisms could have been easily co-opted for hypha-to-hypha adhesion in fruiting bodies, ectomycorrhizae and other 3D structures (Merényi et al., n.d.). This hypothesis entails that adhesion in complex multicellular structures builds on more ancestral mechanisms for the attachment to external (e.g. host) surfaces. There is limited evidence supporting this hypothesis at the moment (e.g. the expression of *Aspergillus* HsbA homologs in fruiting bodies of *Armillaria* (Sipos et al., 2017) or widespread fasciclin expression (Merényi et al., n.d.)), so more research is needed before we can obtain a conclusive answer.

11. Conclusions

Fungi represent one of the several multicellular lineages that evolved on Earth. Single-celled organisms needed to overcome some of the same hurdles to evolve multicellularity, such as the formation of groups (e.g. by adhesion), cell-to-cell communication, the division of labor and cell differentiation, among others. Fungi may be best viewed as a lineage of clonal multicellular organisms, in which clonality, however, should be interpreted at the level of individual nuclei rather than at the level of cells. This derives from the unique, filamentous growth mode of fungi and the mechanisms by which hyphae emerged through evolution. The hyphal thallus has brought about several idiosyncrasies that characterize fungal multicellularity, compared to most other multicellular lineages. For example, while adhesion or the non-separation of daughter cells might have been the first step in the evolution of most multicellular lineages (including animals and green plants), adhesion probably played a minor role in the formation of early fungal hyphae and became important only in lineages that form complex multicellular structures, such as fruiting bodies.

Although significant strides have been made in understanding key multicellular traits of fungi, their evolution and the underlying genetics, several broad questions remain open for further investigations. In our subjective opinion, among the least understood multicellular functions of fungi include cell-to-cell communication systems and gene regulatory networks that underlie the differentiation and development of complex morphological structures, for which we anticipate breakthrough results to be published in the near future.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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REFERENCES

Aanen, D.K., 2019. Germline evolution: sequestered cells or immortal strands? Curr. Biol.. https://doi.org/10.1016/j.cub.2019.07.033.

Aanen, D.K., 2014. How a long-lived fungus keeps mutations in check. Science 80. https://doi.org/10.1126/science.1261401.

Abedin, M., King, N., 2010. Diverse evolutionary paths to cell adhesion. Trends Cell Biol. 20, 734–742. https://doi.org/10.1016/j.tcb.2010.08.002.

Abegglen, L.M., Caulin, A.F., Chan, A., Lee, K., Robinson, R., Campbell, M.S., Kiso, W.K., Schmitt, D.L., Waddell, P.J., Bhaskara, S., Jensen, S.T., Maley, C.C., Schiffman, J.D., 2015. Potential mechanisms for cancer resistance in elephants and comparative cellular response to DNA damage in humans. JAMA. https://doi.org/10.1001/jama.2015.13134.

Adams, T.H., Wieser, J.K., Yu, J.-H., 1998. Asexual sporulation in *Aspergillus nidulans*. Microbiol. Mol. Biol. Rev. https://doi.org/10.1128/mmbr.62.1.35-54.1998.

Albuquerque, P., Casadevall, A., 2012. Quorum sensing in fungi a review. Med. Mycol. https://doi.org/10.3109/13693786.2011.652201.

Albuquerque, P., Nicola, A.M., Nieves, E., Paes, H.C., Williamson, P.R., Silva-Pereira, I., Casadevall, A., 2013. Quorum sensing-mediated, cell density-dependent regulation of growth and virulence in cryptococcus neoformans. mBio. https://doi.org/10.1128/mBio.00986-13.

Álvarez-Escribano, I., Sasse, C., Bok, J.W., Na, H., Amirebrahimi, M., Lipzen, A., Schackwitz, W., Martin, J., Barry, K., Gutiérrez, G., Cea-Sánchez, S., Marcos, A.T., Grigoriev, I.V., Keller, N.P., Braus, G.H., Cánovas, D., 2019. Genome sequencing of evolved aspergilli populations reveals robust genomes, transversions in *A. flavus*, and sexual aberrancy in non-homologous end-joining mutants. BMC Biol. https://doi.org/10.1186/s12915-019-0702-0.

Anderson, J.B., Bruhn, J.N., Kasimer, D., Wang, H., Rodrigue, N., Smith, M.L., 2018. Clonal evolution and genome stability in a 2500-year-old fungal individual. Proc. R. Soc. B Biol. Sci. https://doi.org/10.1098/rspb.2018.2233.

Anderson, J.B., Catona, S., 2014. Genomewide mutation dynamic within a long-lived individual of *Armillaria gallica*. Mycologia. https://doi.org/10.3852/13-367.

Auxier, B., Bazzicalupo, A., Betz, E., Dee, J.M., Le Renard, L., Roushdy, M.M., Schwartz, C., Berbee, M., 2016. No place among the living: phylogenetic considerations place the Palaeozoic fossil *T. protuberans* in Fungi but not in Dikarya. A comment on M. Smith (2016). Bot. J. Linn. Soc. https://doi.org/10.1111/boj.12479.

Bahn, Y.S., Xue, C., Idnurm, A., Rutherford, J.C., Heitman, J., Cardenas, M.E., 2007. Sensing the environment: lessons from fungi. Nat. Rev. Microbiol. https://doi.org/10.1038/nrmicro1578.

Bartlett, A., O'Malley, R.C., Huang, S.S.C., Galli, M., Nery, J.R., Gallavotti, A., Ecker, J.R., 2017. Mapping genome-wide transcription-factor binding sites using DAP-seq. Nat. Protoc. https://doi.org/10.1038/nprot.2017.055.

Bayry, J., Aimanianda, V., Guijarro, J.I., Sunde, M., Latgé, J.P., 2012. Hydrophobins-unique fungal proteins. PLoS Pathog. 8. https://doi.org/10.1371/journal.ppat.1002700.

Berbee, M.L., James, T.Y., Strullu-Derrien, C., 2017. Early diverging fungi: diversity and impact at the dawn of terrestrial life. Annu. Rev. Microbiol. 71, 41–60. https://doi.org/10.1146/annurev-micro-030117-020324.

Berbee, M.L., Taylor, J.W., 2010. Dating the molecular clock in fungi – how close are we? Fungal Biol. Rev. 24, 1–16. https://doi.org/10.1016/j.fbr.2010.03.001.

Bich, L., Pradeu, T., Moreau, J.F., 2019. Understanding multicellularity: the functional organization of the intercellular space. Front. Physiol. https://doi.org/10.3389/fphys.2019.01170.

Bistis, G.N., Perkins, D.D., Read, N.D., 2003. Different cell types in *Neurospora crassa*. Fungal Genet. Rep. https://doi.org/10.4148/1941-4765.1154.

Biswas, S., Van Dijck, P., Datta, A., 2007. environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*. Microbiol. Mol. Biol. Rev.. https://doi.org/10.1128/mmbr.00009-06.

Bloemendal, S., Kück, U., 2013. Cell-to-cell communication in plants, animals, and fungi: a comparative review. Naturwissenschaften. https://doi.org/10.1007/s00114-012-0988-z.

Boddy, L., 1999. Saprotrophic cord-forming fungi: meeting the challenge of heterogeneous environments. In: Mycologia. https://doi.org/10.2307/3761190.

