Convergent evolution of complex multicellularity in fungi

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Abstract

Complex multicellularity comprises the most developmentally integrated morphologies that evolved on Earth. It has evolved in only a few groups of organisms, one of which is fungi, a hyperdiverse kingdom that comprises saprotrophs, mycorrhizal mutualists, pathogens as well as a wide range of model organisms and biotechnological workhorses. Fungi show evidence for convergent transitions in complexity, including the repeated emergence of complex multicellularity and that of reversals to a predominantly unicellular morphology. In this chapter we discuss phylogenetic and genetic evidence that supports these convergent transitions and outline emerging evolutionary models that can provide an explanation for highly convergent yet complex phenotypic traits.

14.1 Introduction - What is complex multicellularity?

The evolution of multicellularity has been one of the most significant transitions in the history of life. Multicellularity comes in many forms, ranging from simple aggregation of cells to the largest macroscopic organisms that dominate the visible world. Some of these are reminiscent of primordial states of multicellularity that hardly go beyond unicellularity in terms of function, whereas others feature sophisticated, highly integrated bodies and structures, structural and functional differentiation, or complex behaviours. Whereas simple cell aggregations, colonies, filaments or other simple solutions to multicellularity have emerged many times during evolution (Grosberg and Strathmann 2007), the highest levels of complexity evolved on only a few occasions. To facilitate discussion, therefore, the continuum of complexity levels is often classified into simple and complex multicellularity. Knoll (Knoll 2011a) provided a detailed but simple, operational distinction between simple and complex multicellularity. Complex multicellular organisms grow 3-dimensional structures or bodies, in which adhesion, and a sophisticated division of labor between cells takes place and the shape and size of the organism is determined by a genetically encoded developmental program. A key trait of complex multicellularity is that not all cells are in direct contact with the environment, necessitating cellular or intercellular mechanisms for transporting oxygen and nutrients to inner cells of the organism. Complex organisms have evolved circulatory and respiratory structures to circumvent this obstacle, a trait that is not seen in simple cell aggregations or filaments.

This chapter focuses on convergent events in multicellular evolution in fungi. Fungi represent one of the most diverse multicellular lineages, with approximately 130,000 described and potentially several times more undescribed species (Willis 2018). The vast majority of fungi are multicellular through most of their life cycle, whereas a minority of species, in particular, early-diverging fungi, are unicellular, similar to related opisthokont protists. Multicellular fungi form tubular, elongate filaments, called hyphae, which grow

apically and branch to form an intricate filamentous thallus. Yeasts, although mostly considered as unicellular, secondarily evolved reduced complexity and are capable of switching to diverse multicellular behaviours (biofilms, hyphae) in response to diverse environmental cues or at given time points in their life cycle. Transitions in levels of complexity show a great deal of convergence in fungi, which is not commonly seen in other frequently studied organisms. Here, we discuss evidence for potential convergent transitions in cellularity level, with a particular focus on complex multicellularity.

14.2 Multicellularity in fungi

14.2.1 The driving force for the evolution of multicellularity in fungi

Multicellularity in fungi differs from that of other lineages in many respects, which is probably a result of the combination of fungi's unique growth mode via hyphae and the unique selection pressures that drove their evolution. As sessile heterotrophic organisms, fungi forage for nutrients by apically growing hyphae, which extend at their apex in response to various chemical cues. It has been speculated that the rigid cell wall, which evolved in early, unicellular fungal ancestors, constrained the trajectory of multicellular evolution (Kiss et al. 2019; Nagy et al. 2020). The cell wall evolved in early fungal ancestors may have offered protection to the cells, but rendered vegetative, feeding cells of early fungi sessile (note that motile cell types in such taxa are restricted to sexual reproduction), which perhaps allowed for efficient feeding in aquatic habitats, where most unicellular fungi live. However, the transition to terrestrial habitats, where nutrient sources are patchy, probably required a strategy that allowed for efficient foraging across larger distances. Under such circumstances and with a rigid cell wall, an apically growing, filamentous thallus might have been the most, or only, optimal solution for fungi, which may explain the evolution of hyphae. Thus, the cell wall and patchy terrestrial nutrient sources together may have led to the evolution of hyphae in fungi (Kiss et al. 2019; Heaton et al. 2020). Of note, very similar tubular hyphae evolved convergently in the Oomycota (Stramenopila), a group that shares a heterotrophic, sessile nature, the decomposition of solid food sources and uptake of nutrients by osmotrophy as well as a cell wall with fungi (Diéguez-Uribeondo et al. 2004; Money et al. 2004).

Compared to the hyphae of extant, early-diverging fungi (e.g. Mucoromycota), early fungal hyphae were likely compartmentalized syncytia, in which the flow of nuclei and organelles were little regulated (Spatafora et al. 2016). Compartmentalization is achieved by cross-walls, called septa, that range from incomplete in early-diverging fungi to highly structured closures between neighboring cells (Jedd 2011; Riquelme et al. 2018). Septation regulates the flow of cytoplasm, organelles and nuclei. Hyphal growth and septation also has a bearing on how intra-organismal conflict is handled in fungi. Conflict naturally arises in the evolution of multicellularity, due to unicells having to align individual behaviours in the interest of a higher, organism-level fitness level (Michod and Roze 2001; Ratcliff et al. 2012; Rainey and Monte 2014). In contrast to most multicellular lineages, that evolved via colonial intermediates (e.g. animals; Sebé-Pedrós et al. 2017, Chapter 13), in fungi multicellular hyphae evolved by what Niklas referred to as a 'direct' route, from siphonous tubular to multicellular (Niklas 2014), which required that fungi handled conflict also in a different way (discussed in Nagy et al. 2020 and Kiss et al. 2019).