Bonneville, S., Delpomdor, F., Preat, A., Chevalier, C., Araki, T., Kazemian, M., Steele, A., Schreiber, A., Wirth, R., Benning, L.G., 2020. Molecular identification of fungi microfossils in a Neoproterozoic shale rock. Sci. Adv. https://doi.org/10.1126/sciadv.aax7599.

Boulianne, R.P., Liu, Y., Aebi, M., Lu, B.C., Kues, U., 2000. Fruiting body development in *Coprinus cinereus*: regulated expression of two galectins secreted by a non-classical pathway. Microbiology 146, 1841–1853. https://doi.org/10.1099/00221287-146-8-1841.

Bowles, A.M.C., Bechtold, U., Paps, J., 2020. the origin of land plants is rooted in two bursts of genomic novelty. Curr. Biol. https://doi.org/10.1016/j.cub.2019.11.090.

Braun, E.J., Howard, R.J., 1994. Adhesion of fungal spores and germlings to host plant surfaces. Protoplasma. https://doi.org/10.1007/BF01666396.

Brown, N.A., Schrevens, S., Van Dijck, P., Goldman, G.H., 2018. Fungal G-protein-coupled receptors: Mediators of pathogenesis and targets for disease control. Nat. Microbiol. https://doi.org/10.1038/s41564-018-0127-5.

Brownlee, C., Jennings, D.H., 1981. Further observations on tear or drop formation by mycelium of Serpula lacrimans. Trans. Br. Mycol. Soc. https://doi.org/10.1016/s0007-1536(81)80176-3.

Brunet, T., King, N., 2017. The origin of animal multicellularity and cell differentiation. Dev. Cell. https://doi.org/10.1016/j.devcel.2017.09.016.

Buenrostro, J.D., Wu, B., Chang, H.Y., Greenleaf, W.J., 2015. ATAC-seq: a method for assaying chromatin accessibility genome-wide. Curr. Protoc. Mol. Biol. 2015, 21.29.1–21.29.9. https://doi.org/10.1002/0471142727.mb2129s109.

Cairney, J.W.G., 1992. Translocation of solutes in ectomycorrhizal and saprotrophic rhizomorphs. Mycol. Res. https://doi.org/10.1016/S0953-7562(09)80928-3.

Cairns, J., 1975. Mutation selection and the natural history of cancer. Nature. https://doi.org/10.1038/255197a0.

Cavalier-Smith, T., 2017. Origin of animal multicellularity: precursors, causes, consequences—the choanoflagellate/sponge transition, neurogenesis and the Cambrian explosion. Philos. Trans. R. Soc. B Biol. Sci. 372. https://doi.org/10.1098/rstb.2015.0476.

Chang, Y., Wang, S., Sekimoto, S., Aerts, A.L., Choi, C., Clum, A., LaButti, K.M., Lindquist, E.A., Yee Ngan, C., Ohm, R.A., Salamov, A.A., Grigoriev, I.V., Spatafora, J.W., Berbee, M.L., 2015. Phylogenomic analyses indicate that early fungi evolved digesting cell walls of algal ancestors of land plants. Genome Biol. Evol. 7, 1590–1601. https://doi.org/10.1093/gbe/evv090.

Claessen, D., Rozen, D.E., Kuipers, O.P., Sogaard-Andersen, L., van Wezel, G.P., 2014. Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies. Nat. Rev. Microbiol. 12, 115–124. https://doi.org/10.1038/nrmicro3178.

Clémencon, H., Emmett, V., Emmett, E.E., 2012. Cytology and plectology of the Hymenomycetes. J. Cramer Gebr. Borntraeger Verlagsbuchhandlung.

Coates, J.C., Aiman, U.-E., Charrier, B., 2015. Understanding green multicellularity: do seaweeds hold the key? Front. Plant Sci. 5, 1–6. https://doi.org/10.3389/fpls.2014.00737.

Cock, J.M., Sterck, L., Rouze, P., Scornet, D., Allen, A.E., Amoutzias, G., Anthouard, V., Artiguenave, F., Aury, J.-M., Badger, J.H., Beszteri, B., Billiau, K., Bonnet, E., Bothwell, J.H., Bowler, C., Boyen, C., Brownlee, C., Carrano, C.J., Charrier, B., Cho, G.Y., Coelho, S.M., Collen, J., Corre, E., Da Silva, C., Delage, L., Delaroque, N., Dittami, S.M., Doulbeau, S., Elias, M., Farnham, G., Gachon, C.M.M., Gschloessl, B., Heesch, S., Jabbari, K., Jubin, C., Kawai, H., Kimura, K., Kloareg, B., Kupper, F.C., Lang, D., Le Bail, A., Leblanc, C., Lerouge, P., Lohr, M., Lopez, P.J., Martens, C., Maumus, F., Michel, G., Miranda-Saavedra, D., Morales, J., Moreau, H., Motomura, T., Nagasato, C., Napoli, C.A., Nelson, D.R., Nyvall-Collen, P., Peters, A.F., Pommier, C., Potin, P., Poulain, J., Quesneville, H., Read, B., Rensing, S.A., Ritter, A., Rousvoal, S., Samanta, M., Samson, G., Schroeder, D.C., Segurens, B., Strittmatter, M., Tonon, T., Tregear, J.W., Valentin, K., von Dassow, P., Yamagishi, T., Van de Peer, Y., Wincker, P., 2010. The Ectocarpus genome and the independent evolution of multicellularity in brown algae. Nature 465, 617–621. https://doi.org/10.3389/fpls.2014.00737.

Coggins, C.R., Jennings, D.H., Clarke, R.W., 1980. Tear or drop formation by mycelium of *Serpula lacrimans*. Trans. Br. Mycol. Soc. https://doi.org/10.1016/s0007-1536(80)80195-1.

Cordero, R.J.B., Casadevall, A., 2017. Functions of fungal melanin beyond virulence. Fungal Biol. Rev. https://doi.org/10.1016/j.fbr.2016.12.003.

Cottier, F., Mühlschlegel, F.A., 2012. Communication in Fungi. Int. J. Microbiol. 2012, 9. https://doi.org/10.1155/2012/351832.

Cowan, M.C., Lewis, B.G., Thain, J.F., 1972. Uptake of potassium by the developing sporangiophore of *Phycomyces blakesleeanus*. Trans. Br. Mycol. Soc. https://doi.org/10.1016/s0007-1536(72)80077-9.

Daly, P., Peng, M., Mitchell, H.D., Kim, Y.M., Ansong, C., Brewer, H., de Gijsel, P., Lipton, M.S., Markillie, L.M., Nicora, C.D., Orr, G., Wiebenga, A., Hildén, K.S., Kabel, M.A., Baker, S.E., Mäkelä, M.R., de Vries, R.P., 2020. Colonies of the fungus *Aspergillus niger* are highly differentiated to adapt to local carbon source variation. Environ. Microbiol. https://doi.org/10.1111/1462-2920.14907.

Daskalov, A., Heller, J., Herzog, S., Fleißner, A., Glass, N.L., 2017. Molecular mechanisms regulating cell fusion and heterokaryon formation in filamentous fungi. Microbiol. Spectr. https://doi.org/10.1128/microbiolspec.funk-0015-2016.