14.2.2 Simple versus complex multicellularity in fungi

In terms of complexity, hyphal growth resembles other lineages in which the predominant multicellular forms are filamentous (e.g. algae, cyanobacteria) or colonial (e.g.

choanoflagellates). This is often referred to as simple multicellularity, which is defined as the level of organization in which all cells are in contact with the environment and cell-to-cell differentiation is limited (Knoll 2011b; Nagy et al. 2018; Kiss et al. 2019). Fungi also evolved complex multicellular structures, however, these evolved significantly later than hyphal multicellularity (Kiss et al. 2019). Complex multicellularity in fungi is most frequently discussed in the context of sexual fruiting bodies, structures that aid the protection, development, and dispersal of sexual spores (Fig. 14.1). Fruiting bodies are 3-dimensional structures whose development follows a genetically encoded program and results in species-specific shapes and sizes. Fruiting bodies come in several shapes and sizes, from simple, crust-like morphologies to the most complex mushroom shapes known in fungi (Figure 1/d,f). The evolution of fruiting body morphologies follows some well-known trends. For example, Basidiomycota fruiting bodies evolved from crust-like forms towards the typical 'toadstool' morphology, i.e. those with cap, stipe and gills (Varga et al. 2019). In the Ascomycota, fruiting bodies evolved from open types, which bear the spore-producing surface on the upper side open to the environment (called apothecium-type), towards closed forms in which spore-producing cells (asci) develop internally and shoot spores into the air through various pores/channels (called perithecium-type; Liu and Hall 2004).

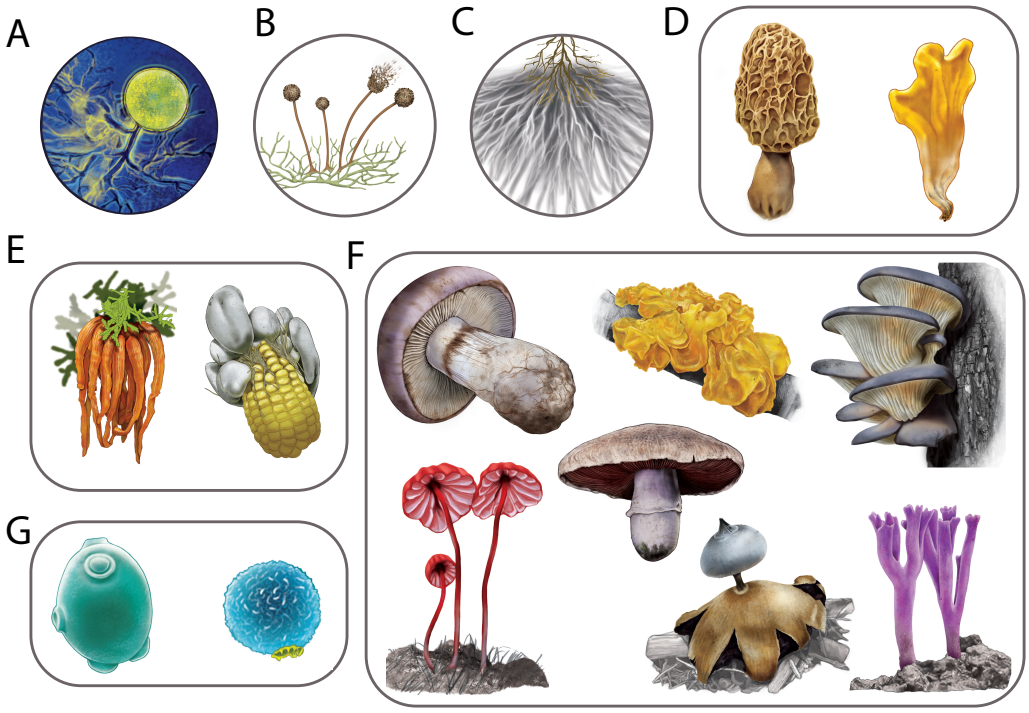

Figure 14.1. Example representatives of complexity levels in fungi. Fungi produce a plethora of different structures that are plesiomorphically unicellular (a, *Spizellomyces punctatus*), simple multicellular (b: hyphal colony with conidiophores; c: hyphal network), complex multicellular (d: a morel fungus, *Morchella* and *Neolecta irregularis*; e: fruiting bodies (*Gymnosporangium* sp.) and galls (*Ustilago maydis*) of complex multicellular rust and smut fungi, respectively; f: representatives of mushroom-forming fungi, Agaricomycotina left to right, top to bottom: *Cortinarius sp, Tremella mesenterica, Pleurotus ostreatus, Agaricus bisporus, Mycena sp, Geastrum sp, Clavulinopsis helvola*) and secondarily unicellular yeasts (g: *Saccharomyces cerevisiae* and *Cryptococcus neoformans*).

It should be noted, however, that a large number of other complex multicellular structures also evolved in fungi, these include sclerotia, ectomycorrhizae or asexual fruiting bodies, to name a few (Nagy et al. 2018). It is important to note that growth remains hyphal even in fruiting bodies, but cell shape is modified, in some cases to an extent that resembles isodiametric (polyhedral) cell morphologies (Lord and Read 2011).

14.2.3 Blurred lines between simple and complex multicellularity in fungi

Whereas the distinction between simple and complex multicellularity is relatively straightforward in most lineages (Knoll 2011b), the diversity of fungal morphologies often blurs lines between these categories. Fungi evolved a range of forms that lie intermediate between simple and complex multicellularity and which has also led to confusion about the use of these terms in the mycological literature (see Nagy et al 2020 for more discussion). For example, asexual spores are produced on specialized structures, called conidiophores (Fig 1b), that grow out of vegetative mycelia and are made up of individual cells that are in contact with the environment but follow a genetically determined developmental program and grow determinately. Thus, while conidiophores are not real 3-dimensional structures, they do show several attributes of complex multicellularity as defined by Knoll (2011).

Similar, but less well-known cases can be encountered in sexual reproductive structures also. In both the Asco- and Basidiomycota, early-diverging species produce sporogenous cells called asci and basidia, respectively, on the bare surface of the substrate, without an enclosing fleshy fruiting body. Asci and basidia are sexual sporangia and resemble conidiophores, in that their shapes and sizes are species-specific and genetically encoded and, in the simplest cases, they grow naked on the substrate. It is conceivable that such ‘naked’ asci and basidia represent the ancestral condition in the Asco- and Basidiomycota, respectively (Hibbett 2004; Varga et al. 2019), although reductive evolution has not been completely ruled out in many of the cases (Wynns 2015). Nevertheless, a gradient in complexity levels can be found in early-diverging Asco- and Basidiomycota, with a gradual thickening of hyphal layers that eventually enclose asci and basidia into a thick, protective tissue that supports spore production. These evolutionary processes might explain some of the pervasiveness of convergence fungal fruiting bodies (see below).