De Clerck, O., Kao, S.M., Bogaert, K.A., Blomme, J., Foflonker, F., Kwantes, M., Vancaester, E., Vanderstraeten, L., Aydogdu, E., Boesger, J., Califano, G., Charrier, B., Clewes, R., Del Cortona, A., D'Hondt, S., Fernandez-Pozo, N., Gachon, C.M., Hanikenne, M., Lattermann, L., Leliaert, F., Liu, X., Maggs, C.A., Popper, Z.A., Raven, J.A., Van Bel, M., Wilhelmsson, P.K.I., Bhattacharya, D., Coates, J.C., Rensing, S.A., Van Der Straeten, D., Vardi, A., Sterck, L., Vandepoele, K., Van de Peer, Y., Wichard, T., Bothwell, J.H., 2018. Insights into the evolution of multicellularity from the Sea lettuce genome. Curr. Biol. https://doi.org/10.1016/j.cub.2018.08.015.

de Groot, P.W.J., Bader, O., de Boer, A.D., Weig, M., Chauhan, N., 2013. Adhesins in human fungal pathogens: glue with plenty of stick. Eukaryot. Cell 12, 470–481. https://doi.org/10.1128/EC.00364-12.

de Mendoza, A., Sebé-Pedrós, A., Ruiz-Trillo, I., 2014. The evolution of the GPCR signaling system in eukaryotes: modularity, conservation, and the transition to metazoan multicellularity. Genome Biol. Evol. 6, 606–619. https://doi.org/10.1093/gbe/evu038.

De Schepper, V., De Swaef, T., Bauweraerts, I., Steppe, K., 2013. Phloem transport: a review of mechanisms and controls. J. Exp. Bot. https://doi.org/10.1093/jxb/ert302.

Dee, J.M., Landry, B.R., Berbee, M.L., 2019. Actin guides filamentous rhizoid growth and morphogenesis in the zoosporic fungus *Chytriomyces hyalinus*. Mycologia. https://doi.org/10.1080/00275514.2019.1669999.

Dee, J.M., Mollicone, M., Longcore, J.E., Roberson, R.W., Berbee, M.L., 2015. Cytology and molecular phylogenetics of Monoblepharidomycetes provide evidence for multiple independent origins of the hyphal habit in the fungi. Mycologia. https://doi.org/10.3852/14-275.

Dilks, T., Halsey, K., De Vos, R.P., Hammond-Kosack, K.E., Brown, N.A., 2019. Non-canonical fungal G-protein coupled receptors promote Fusarium head blight on wheat. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1007666.

Drake, J.W., 1991. A constant rate of spontaneous mutation in DNA-based microbes. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.88.16.7160.

Du, Q., Kawabe, Y., Schilde, C., Chen, Z.H., Schaap, P., 2015. the evolution of aggregative multicellularity and cell-cell communication in the dictyostelia. J. Mol. Biol. https://doi.org/10.1016/j.jmb.2015.08.008.

Dyer, P.S., O'Gorman, C.M., 2012. Sexual development and cryptic sexuality in fungi: Insights from Aspergillus species. FEMS Microbiol. Rev. https://doi.org/10.1111/j.1574-6976.2011.00308.x.

Elramli, N., Karahoda, B., Sarikaya-Bayram, O., Frawley, D., Ulas, M., Oakley, C.E., Oakley, B.R., Seiler, S., Bayram, O., 2019. Assembly of a heptameric STRIPAK complex is required for coordination of light-dependent multicellular fungal development with secondary metabolism in aspergillus nidulans. PLoS Genet. https://doi.org/10.1371/journal.pgen.1008053.

Etxebeste, O., Otamendi, A., Garzia, A., Espeso, E.A., Cortese, M.S., 2019. Rewiring of transcriptional networks as a major event leading to the diversity of asexual multicellularity in fungi. Crit. Rev. Microbiol. https://doi.org/10.1080/1040841X.2019.1630359.

Fischer, M.S., Glass, N.L., 2019. Communicate and fuse: how filamentous fungi establish and maintain an interconnected mycelial network. Front. Microbiol. https://doi.org/10.3389/fmicb.2019.00619.

Fisher, R.M., Cornwallis, C.K., West, S.A., 2013. Group formation, relatedness, and the evolution of multicellularity. Curr. Biol. https://doi.org/10.1016/j.cub.2013.05.004.

Fleißner, A., Glass, N.L., 2007. SO, a protein involved in hyphal fusion in Neurospora crassa, localizes to septal plugs. Eukaryot. Cell. https://doi.org/10.1128/EC.00268-06.

Fleißner, A., Simonin, A.R., Glass, N.L., 2008. Cell fusion in the filamentous fungus, *Neurospora crassa*. Methods Mol. Biol. https://doi.org/10.1007/978-1-59745-250-2_2.

Fricker, M.D., Heaton, L.L.M., Jones, N.S., Boddy, L., 2017. The mycelium as a network. Microbiol. Spectr. 5. https://doi.org/10.1128/microbiolspec.FUNK-0033-2017.

Fricker, M.D., Tlalka, M., Bebber, D., Tagaki, S., Watkinson, S.C., Darrah, P.R., 2007. Fourier-based spatial mapping of oscillatory phenomena in fungi. Fungal Genet. Biol. https://doi.org/10.1016/j.fgb.2007.02.012.

Geer, G.A., Wold, M.P., Böttger, B., Gamow, R.I., 1990. Phycomyces: control of transpiration and the anemotropic reversal. Exp. Mycol. https://doi.org/10.1016/0147-5975(90)90024-N.

Gessler, N.N., Filippovich, S.Y., Bachurina, G.P., Kharchenko, E.A., Groza, N.V., Belozerskaya, T.A., 2017. Oxylipins and oxylipin synthesis pathways in fungi. Appl. Biochem. Microbiol. 53, 628–639. https://doi.org/10.1134/S0003683817060060.

Glass, N.L., Dementhon, K., 2006. Non-self recognition and programmed cell death in filamentous fungi. Curr. Opin. Microbiol.. https://doi.org/10.1016/j.mib.2006.09.001.

Gonçalves, A.P., Heller, J., Daskalov, A., Videira, A., Glass, N.L., 2017. Regulated forms of cell death in fungi. Front. Microbiol. https://doi.org/10.3389/fmicb.2017.01837.

Gonçalves, A.P., Heller, J., Span, E.A., Rosenfield, G., Do, H.P., Palma-Guerrero, J., Requena, N., Marletta, M.A., Glass, N.L., 2019. Allorecognition upon fungal cell-cell contact determines social cooperation and impacts the acquisition of multicellularity. Curr. Biol. https://doi.org/10.1016/j.cub.2019.07.060.

Grau-Bové, X., Torruella, G., Donachie, S., Suga, H., Leonard, G., Richards, T.A., Ruiz-Trillo, I., 2017. Dynamics of genomic innovation in the unicellular ancestry of animals. Elife. https://doi.org/10.7554/eLife.26036.

Grosberg, R.K., Strathmann, R.R., 2007. The evolution of multicellularity: a minor major transition? Annu. Rev. Ecol. Evol. Syst. 38, 621–654. https://doi.org/10.1146/annurev.ecolsys.36.102403.114735.

Gulati, M., Nobile, C.J., 2016. Candida albicans biofilms: development, regulation, and molecular mechanisms. Microbes Infect. https://doi.org/10.1016/j.micinf.2016.01.002.

Harris, S.D., 2019. Hyphal branching in filamentous fungi. Dev. Biol. https://doi.org/10.1016/j.ydbio.2019.02.012.

Harris, S.D., 2011. Hyphal morphogenesis: an evolutionary perspective. Fungal Biol. 115, 475–484. https://doi.org/10.1016/j.funbio.2011.02.002.

Harris, S.D., 2008. Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems. Mycologia 100, 823–832. https://doi.org/10.3852/08-177.

Hartmann, A.M., Tesch, D., Nothwang, H.G., Bininda-Emonds, O.R.P., 2014. Evolution of the cation chloride cotransporter family: ancient origins, gene losses, and subfunctionalization through duplication. Mol. Biol. Evol. https://doi.org/10.1093/molbev/mst225.

Hassan, M., Rouf, R., Tiralongo, E., May, T., Tiralongo, J., 2015. Mushroom lectins: specificity, structure and bioactivity relevant to human disease. Int. J. Mol. Sci. 16, 7802–7838. https://doi.org/10.3390/ijms16047802.

Heaton, L.M., Jones, N.S., Fricker, M.D., 2020. A mechanistic explanation of the transition to simple multicellularity in fungi. Nat. Commun. 11, 2594. https://doi.org/10.1038/s41467-020-16072-4. https://www.nature.com/articles/s41467-020-16072-4.

Heaton, L.L.M., López, E., Maini, P.K., Fricker, M.D., Jones, N.S., 2010. Growth-induced mass flows in fungal networks. Proc. R. Soc. B Biol. Sci. https://doi.org/10.1098/rspb.2010.0735.

Hernandez-Lauzardo, A.-N., Bautista-Banos, S., Velazquez-Del-Valle, M.-G., Trejo-Espino, J.-L., Hernandez-Lauzardo, A.-N., Bautista-Banos, S., Velazquez-Del-Valle, M.-G., Trejo-Espino, J.-L., 2006. Rev. Mex. Fitopatol. 24, 65–69.

Herron, M.D., Borin, J.M., Boswell, J.C., Walker, J., Chen, I.C.K., Knox, C.A., Boyd, M., Rosenzweig, F., Ratcliff, W.C., 2019. De novo origins of multicellularity in response to predation. Sci. Rep. https://doi.org/10.1038/s41598-019-39558-8.

Hiltunen, M., Grudzinska-Sterno, M., Wallerman, O., Ryberg, M., Johannesson, H., 2019. Maintenance of high genome integrity over vegetative growth in the fairy-ring mushroom *Marasmius oreades*. Curr. Biol. https://doi.org/10.1016/j.cub.2019.07.025.

Hogan, D.A., 2006. Talking to themselves: autoregulation and quorum sensing in fungi. Eukaryot. Cell. https://doi.org/10.1128/EC.5.4.613-619.2006.

Ispolatov, I., Ackermann, M., Doebeli, M., 2012. Division of labour and the evolution of multicellularity. Proc. R. Soc. B Biol. Sci. https://doi.org/10.1098/rspb.2011.1999.

Reschka, E.J., Nordzieke, S., Valerius, O., Braus, G.H., Pöggeler, S., 2018. A novel STRIPAK complex component mediates hyphal fusion and fruiting-body development in filamentous fungi. Mol. Microbiol. https://doi.org/10.1111/mmi.14106.

Jedd, G., 2011. Fungal evo-devo: organelles and multicellular complexity. Trends Cell Biol.. https://doi.org/10.1016/j.tcb.2010.09.001.

Jones, E.B.G., 1994. Fungal adhesion. Mycol. Res. https://doi.org/10.1016/S0953-7562(09)80421-8.

King, N., Carroll, S.B., 2001. A receptor tyrosine kinase from choanoflagellates: molecular insights into early animal evolution. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.261477698.

Kiss, E., Hegedüs, B., Virágh, M., Varga, T., Merényi, Z., Kószó, T., Bálint, B., Prasanna, A.N., Krizsán, K., Kocsubé, S., Riquelme, M., Takeshita, N., Nagy, L.G., 2019. Comparative genomics reveals the origin of fungal hyphae and multicellularity. Nat. Commun.. https://doi.org/10.1038/s41467-019-12085-w.

Knoll, A.H., 2011. The multiple origins of complex multicellularity. Annu. Rev. Earth Planet. Sci. https://doi.org/10.1146/annurev.earth.031208.100209.

Knoll, A.H., Hewitt, D., 2013. Phylogenetic, functional, and geological perspectives on complex multicellularity. In: The Major Transitions in Evolution Revisited. https://doi.org/10.7551/mitpress/9780262015240.003.0013.

Krijgsheld, P., Bleichrodt, R., van Veluw, G.J., Wang, F., Müller, W.H., Dijksterhuis, J., Wösten, H.A.B., 2013. Development in aspergillus. Stud. Mycol. https://doi.org/10.3114/sim0006.

Krishnan, A., Almén, M.S., Fredriksson, R., Schiöth, H.B., 2012. The origin of GPCRs: identification of mammalian like rhodopsin, adhesion, glutamate and frizzled GPCRs in fungi. PLoS One. https://doi.org/10.1371/journal.pone.0029817.

Krizsan, K., Almasi, E., Merenyi, Z., Sahu, N., Viragh, M., Koszo, T., Mondo, S., Kiss, B., Balint, B., Kues, U., Barry, K., Cseklye, J., Hegedus, B., Henrissat, B., Johnson, J., Lipzen, A., Ohm, R.A., Nagy, I., Pangilinan, J., Yan, J., Xiong, Y., Grigoriev, I.V., Hibbett, D.S., Nagy, L.G., 2019. Transcriptomic atlas of mushroom development highlights an independent origin of complex multicellularity. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1817822116.

Kruppa, M., 2009. Quorum sensing and *Candida albicans*. Mycoses. https://doi.org/10.1111/j.1439-0507.2008.01626.x.

Kück, U., Beier, A.M., Teichert, I., 2016. The composition and function of the striatin-interacting phosphatases and kinases (STRIPAK) complex in fungi. Fungal Genet. Biol. https://doi.org/10.1016/j.fgb.2015.10.001.

Kües, U., 2000. Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol. Mol. Biol. Rev. 64, 316–353. https://doi.org/10.1128/MMBR.64.2.316-353.2000.

Kües, U., Khonsuntia, W., Subba, S., 2018a. Complex fungi. Fungal Biol. Rev. 32, 205–218. https://doi.org/10.1016/J.FBR.2018.08.001.

Kües, U., Khonsuntia, W., Subba, S., Dörnte, B., 2018b. Volatiles in communication of Agaricomycetes. In: Physiology and Genetics. https://doi.org/10.1007/978-3-319-71740-1_6.

Kües, U., Liu, Y., 2000. Fruiting body production in basidiomycetes. Appl. Microbiol. Biotechnol. 54, 141–152. https://doi.org/10.1007/s002530000396.

Kües, U., Navarro-González, M., 2015. How do Agaricomycetes shape their fruiting bodies? 1. Morphological aspects of development. Fungal Biol. Rev. https://doi.org/10.1016/j.fbr.2015.05.001.

Lanfear, R., 2018. Do plants have a segregated germline? PLoS Biol. https://doi.org/10.1371/journal.pbio.2005439.