14.2.4 Reduction of multicellularity in fungi

Along with the emergence of multicellularity, fungi display repeated reductions in complexity level (Fig. 14.2). The best-known examples are yeasts, which are secondarily simplified organisms that spend much of their life cycle in a unicellular form. This is a remarkable case of reduced complexity, given that most lineages seem to evolve towards increased complexity (O’Malley et al. 2016) or that most research attention is directed towards that. Losses of multicellularity are rare across the tree of life (but seen in cancerous cells; Chen et al. 2015), especially in lineages, like fungi, which transitioned to stable multicellular organization hundreds of millions of years ago (losses were less surprising in lineages that show transient or primordial forms of multicellularity). The loss of multicellularity also may seem surprising given that it is considered to offer selective advantages to the organisms in diverse environments, for example, through increased cell size which helps to avoid predation (Rokas 2008). How can we then explain the unicellularity of yeasts?

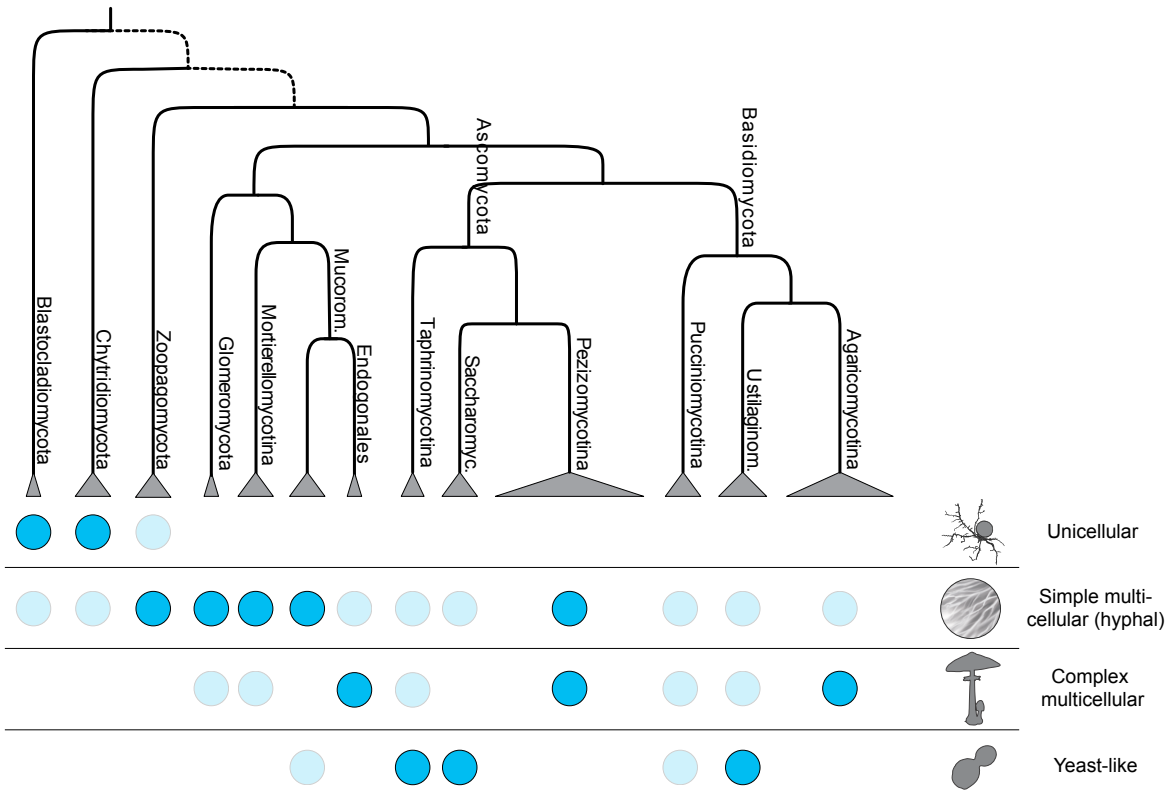

Figure 14.2. The diversity and patchiness of complexity levels in fungi. A schematic representation of the fungal tree is shown with main complex multicellular and yeast-like clades highlighted. The phylogenetic distribution of unicellular, simple and complex multicellular as well as yeast-like fungi is shown on the bottom panel.

The term yeasts refers to a polyphyletic assemblage of fungi that are observed primarily as unicells (Nagy et al. 2017) and that evolved convergently from more complex, hyphal ancestors. The best-known yeasts are those found in the Saccharomycotina subphylum, such as baker's yeast *Saccharomyces cerevisiae* or *Candida* spp, which are causative agents of often life-threatening mycoses. Another widely known yeast is the fission yeast *Schizosaccharomyces pombe* (Taphrinomycotina subphylum), which is best known for its role as a model organism. However, there are several other yeast-like lineages in fungi as well (JW et al. 2017). The term yeast-like fungus is used in this review for species that spend the majority of their life cycle as walled unicells that feed by osmotrophy and divide by budding or fission. The Basidiomycota comprises three yeast-like lineages, the class Tremellomycetes, which includes the opportunistic pathogen *Cryptococcus neoformans*, the Ustilaginomycotina subphylum, which includes plant pathogenic smuts (e.g. *Ustilago maydis*) and the Pucciniomycotina, which comprises several groups rust species and red yeasts (e.g. *Rhodotorula*). Fungi further include several dimorphic fungi, which, by definition are able to switch between unicellular and multicellular morphologies (Boyce and Andrianopoulos 2015).