Laundon, D., Chrismas, N., Wheeler, G., Cunliffe, M., 2019. Chytrid rhizoid morphogenesis is adaptive and resembles hyphal development in 'higher' fungi. bioRxiv. https://doi.org/10.1101/735381.

Leonard et al., 2018Leonard, G., Labarre, A., Milner, D.S., Monier, A., Soanes, D., Wideman, J.G., Maguire, F., Stevens, S., Sain, D., Grau-Bové, X., Sebé-Pedrós, A., Stajich, J.E., Paszkiewicz, K., Brown, M.W., Hall, N., Wickstead, B., Richards, T.A., 2018. Comparative genomic analysis of the 'pseudofungus' Hyphochytrium catenoides. Open Biol. https://doi.org/10.1098/rsob.170184.

Levine, M., Davidson, E.H., 2005. Gene regulatory networks for development. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.0408031102.

Lichius, A., Lord, K.M., Jeffree, C.E., Oborny, R., Boonyarungsrit, P., Read, N.D., 2012. Importance of MAP kinases during proto-perithecial morphogenesis in *Neurospora crassa*. PLoS One 7, e42565. https://doi.org/10.1371/journal.pone.0042565.

Lipke, P.N., 2018. What we do not know about fungal cell adhesion molecules. J. Fungi. https://doi.org/10.3390/jof4020059.

Liu, L., He, G.J., Chen, L., Zheng, J., Chen, Y., Shen, L., Tian, X., Li, E., Yang, E., Liao, G., Wang, L., 2018. Genetic basis for co-ordination of meiosis and sexual structure maturation in cryptococcus neoformans. Elife. https://doi.org/10.7554/eLife.38683.

Lord, K.M., Read, N.D., 2011. Perithecium morphogenesis in *Sordaria macrospora*. Fungal Genet. Biol. 48, 388–399. https://doi.org/10.1016/j.fgb.2010.11.009.

Loron, C.C., François, C., Rainbird, R.H., Turner, E.C., Borensztajn, S., Javaux, E.J., 2019. Early fungi from the proterozoic era in arctic Canada. Nature. https://doi.org/10.1038/s41586-019-1217-0.

Lugones, L.G., Wösten, H.A.B., Birkenkamp, K.U., Sjollema, K.A., Zagers, J., Wessels, J.G.H., 1999. Hydrophobins line air channels in fruiting bodies of *Schizophyllum commune* and *Agaricus bisporus*. Mycol. Res. 103, 635–640. https://doi.org/10.1017/S0953756298007552.

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., Hibbett, D.S., 2016. Unearthing the roots of ectomycorrhizal symbioses. Nat. Rev. Microbiol. https://doi.org/10.1038/nrmicro.2016.149.

Merényi Z, Prasanna AN, Wang Z, Kovács K, Hegedüs B, Bálint B, Papp B, Townsend JP, Nagy LG. Unmatched level of molecular convergence among deeply divergent complex multicellular fungi. https://doi.org/10.1093/molbev/msaa077/5810091.

Michod, R.E., Roze, D., 2001. Cooperation and conflict in the evolution of multicellularity. Heredity. https://doi.org/10.1046/j.1365-2540.2001.00808.x.

Milholland, B., Dong, X., Zhang, L., Hao, X., Suh, Y., Vijg, J., 2017. Differences between germline and somatic mutation rates in humans and mice. Nat. Commun. https://doi.org/10.1038/ncomms15183.

Miller, W.T., 2012. Tyrosine kinase signaling and the emergence of multicellularity. Biochim. Biophys. Acta Mol. Cell Res. 1823, 1053–1057. https://doi.org/10.1016/j.bbamcr.2012.03.009.

Mitchell, K.F., Zarnowski, R., Andes, D.R., 2016a. The extracellular matrix of fungal biofilms. Adv. Exp. Med. Biol. https://doi.org/10.1007/5584_2016_6.

Mitchell, K.F., Zarnowski, R., Andes, D.R., 2016b. Fungal super glue: the biofilm matrix and its composition, assembly, and functions. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1005828.

Miyazaki, Y., Kaneko, S., Sunagawa, M., Shishido, K., Yamazaki, T., Nakamura, M., Babasaki, K., 2007. The fruiting-specific Le.flp1 gene, encoding a novel fungal fasciclin-like protein, of the basidiomycetous mushroom *Lentinula edodes*. Curr. Genet. 51, 367–375. https://doi.org/10.1007/s00294-007-0133-2.

Monahan-Earley, R., Dvorak, A.M., Aird, W.C., 2013. Evolutionary origins of the blood vascular system and endothelium. J. Thromb. Haemost. https://doi.org/10.1111/jth.12253.

Money, N.P., 2002. Mushroom stem cells. BioEssays. https://doi.org/10.1002/bies.10160.

Morin, E., Kohler, A., Baker, A.R., Foulongne-Oriol, M., Lombard, V., Nagy, L.G., Ohm, R.A., Patyshakuliyeva, A., Brun, A., Aerts, A.L., Bailey, A.M., Billette, C., Coutinho, P.M., Deakin, G., Doddapaneni, H., Floudas, D., Grimwood, J., Hildén, K., Kües, U., Labutti, K.M., Lapidus, A., Lindquist, E.A., Lucas, S.M., Murat, C., Riley, R.W., Salamov, A.A., Schmutz, J., Subramanian, V., Wösten, H.A.B., Xu, J., Eastwood, D.C., Foster, G.D., Sonnenberg, A.S.M., Cullen, D., de Vries, R.P., Lundell, T., Hibbett, D.S., Henrissat, B., Burton, K.S., Kerrigan, R.W., Challen, M.P., Grigoriev, I.V., Martin, F., 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc. Natl. Acad. Sci. U. S. A. 109, 17501–17506. https://doi.org/10.1073/pnas.1206847109.

Nagy, L.G., 2017. Evolution: complex multicellular life with 5,500 genes. Curr. Biol. 27, R609–R612. https://doi.org/10.1016/J.CUB.2017.04.032.

Nagy, L.G., Kovács, G.M., Krizsán, K., 2018. Complex multicellularity in fungi: evolutionary convergence, single origin, or both? Biol. Rev.. https://doi.org/10.1111/brv.12418.

Nagy, L.G., Ohm, R.A., Kovács, G.M., Floudas, D., Riley, R., Gácser, A., Sipiczki, M., Davis, J.M., Doty, S.L., de Hoog, G.S., Lang, B.F., Spatafora, J.W., Martin, F.M., Grigoriev, I. V, Hibbett, D.S., 2014. Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts. 5, 4471. https://doi.org/10.1038/ncomms5471https://www.nature.com/articles/ncomms5471#supplementary-information.

Nagy, L.G., Tóth, R., Kiss, E., Slot, J., Gácser, A., Kovács, G.M., 2017. Six key traits of fungi: their evolutionary origins and genetic bases. In: The Fungal Kingdom. American Society of Microbiology, pp. 35–56. https://doi.org/10.1128/microbiolspec.FUNK-0036-2016.

Nguyen, T.A., Cissé, O.H., Yun Wong, J., Zheng, P., Hewitt, D., Nowrousian, M., Stajich, J.E., Jedd, G., 2017. Innovation and constraint leading to complex multicellularity in the *Ascomycota*. 8, 14444. https://doi.org/10.1038/ncomms14444https://www.nature.com/articles/ncomms14444#supplementary-information.