To understand how surprising the reduction of multicellularity in yeasts is, it is important to reiterate that for fungi, the selective advantage of multicellularity might have been different, perhaps to move from liquid niches to terrestrial habitats. In view of this, if we examine the niche that yeasts fill, we find that they share properties with aquatic niches of plesiomorphically unicellular fungi. Yeast-like fungi are often found in liquid niches, such as, for example, nectars, insect guts, or various external or internal surfaces of mammals,

including humans. Simple sugars are readily available in these niches, which makes active foraging for spatially heterogeneous nutrients dispensable. It is, thus, not surprising that yeasts produce unicellular morphologies that divide mitotically either by budding or fission. A widely held view assumes that, collaterally, the ability to form multicellular hyphae has also been lost in yeast species. In support of this hypothesis, yeasts experienced rampant gene loss during their evolution (Nagy et al. 2014) and recent analyses of yeast genomes suggested a complex ancestor of the budding yeast lineage (Saccharomycotina; Shen et al. 2018). To test this hypothesis, we tested whether the massive gene loss event that 5 independent yeast-like lineages experienced in their evolution affected hypha morphogenesis genes more, less, or to a similar extent than it did metabolic and other gene functions (Kiss et al. 2019). Surprisingly, genes related to hyphal growth were significantly depleted among lost genes, suggesting that such genes are preferentially retained by yeasts despite having lost ~3-5,000 genes since their last common ancestors. This argues against the presumed losses, or reductions in multicellularity and, rather, reinforces the view that multicellularity, once acquired, is maintained through evolution.

It is important to note that yeasts display a number of multicellular traits, such as communication between cells (e.g. via quorum sensing), synchronization of cellular behaviours or cell differentiation. Most yeast species are capable of producing (reduced) hyphae and they can switch between unicellular and hyphal growth in response to environmental stimuli. For example, opportunistic pathogen *Candida albicans*, which is a commensal resident of the mucosal and genital surfaces in healthy human individuals, can form true hyphae and invade tissues upon changing environmental or host conditions. This, again, underscores the finding that yeasts, despite their most commonly seen morphology, retained multicellular growth form in their evolution.

14.2.5 Complex multicellular fungi are phylogenetically scattered

Complex multicellularity represents the highest level of morphological organization that evolved across the tree of life. It refers to organisms or structures in which cells are tightly packed, show extensive morphological and functional differentiation and are organized into higher-level functional units, such as tissues or organs (Knoll 2011b). In contrast to simple multicellularity, which evolved in >25 lineages (Grosberg and Strathmann 2007), complex multicellularity is limited to 5 major groups: metazoans, green plants, brown and red algae as well as fungi. Species in these groups dominate the visible world and have evolved to fill diverse niches on Earth. Whereas research on multicellular behaviours of metazoans and green plants has deep roots and goes back to over a century, our knowledge on complex multicellularity and how it evolved in fungi is more limited. For example, while cell type diversity is well-understood in several animal and plant species, and current research aims to refine cell type definitions and classifications (e.g. by single-cell transcriptomics), our estimates of cell-type diversity in complex multicellular fungi are based on the counting of morphologically distinct cellular morphologies. Even using such simple approaches, up to 28 - 30 morphologically distinct cell types were recognized in sexual fruiting bodies of fungi (Bistis et al. 2003; Lord and Read 2011; Kües and Navarro-González 2015), but this number is expected to rise significantly as single-cell approaches get deployed for complex multicellularity fungi.

Complex multicellularity is rare across the tree of life; this accords well with the hypothesis that lineages need to overcome a significant number of genetic obstacles to evolve it (Knoll 2011b). Complex multicellular fungi are not monophyletic, rather, they display a patchy phylogenetic distribution. The best-known complex multicellularity fungi are found in

the Agarico- and Pezizomycetes, which belong to the Basidio- and Ascomycota respectively and are separated by at least 600 million years from each other (Kohler et al. 2015). These include the best-known mushroom-forming fungi, such as truffles and morels in the Pezizomycotina and agarics, puffballs, bracket fungi, among others, in the Agaricomycotina (Figure 1/f). However, a number of other complex multicellular lineages are also found in fungi, some of which comprise a single genus with less than a handful of species (*Neolecta*, Taphrinomycotina or *Modicella*, Mortierellomycotina, see Fig. 14.1/d), whereas others are highly diverse. Estimates for the species diversity and age of these lineages range from 2 to over 32,000 species and from very recent origins to clades that are >500 million years old (Nagy et al. 2018). Altogether, we identified 8-11 clades of complex multicellular clades across fungi, although their number may change slightly in the future as some phylogenetic uncertainties in the fungal tree of life get resolved. These clades are separated from each other by simple multicellular and facultatively unicellular, yeast-like lineages. This scattered nature of complex multicellular fungal lineages suggests an intricate evolutionary history either with multiple origins or with a single origin and several losses.

14.2.6 Fungi offer an unparalleled model system for studying complex multicellularity

Given the disparate occurrence of complex multicellularity in fungi, we were interested in what genetic mechanisms might underlie its evolution. To this end, we used developmental transcriptomes - gene expression data that can reveal multicellularity-related genes in fungi. Fungi offer a great model system to study transitions in multicellularity because they undergo a transition from simple to complex multicellularity as part of their life cycle. The vegetative mycelium, which is an underground fungal network of filaments that is adapted to explore the substrate and assimilate nutrients, is simple multicellular, whereas sexual fruiting bodies, which enclose developing spores into a resistant, reproductive organ, are complex multicellular (Fig. 14.1/a-f). Because the transition from simple to complex multicellularity happens within the same, extant fungal species, it allows us to assay what genes become activated upon the onset of the complex multicellular stage of the life cycle, offering a window into the genetic bases of complex multicellularity.

Fruiting body formation can be induced under laboratory conditions by changes in environmental variables (nutrient availability, light). Accompanying changes in gene expression can be assayed real-time for example, by RNA-Seq, providing an unparalleled model system not available for studying complex multicellularity in other lineages. This is not available in animal or plant model systems that evolved complex multicellularity once and exist in that state throughout their life cycle (except for a unicellular bottleneck in gametes) and therefore, research on the evolution of their multicellularity has to rely on looking into the past, using comparative genomic techniques (Sebé-Pedrós et al. 2017). The existence of facultative multicellular developmental stages in fungi is surprisingly similar to that seen in aggregative multicellular amoebozoans, such as the slime mold *Dictyostelium*, which produces very simple fruiting bodies and has been subject to multicellularity research for decades (Eichinger et al. 2005; Du et al. 2015; Glöckner et al. 2016). Notably, both fungi and slime molds respond to starvation and use conserved cAMP signal transduction pathways during this process. However, this probably reflects conserved genetic circuits underlying sexual reproduction - which is induced when nutrients become limiting - rather than homologies among fungal and slime mold fruiting bodies, given the clearly independent origins of the latter.

Researchers have taken advantage of the ability of fungi to produce fruiting bodies and used high-throughput -omics techniques in both the Asco- and Basidiomycota to unravel

the genes that get activated or downregulated upon the start of complex multicellular development. For this, RNA-Seq offers a suitable approach (Nowrousian 2018), which, combined with the ever increasing number of sequenced fungal genomes (Grigoriev et al. 2014) provides a window into how complex multicellular development takes place in fungi. RNA-Seq data on sexual fruiting bodies started to accumulate from the two largest complex multicellular clades. In the Ascomycota, some classic model systems of sexual fruiting body development, for which RNA-Seq data have been published, include *Neurospora crassa* (Lehr et al. 2014; Wang et al. 2014; Trail et al. 2017)*, Aspergillus nidulans* (Han 2009)*, Sordaria macrospora* (Nowrousian et al. 2010; Teichert et al. 2020) along with more recently emerging models such as *Pyronema confluens* (Traeger et al. 2013)*, Fusarium* spp (Kim et al. 2019) or *Botrytis cinerea* (Rodenburg et al. 2018)*.* In the Agaricomycotina, the most researched model species are *Coprinopsis cinerea* (Kues 2000; Stajich et al. 2010; Krizsan et al. 2019) and *Schizophyllum commune* (Ohm et al. 2010), with fruiting body transcriptomes available for both (Ohm et al. 2011; Muraguchi et al. 2015; Almási et al. 2019; Krizsan et al. 2019). Fruiting body development of a number of other Agaricomycotina species has also been examined, including that of *Lentinula edodes* (Sakamoto et al. 2017), *Flammulina velutipes* (Park et al. 2014), among others, to a large extent motivated by interest in informing rational strain development for commercially produced mushroom species.

14.3 The genetic bases of transitions to complex multicellularity

14.3.1 Transitions to complex multicellularity may not be so hard for fungi

It has been hypothesised that complex multicellularity is rare across the tree of life because organisms have to overcome a significant number of genetic/physiological obstacles to evolve it (Knoll 2011b). This is in line with complex multicellularity being limited to animals, plants, green and brown algae, as well as fungi. However, it appears that in fungi, a large number of independent complex multicellular clades exist, raising questions on convergent evolution and on to how complicated it might be for fungi to evolve complex multicellularity.

A particularly relevant group of organisms for examining this question is the genus *Neolecta*, which contains species that form yellow, tongue-like fruiting bodies in temperate forests. Phylogenetically, this genus is nested in the Taphrinomycotina, which primarily contains simplified, yeast-like or filamentous fungi, such as the fission yeast *Schizosaccharomyces pombe*. The genome of *Neolecta irregularis* has recently been published (Nguyen et al. 2017). This has revealed that its genome contains ~5,500 genes, which is an unusually low gene count for a complex multicellular fungus (Nagy 2017). Most filamentous and complex multicellular fungal genomes encode on average 12,000-15,000 protein coding genes, whereas yeasts and yeast-like fungi have small, highly streamlined genomes, containing 5,000 to 7,000 protein coding genes. Further evidence for the similarity between *Neolecta* and yeast genomes is that yeast genomes and that of *Neolecta* evolved predominantly by gene loss and are depleted in introns (Nguyen et al. 2017), whereas genomes of complex multicellular fungi did not experience massive gene loss events, are rich in intron/exon boundaries and are known to be alternatively spliced (Marshall et al. 2013; Gehrmann et al. 2016).

The finding that *Neolecta* is able to produce complex multicellular fruiting bodies suggested, first, that the gene count difference between yeast-like and complex multicellular fungi may not be relevant to differences in complexity level, (ii) that complex multicellularity can exist in organisms with highly streamlined, small genomes, and (iii) that, in terms of

protein coding capacity, it does not require a lot more to build complex multicellular fruiting bodies than to build hyphae or yeast cells. A broader look across complex multicellular fungi revealed that there are several complex multicellular clades with reduced genomes, particularly in the Basidiomycota (Nagy 2017). Several species in the reduced-genome Puccinio- and Ustilaginomycotina, as well as the Tremellomycetes produce complex multicellular fruiting bodies. For example, the golden jelly fungus (*Tremella mesenterica*), which forms gelatinous, yellow fruiting bodies on dead branches of trees, possesses ca. 8,000 genes and, like *Neolecta*, is a close relative of morphologically secondarily simplified yeast like fungi, such as *Cryptococcus neoformans*.

The streamlined genomes and close phylogenetic affinity of certain complex multicellular fungi to yeast-like species, combined with the phylogenetic patchiness of complex multicellularity in fungi suggests that complex multicellularity might be easy to evolve for fungi. Given the multiple origins model of fungal complex multicellularity, these fruiting body forming fungi must have evolved in the middle of otherwise simplified fungi that have already undergone severe genome contractions (Nagy et al. 2014). It also follows from these that the genome contractions that yeasts have experienced must have left much of the genetic toolkit required for complex multicellularity largely intact, otherwise, these complex multicellular fungi could not have evolved from within yeast-like clades. It may seem imperative to revisit, in light of these observations, the single origin model, which could more logically explain how complex multicellular fungi can be nested in secondarily simplified clades. In that scenario, complex multicellularity would be the ancestral condition, which was retained by some species (Fig. 14.3), whereas others have lost complex multicellularity (and even hyphae) and reversed to a facultatively unicellular lifestyle. However, species richness data and topologies of phylogenetic trees speak against the single origin model in both the Tremellomycetes and the Taphrinomycotina, where *Tremella* and *Neolecta* belong, respectively. Both clades are composed primarily of simple organisms, with an estimated diversity of 400 and 200 species in the Tremellomycetes and the Taphrinomycotina, of which only <100 and 3 are complex multicellular, respectively. Thus, considering trait gain/loss at higher resolution, it becomes clear that a complex multicellular ancestry in these clades would entail an excessively high number of loss events, which makes the single origin model even less likely.