Nicholson, R.L., 1996. Adhesion of Fungal Propagules. Springer, Dordrecht, pp. 117–134. https://doi.org/10.1007/978-94-009-0189-6_7.

Niklas, K.J., 2014. The evolutionary-developmental origins of multicellularity. Am. J. Bot. 101, 6–25. https://doi.org/10.3732/ajb.1300314.

Niklas, K.J., Newman, S.A., 2013. The origins of multicellular organisms. Evol. Dev. 15, 41–52. https://doi.org/10.1111/ede.12013.

Niklas, K.J., Stuart, N.A., 2019. The many roads to and from multicellularity. J. Exp. Bot. https://doi.org/10.1093/jxb/erz547.

Nowrousian, M., 2018. Genomics and transcriptomics to study fruiting body development: an update. Fungal Biol. Rev. https://doi.org/10.1016/j.fbr.2018.02.004.

Nowrousian, M., Teichert, I., Masloff, S., Kück, U., 2012. Whole-genome sequencing of sordaria macrospora mutants identifies developmental genes. G3. https://doi.org/10.1534/g3.111.001479.

Obert, M., Pfeifer, P., Sernetz, M., 1990. Microbial growth patterns described by fractal geometry. J. Bacteriol. https://doi.org/10.1128/jb.172.3.1180-1185.1990.

Ohm, R.A., de Jong, J.F., de Bekker, C., Wösten, H.A.B., Lugones, L.G., 2011. Transcription factor genes of Schizophyllum commune involved in regulation of mushroom formation. Mol. Microbiol. 81, 1433–1445. https://doi.org/10.1111/j.1365-2958.2011.07776.x.

Ohm, R.A., De Jong, J.F., Lugones, L.G., Aerts, A., Kothe, E., Stajich, J.E., De Vries, R.P., Record, E., Levasseur, A., Baker, S.E., Bartholomew, K.A., Coutinho, P.M., Erdmann, S., Fowler, T.J., Gathman, A.C., Lombard, V., Henrissat, B., Knabe, N., Kües, U., Lilly, W.W., Lindquist, E., Lucas, S., Magnuson, J.K., Piumi, F., Raudaskoski, M., Salamov, A., Schmutz, J., Schwarze, F.W.M.R., Vankuyk, P.A., Horton, J.S., Grigoriev, I.V., Wösten, H.A.B., 2010. Genome sequence of the model mushroom *Schizophyllum commune*. Nat. Biotechnol. 28, 957–963. https://doi.org/10.1038/nbt.1643.

Ojeda-López, M., Chen, W., Eagle, C.E., Gutiérrez, G., Jia, W.L., Swilaiman, S.S., Huang, Z., Park, H.S., Yu, J.H., Cánovas, D., Dyer, P.S., 2018. Evolution of asexual and sexual reproduction in the aspergilli. Stud. Mycol. https://doi.org/10.1016/j.simyco.2018.10.002.

Olsson, S., 2001. Colonial growth of fungi. In: Biology of the Fungal Cell. https://doi.org/10.1007/978-3-662-06101-5_6.

Ossowski, S., Schneeberger, K., Lucas-Lledó, J.I., Warthmann, N., Clark, R.M., Shaw, R.G., Weigel, D., Lynch, M., 2010. The rate and molecular spectrum of spontaneous mutations in arabidopsis thaliana. Science 80. https://doi.org/10.1126/science.1180677.

Padder, S.A., Prasad, R., Shah, A.H., 2018. Quorum sensing: a less known mode of communication among fungi. Microbiol. Res.. https://doi.org/10.1016/j.micres.2018.03.007.

Papagianni, 2006Papagianni, M., 2006. Quantification of the fractal nature of mycelial aggregation in *Aspergillus niger* submerged cultures. Microb. Cell Fact. https://doi.org/10.1186/1475-2859-5-5.

Parfrey, L.W., Lahr, D.J.G., 2013. Multicellularity arose several times in the evolution of eukaryotes. BioEssays. https://doi.org/10.1002/bies.201200143.

Pincus, D., Letunic, I., Bork, P., Lim, W.A., 2008. Evolution of the phospho-tyrosine signaling machinery in premetazoan lineages. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.0803161105.

Pöggeler, S., Nowrousian, M., Kück, U., 2006. 16 Fruiting-Body Development in Ascomycetes.

Pöggeler, S., Nowrousian, M., Teichert, I., Beier, A., Kück, U., 2018. Fruiting-body development in ascomycetes. In: Physiology and Genetics. Springer International Publishing, Cham, pp. 1–56. https://doi.org/10.1007/978-3-319-71740-1_1.

Prosser, J.I., 1995. Kinetics of filamentous growth and branching. In: The Growing Fungus. https://doi.org/10.1007/978-0-585-27576-5_14.

Ratcliff, W.C., Denison, R.F., Borrello, M., Travisano, M., 2012. Experimental evolution of multicellularity. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1115323109.

Raven, J.A., 2018. Evolution and palaeophysiology of the vascular system and other means of long-distance transport. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2016.0497.

Read, N.D., Lichius, A., Shoji, J. ya, Goryachev, A.B., 2009. Self-signalling and self-fusion in filamentous fungi. Curr. Opin. Microbiol.. https://doi.org/10.1016/j.mib.2009.09.008.

Richards, T.A., Leonard, G., Wideman, J.G., 2017. What defines the "Kingdom" fungi? Microbiol. Spectr. https://doi.org/10.1128/-microbiolspec.funk-0044-2017.

Richards, T.A., Talbot, N.J., 2018. Current biology, current biology. https://doi.org/10.1016/j.cub.2018.07.069.

Riquelme, M., Aguirre, J., Bartnicki-García, S., Braus, G.H., Feldbrügge, M., Fleig, U., Hansberg, W., Herrera-Estrella, A., Kämper, J., Kück, U., Mouriño-Pérez, R.R., Takeshita, N., Fischer, R., 2018. Fungal morphogenesis, from the polarized growth of hyphae to complex reproduction and infection structures. Microbiol. Mol. Biol. Rev. 82. https://doi.org/10.1128/MMBR.00068-17 e00068–17.

Rodenburg, S.Y.A., Terhem, R.B., Veloso, J., Stassen, J.H.M., Van Kan, J.A.L., 2018. Functional analysis of mating type genes and transcriptome analysis during fruiting body development of *botrytis cinerea*. mBio 9. https://doi.org/10.1128/mBio.01939-17.

Rokas, A., 2008. The origins of multicellularity and the early history of the genetic toolkit for animal development. Annu. Rev. Genet. 42, 235–251. https://doi.org/10.1146/annurev.genet.42.110807.091513.

Roper, M., Lee, C.H., Hickey, P.C., Gladfelter, A.S., 2015. Life as a moving fluid: fate of cytoplasmic macromolecules in dynamic fungal syncytia. Curr. Opin. Microbiol. https://doi.org/10.1016/j.mib.2015.07.001.

Rosenberger, R.F., Kessel, M., 1968. Nonrandom sister chromatid segregation and nuclear migration in hyphae of *Aspergillus nidulans*. J. Bacteriol. https://doi.org/10.1128/jb.96.4.1208-1213.1968.