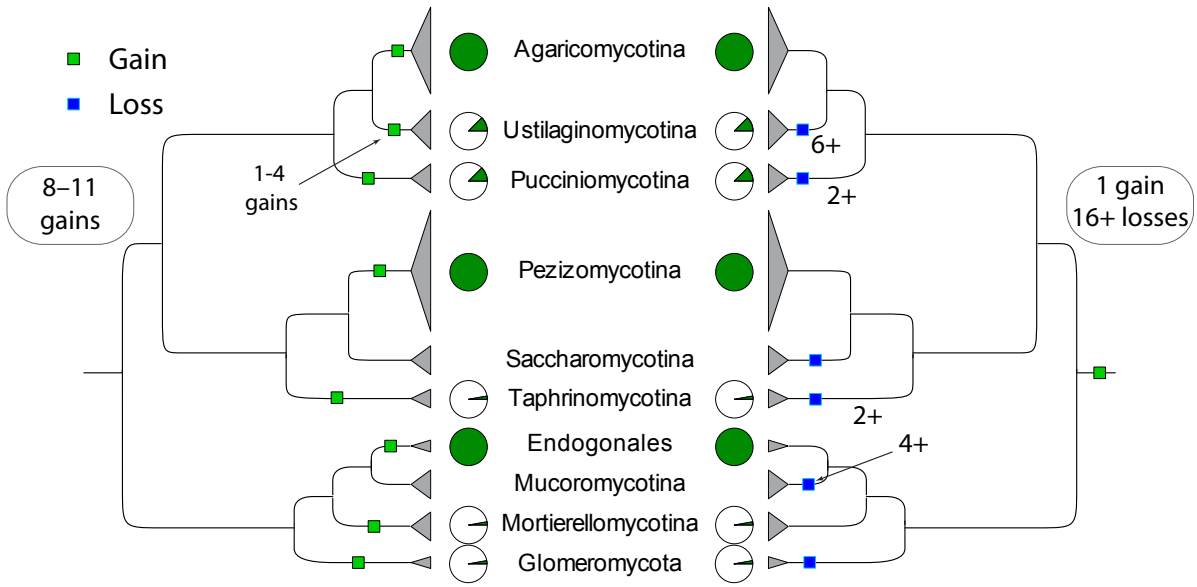

Figure 14.3. Contrasting scenarios of the multiple and single origin model describing the evolution of complex multicellularity in fungi. Acquisitions and losses of complex multicellularity under two contrasting models are shown by green and blue squares, respectively. The the multiple (convergent) origins model (left) requires 8–11 steps to explain the phylogenetic distribution of complex multicellular fungi, whereas the single origin model (right) implies a single origin and at least 16 losses. Pie charts mark clades containing complex multicellular species; solid section denotes the approximate proportion of complex multicellular species.

14.3.2 Repeated co-option of genes in complex multicellular fungi

As explained above, developmental transcriptomes offer a window into the genetic bases of the formation of complex multicellular fruiting bodies in fungi. These can be used to address questions about the origins of fruiting body formation as well. We used transcriptomes from five Asco- and four Basidiomycota species to test plausible models of the evolution of complex multicellularity in fungi (Merényi et al. 2020). More specifically, in the Ascomycota, we focused on the Pezizomycotina, whereas in the Basidiomycota we sampled species from the Agaricomycotina. These two subphyla represent the largest complex multicellular clades in fungi and include several species that can complete their life cycle under laboratory conditions.

Asco- and Basidiomycota fruiting bodies show no evidence for homology, which could be because of independent origins, or because, after a single origin, they have diverged so much that traces of homology are not detectable anymore. In line with the lack of discernible homology, ancestral character state reconstructions unequivocally supported the independent origins model, with fruiting bodies having originated independently in the most recent common ancestor of the Agaricomycotina and in that of the Pezizomycotina. However, phylogenetic methods have little power for distinguishing between potential models of trait evolution. While phylogenetic methods build on homology hypotheses at the phenotypic level, looking for homologies at the level of the genetic toolkit behind complex multicellularity might provide a more resolved view on how complex multicellularity evolved and helps the evaluation of alternative models of trait evolution.

We analyzed the extent to which developmentally regulated genes were shared among Asco- and Basidiomycota fruiting bodies. After reanalyzing published developmental transcriptome data, we identified developmentally regulated genes, i.e. genes that show considerable (in this case ≥4 fold) expression dynamics throughout fruiting body development. Given the convergent nature of fruiting bodies in these clades, we expected little overlap among developmentally regulated genes. Yet, we identified 1,026 gene families that were developmentally regulated in ≥75% of the species in either the Asco-, the Basidiomycota or both. These break down to 314, 439, and 273 families that were developmentally regulated in ≥7 of 9 species overall, ≥3 of 4 Basidiomycota, and ≥4 of 5 Ascomycota species, respectively (Fig. 14.4).

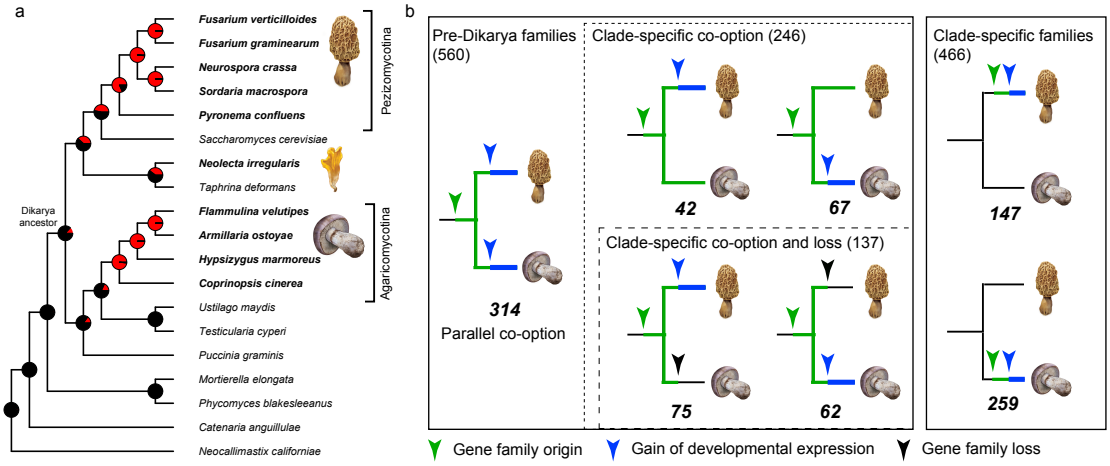

Figure 14.4. Phylogenetic relationships of complex multicellularity (a) and the fate of conserved developmentally regulated gene families (b). (a) Two independent clades of complex multicellular species are marked, and typical fruiting body morphologies are shown. Pie charts at nodes indicate the ancestral states inferred by Maximum Likelihood (proportional likelihoods of complex multicellular (red) and non-complex multicellular (black)). Complex multicellular species are shown by boldface font. (b) Developmentally regulated gene families grouped by evolutionary conservation and history. Adapted from Merenyi et al 2020(Merényi et al. 2020).

To understand the evolution of complex multicellularity at a higher resolution, we reconstructed the evolution of developmental gene families along the phylogeny (see Fig. 14.4/a). Of the 1,026 conserved developmental families, 297 and 169 families that are taxonomically restricted to the Agarico- and Pezizomycotina, respectively. On the other hand, 560 families predate the origin of complex multicellularity, indicating that they were likely co-opted for multicellularity-related functions (Fig 4/b). These ancient families were significantly more often developmentally regulated in both clades than expected by chance (314 out of 560 ancient families, $P < 10^{-4}$, permutation-test), indicating that parallel co-option of these families has been rampant. On the contrary, the frequency of clade-specific co-option was low, with only about 7.5% and 12% in the Agarico- and Pezizomycotina (42 and 67 families). The combination of limited clade-specific co-option with rampant parallel co-option suggests that genes with suitable biochemical properties were predominantly recruited into the genetic toolkit underlying fungal complex multicellularity. It is also consistent with the hypothesis that genes suitable for any given trait are rare and thus they mostly end up being recruited for convergent traits (Christin et al. 2010).

The observed evolutionary patterns of developmental gene families can be explained by two hypotheses. First, the simplest model of convergent evolution can explain gene families with clade-specific taxonomic distribution or clade-specific co-option. Under this scenario, complex multicellularity emerged independently in the Agarico- and Pezizomycotina and different genes evolved *de novo*, or were co-opted into the genetic toolkit. Similarly, shared developmentally regulated gene families could have been parallelly co-opted for complex multicellularity in the Agarico- and Pezizomycotina. However, this assumes a large number of parallel co-option events. As a more parsimonious explanation, these families could also encode functions that the Dikarya ancestor possessed and that were integrated into complex multicellular fruiting bodies of the Agarico- and Pezizomycotina as

single units or developmental modules. This scenario does not assume independent co-option events for each of those genes and is thus more parsimonious, from a purely phylogenetic perspective, than a simple model that assumes convergence at all levels. Processes related to sexual spore formation represent probable candidates for such functions: they are conserved in sporulating tissues across fungi, and fruiting bodies evolved to provide a protected environment to sporulating tissues. As a consequence, genes associated with spore formation show expression peaks within fruiting bodies and will show up among the developmentally regulated genes we identified. There are other plesiomorphic functions that may have been present in the last common ancestor of the Agarico- and Pezizomycotina, such as adhesion or defense-related gene families that may have proven useful for complex multicellularity, explaining their parallel co-option.

14.3.3 Plausible genetic models for phylogenetically patchy traits

Complex multicellularity in fungi is a typical phylogenetically patchy trait: it occurs in disparate clades across the tree, yet certain components of its genetic bases show homologies across independent occurrences. Such traits are not uncommon in biology. For example, the formation of nodules that harbor nitrogen-fixing bacteria occurs in several clades of leguminous plants and its phylogenetic distribution suggests convergent origins. Yet, nodule development shares a surprisingly high fraction of its genetic components across clades (van Velzen et al. 2018). Griesmann et al addressed the origins of root nodule symbiosis with nitrogen-fixing bacteria using a broad sampling of plant genomes (Griesmann et al. 2018) and found that, although the NODULE INCEPTION (NIN) gene, which is a key regulator of nodule development, was shared across a wide phylogenetic spectrum of plants and was lost repeatedly in multiple non-nodulating leguminous plants, its conservation can’t explain the convergent origins of nodule formation in plants.

Traits with patchy phylogenetic distributions pose a challenge for evolutionary biologists to explain. They often display homologies in terms of genetic composition, in conflict with the classic model of convergent evolution, which implies that traits arose independently via different genetic changes to non-homologous genetic precursors (Fig. 14.5). On the other hand, their patchiness across the phylogenetic tree speaks against homology because explaining their contemporary distribution on the tree would require an excessive number of gene losses to be assumed (cf. Fig. 14.3).