Rossetti, V., Filippini, M., Svercel, M., Barbour, A.D., Bagheri, H.C., 2011. Emergent multicellular life cycles in filamentous bacteria owing to density-dependent population dynamics. J. R. Soc. Interface. https://doi.org/10.1098/rsif.2011.0102.

Ruiz-Trillo, I., Burger, G., Holland, P.W.H., King, N., Lang, B.F., Roger, A.J., Gray, M.W., 2007. The origins of multicellularity: a multi-taxon genome initiative. Trends Genet. 23, 113–118. https://doi.org/10.1016/j.tig.2007.01.005.

Schmieder, S.S., Stanley, C.E., Rzepiela, A., van Swaay, D., Sabotič, J., Nørrelykke, S.F., deMello, A.J., Aebi, M., Künzler, M., 2019. bidirectional propagation of signals and nutrients in fungal networks via specialized hyphae. Curr. Biol. https://doi.org/10.1016/j.cub.2018.11.058.

Schuster, M., Kahmann, R., 2019. CRISPR-Cas9 genome editing approaches in filamentous fungi and oomycetes. Fungal Genet. Biol. https://doi.org/10.1016/j.fgb.2019.04.016.

Sebé-Pedrós, A., Ballaré, C., Parra-Acero, H., Chiva, C., Tena, J.J., Sabidó, E., Gómez-Skarmeta, J.L., Di Croce, L., Ruiz-Trillo, I., 2016. The dynamic regulatory genome of capsaspora and the origin of animal multicellularity. Cell. https://doi.org/10.1016/j.cell.2016.03.034.

Sebé-Pedrós, A., Chomsky, E., Pang, K., Lara-Astiaso, D., Gaiti, F., Mukamel, Z., Amit, I., Hejnol, A., Degnan, B.M., Tanay, A., 2018. Early metazoan cell type diversity and the evolution of multicellular gene regulation. Nat. Ecol. Evol. https://doi.org/10.1038/s41559-018-0575-6.

Sebé-Pedrós, A., Degnan, B.M., Ruiz-Trillo, I., 2017. The origin of metazoa: a unicellular perspective. Nat. Rev. Genet.. https://doi.org/10.1038/nrg.2017.21.

Sebé-Pedrós, A., Irimia, M., del Campo, J., Parra-Acero, H., Russ, C., Nusbaum, C., Blencowe, B.J., Ruiz-Trillo, I., 2013. Regulated aggregative multicellularity in a close unicellular relative of metazoa. Elife 2, e01287. https://doi.org/10.7554/eLife.01287.

Sebé-Pedrós, A., Roger, A.J., Lang, F.B., King, N., Ruiz-Trillo, I., 2010. Ancient origin of the integrin-mediated adhesion and signaling machinery. Proc. Natl. Acad. Sci. U. S. A. 107, 10142–10147. https://doi.org/10.1073/pnas.1002257107.

Sekimoto, S., Rochon, D., Long, J.E., Dee, J.M., Berbee, M.L., 2011. A multigene phylogeny of Olpidium and its implications for early fungal evolution. BMC Evol. Biol. 11, 331. https://doi.org/10.1186/1471-2148-11-331.

Sherman, T.F., 1981. On connecting large vessels to small. The meaning of Murray's law. J. Gen. Physiol. https://doi.org/10.1085/jgp.78.4.431.

Shiu, S.H., Bleecker, A.B., 2001. Plant receptor-like kinase gene family: diversity, function, and signaling. Sci. STKE 2001, re22. https://doi.org/10.1126/stke.2001.113.re22.

Sil, A., 2019. Molecular regulation of Histoplasma dimorphism. Curr. Opin. Microbiol. 52, 151–157. https://doi.org/10.1016/j.mib.2019.10.011.

Simonin, A., Palma-Guerrero, J., Fricker, M., Louise Glass, N., 2012. Physiological significance of network organization in fungi. Eukaryot. Cell. https://doi.org/10.1128/EC.00213-12.

Sipos, G., Prasanna, A.N., Walter, M.C., O'Connor, E., Bálint, B., Krizsán, K., Kiss, B., Hess, J., Varga, T., Slot, J., Riley, R., Bóka, B., Rigling, D., Barry, K., Lee, J., Mihaltcheva, S., LaButti, K., Lipzen, A., Waldron, R., Moloney, N.M., Sperisen, C., Kredics, L., Vágvölgyi, C., Patrignani, A., Fitzpatrick, D., Nagy, I., Doyle, S., Anderson, J.B., Grigoriev, I.V., Güldener, U., Münsterkötter, M., Nagy, L.G., 2017. Genome expansion and lineage-specific genetic innovations in the forest pathogenic fungi Armillaria. Nat. Ecol. Evol. 1, 1931–1941. https://doi.org/10.1038/s41559-017-0347-8.

Smith, M.R., 2016. Cord-forming Palaeozoic fungi in terrestrial assemblages. Bot. J. Linn. Soc. https://doi.org/10.1111/boj.12389.

Sogabe, S., Hatleberg, W.L., Kocot, K.M., Say, T.E., Stoupin, D., Roper, K.E., Fernandez-Valverde, S.L., Degnan, S.M., Degnan, B.M., 2019. Pluripotency and the origin of animal multicellularity. Nature. https://doi.org/10.1038/s41586-019-1290-4.

Stajich, J.E., Berbee, M.L., Blackwell, M., Hibbett, D.S., James, T.Y., Spatafora, J.W., Taylor, J.W., 2009. The fungi. Curr. Biol. 19, R840–R845. https://doi.org/10.1016/j.cub.2009.07.004.

Stajich, J.E., Wilke, S.K., Ahrén, D., Au, C.H., Birren, B.W., Borodovsky, M., Burns, C., Canbäck, B., Casselton, L.A., Cheng, C.K., Deng, J., Dietrich, F.S., Fargo, D.C., Farman, M.L., Gathman, A.C., Goldberg, J., Guigó, R., Hoegger, P.J., Hooker, J.B., Huggins, A., James, T.Y., Kamada, T., Kilaru, S., Kodira, C., Kües, U., Kupfer, D., Kwan, H.S., Lomsadze, A., Li, W., Lilly, W.W., Ma, L.-J., Mackey, A.J., Manning, G., Martin, F., Muraguchi, H., Natvig, D.O., Palmerini, H., Ramesh, M.A., Rehmeyer, C.J., Roe, B.A., Shenoy, N., Stanke, M., Ter-Hovhannisyan, V., Tunlid, A., Velagapudi, R., Vision, T.J., Zeng, Q., Zolan, M.E., Pukkila, P.J., 2010. Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). Proc. Natl. Acad. Sci. U. S. A. 107, 11889–11894. https://doi.org/10.1073/pnas.1003391107.

Steinberg, G., 2007. Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. Eukaryot. Cell 6, 351–360. https://doi.org/10.1128/EC.00381-06.

Steinberg, G., Peñalva, M.A., Riquelme, M., Wosten, H., Harris, S.D., 2017. Cell biology of hyphal growth. Microbiol. Spectr. 5, 1–34. https://doi.org/10.1128/microbiolspec.funk-0034-2016.

Strullu-Derrien, C., Spencer, A.R.T., Goral, T., Dee, J., Honegger, R., Kenrick, P., Longcore, J.E., Berbee, M.L., 2018. New insights into the evolutionary history of fungi from a 407 Ma Blastocladiomycota fossil showing a complex hyphal thallus. Philos. Trans. R. Soc. B Biol. Sci. 373 (20160502). https://doi.org/10.1098/rstb.2016.0502.

Sudbery, P.E., 2011. Growth of *Candida albicans* hyphae. Nat. Rev. Microbiol. https://doi.org/10.1038/nrmicro2636.

Suga, H., Dacre, M., De Mendoza, A., Shalchian-Tabrizi, K., Manning, G., Ruiz-Trillo, I., 2012. Genomic survey of premetazoans shows deep conservation of cytoplasmic tyrosine kinases and multiple radiations of receptor tyrosine kinases. Sci. Signal. 5. https://doi.org/10.1126/scisignal.2002733.

Szathmáry, E., Smith, J.M., 1995. The major evolutionary transitions. Nature 374, 227–232. https://doi.org/10.1038/374227a0.

Taylor, J.W., Ellison, C.E., 2010. Mushrooms: morphological complexity in the fungi. Proc. Natl. Acad. Sci. U. S. A. 107, 11655–11656. https://doi.org/10.1073/pnas.1006430107.

Taylor, T.N., Remy, W., Haas, H., Kerp, H., 1995. Fossil arbuscular mycorrhizae from the early Devonian. Mycologia. https://doi.org/10.2307/3760776.

Teichert, I., Nowrousian, M., Pöggeler, S., Kück, U., 2014. The filamentous fungus Sordaria macrospora as a genetic model to study fruiting body development. In: Advances in Genetics. https://doi.org/10.1016/B978-0-12-800149-3.00004-4.

Thurston, C.F., 1994. The structure and function of fungal laccases. Microbiology. https://doi.org/10.1099/13500872-140-1-19.

Tian, X., He, G.J., Hu, P., Chen, L., Tao, C., Cui, Y.L., Shen, L., Ke, W., Xu, H., Zhao, Y., Xu, Q., Bai, F., Wu, B., Yang, E., Lin, X., Wang, L., 2018. Cryptococcus neoformans sexual reproduction is controlled by a quorum sensing peptide. Nat. Microbiol. https://doi.org/10.1038/s41564-018-0160-4.

Tlalka, M., Hensman, D., Darrah, P.R., Watkinson, S.C., Fricker, M.D., 2003. Noncircadian oscillations in amino acid transport have complementary profiles in assimilatory and foraging hyphae of *Phanerochaete velutina*. New Phytol. https://doi.org/10.1046/j.1469-8137.2003.00737.x.

Tong, K., Wang, Y., Su, Z., 2017. Phosphotyrosine signalling and the origin of animal multicellularity. Proc. R. Soc. B Biol. Sci. https://doi.org/10.1098/rspb.2017.0681.

Traeger, S., Altegoer, F., Freitag, M., Gabaldon, T., Kempken, F., Kumar, A., Marcet-Houben, M., Pöggeler, S., Stajich, J.E., Nowrousian, M., 2013. The genome and development-dependent transcriptomes of pyronema confluens: a window into fungal evolution. PLoS Genet. 9, e1003820. https://doi.org/10.1371/journal.pgen.1003820.

Trail, F., 2013. Sex and fruiting in Fusarium. In: Daren, W., Brown Robert, H. Proctor (Eds.), Fusarium Genomics, Mol. Cell. Biol. Caister Acad. Press, U.K.

Trail, F., Wang, Z., Stefanko, K., Cubba, C., Townsend, J.P., 2017. The ancestral levels of transcription and the evolution of sexual phenotypes in filamentous fungi. PLoS Genet. 13, e1006867. https://doi.org/10.1371/journal.pgen.1006867.

Tronchin, G., Pihet, M., Lopes-Bezerra, L., Bouchara, J.P., 2008. Adherence mechanisms in human pathogenic fungi. Med. Mycol. https://doi.org/10.1080/13693780802206435.

Tsitsigiannis, D.I., Keller, N.P., 2007. Oxylipins as developmental and host-fungal communication signals. Trends Microbiol. https://doi.org/10.1016/j.tim.2007.01.005.

Ugalde, U., 2006. Autoregulatory signals in mycelial fungi. In: Growth, Differentiation and Sexuality. https://doi.org/10.1007/3-540-28135-5_11.

Umen, J.G., 2014. Green algae and the origins of multicellularity in the Plant Kingdom. Cold Spring Harb. Perspect. Biol. 6, a016170. https://doi.org/10.1101/cshperspect.a016170.

Váchová, L., Palková, Z., 2018. How structured yeast multicellular communities live, age and die? FEMS Yeast Res. https://doi.org/10.1093/femsyr/foy033.

van Peer, A.F., Müller, W.H., Boekhout, T., Lugones, L.G., Wösten, H.A.B., 2009. Cytoplasmic continuity revisited: closure of septa of the filamentous fungus Schizophyllum commune in response to environmental conditions. PLoS One. https://doi.org/10.1371/journal.pone.0005977.

Walbot, V., 1985. On the life strategies of plants and animals. Trends Genet. https://doi.org/10.1016/0168-9525(85)90071-X.

Walser, P.J., Kües, U., Aebi, M., Künzler, M., 2005. Ligand interactions of the *Coprinopsis cinerea* galectins. Fungal Genet. Biol. https://doi.org/10.1016/j.fgb.2004.12.004.

Wang, Z., Gudibanda, A., Ugwuowo, U., Trail, F., Townsend, J.P., 2018. Using evolutionary genomics, transcriptomics, and systems biology to reveal gene networks underlying fungal

development. Fungal Biol. Rev. https://doi.org/10.1016/J.FBR.2018.02.001.
Weismann, A., 1893. The Germ-Plasm: a Theory of Heredity. Translated by W. Newton Parker and Harriet Rönnfeldt., the Germ-Plasm: a Theory of Heredity. Translated by W. Newton Parker and Harriet Rönnfeldt. Scribner, New York. https://doi.org/10.5962/bhl.title.25196.
Werner, B., Sottoriva, A., 2018. Variation of mutational burden in healthy human tissues suggests non-random strand segregation and allows measuring somatic mutation rates. PLoS Comput. Biol. https://doi.org/10.1371/journal.pcbi.1006233.
Whiteside, M.D., Werner, G.D.A., Caldas, V.E.A., van't Padje, A., Dupin, S.E., Elbers, B., Bakker, M., Wyatt, G.A.K., Klein, M., Hink, M.A., Postma, M., Vaitla, B., Noë, R., Shimizu, T.S., West, S.A., Kiers, E.T., 2019. Mycorrhizal fungi respond to resource inequality by moving phosphorus from rich to poor patches across networks. Curr. Biol. https://doi.org/10.1016/j.cub.2019.04.061.
Xue, C., Hsueh, Y.P., Heitman, J., 2008. Magnificent seven: roles of G protein-coupled receptors in extracellular sensing in fungi. FEMS Microbiol. Rev. https://doi.org/10.1111/j.1574-6976.2008.00131.x.
Yafetto, L., 2018. The structure of mycelial cords and rhizomorphs of fungi: a mini-review. Mycosphere. https://doi.org/10.5943/mycosphere/9/5/3.
Zhao, Z., Jin, Q., Xu, J.R., Liu, H., 2014. Identification of a fungi-specific lineage of protein kinases closely related to tyrosine kinases. PLoS One 9. https://doi.org/10.1371/journal.pone.0089813.