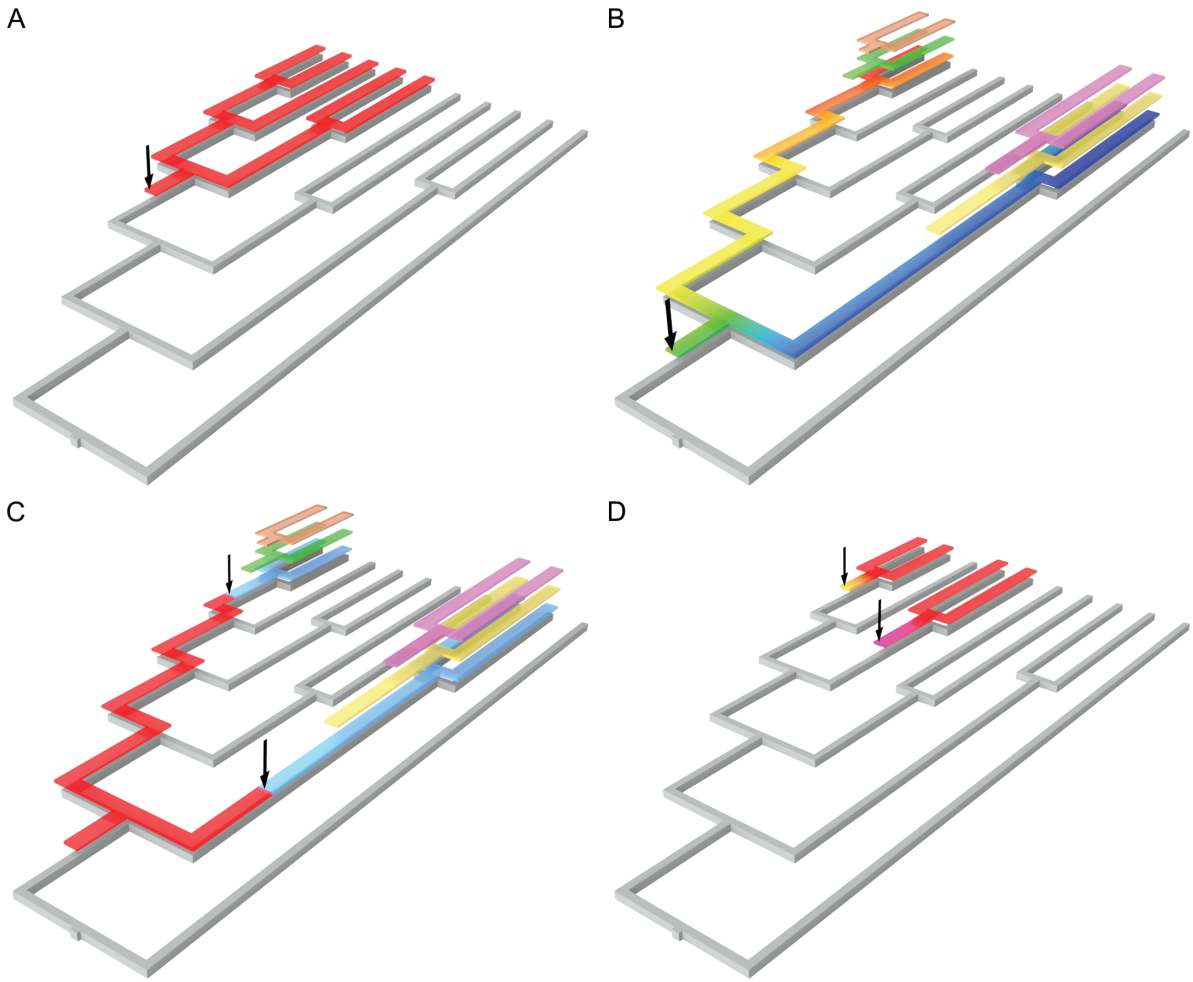

Figure 14.5. Models of trait evolution. The simplest models include a single origin followed by divergence (a). Traits share deep homology if divergence blurs similarity and common origins completely (b). Phylogenetically patchy traits can evolve convergently, building on non-homologous genetic bases (d) or via latent homologies (c) where similar function arises convergently by independent co-option of the homologous genes or genetic modules for the same, new function. Black arrows mark the origin of similar traits.

Intermediate models that incorporate elements of both homology and independent origins have been proposed recently (Fig. 14.5/b-c). The latent homology model posits that precursor traits underlie several phylogenetically patchy or convergent characters (Nagy et al. 2014; Nagy 2018). Precursors are traits that have been in place in the common ancestors of species that show the trait and were convergently modified, resulting in the repeated emergence of the patchy trait. Such conserved precursor traits can underlie the convergent emergence of new traits if they can easily be co-opted for new functions by simple genetic changes. Such changes could include, but are not limited to, tweaks to the regulation of the precursor, which results in its expression in a new context. For example, a few mutations affecting the regulation of a master regulator that orchestrates the expression of an entire developmental module should be sufficient to cause the expression of this module in a new context. Precursor traits could be developmental modules, gene regulatory circuits or other modular gene assemblages, among others, that are mutationally accessible for being deployed for a new function. Thus, latent homologies can reduce the mutational target size for evolution, by providing ready access for evolution to new cellular outputs via a few

mutations. This increases the likelihood of convergence and makes convergent scenarios more parsimonious than if we had to assume convergent or parallel mutations to each of the member genes in that module. Examples where a genetic module is only a few mutations away from being expressed in a new context are abundant in the literature, and there is a documented history of such traits in developmental biology (Carroll 2008); however, how frequently such cases underlie convergent traits has not been explored yet. This way, latent homologies can increase the likelihood of convergent evolution without assuming highly unparsimonious evolutionary scenarios.

The latent homology model was first proposed to explain the convergent evolution of yeasts and yeast-like fungi (Nagy et al. 2014). As explained above, yeast-like fungi are secondarily and facultatively unicellular organisms that spend much of their life cycle in a unicellular form. They emerged independently in fungi several times and include some of the most important human pathogens. They evolved presumably as adaptations to liquid niches, such as nectars, insect intestinal tracts, or other habitats rich in simple sugars. Based on the phylogenetically widespread occurrence of yeast-like fungi, we suggested that some part of the genetic toolkit for yeast-like growth, the putative precursor trait, is ancient and conserved in fungi. In support of this hypothesis, we reconstructed convergent changes in transcription factor families along stem branches leading to yeast-like clades (Nagy et al 2014). Although the identity of the precursor trait remains unknown, the latent homology model can explain how the yeast morphology may have evolved repeatedly at various phylogenetic depths and clades and why the morphology of yeast-like fungi show surprisingly high similarity across clades.

In light of these considerations, it is imperative to examine if precursor traits might underlie the convergent evolution of complex multicellularity as well in fungi. The presence of such precursor traits in non-complex multicellular ancestors would have predisposed lineages for evolving complex multicellularity by providing stepping stones for evolution, leading to a higher likelihood of phenotypic convergence and explaining why complex multicellularity is so common across fungi. To understand if any functions and which might have served as precursors to complex multicellularity, we looked at the genetic makeup of the last common ancestor of the Agarico- and Pezizomycotina. Although this ancestral species most likely did not have fruiting bodies, we reasoned that its ancestral genome composition could shed light on whether predisposition by precursor traits provides a plausible hypothesis for the evolution of complex multicellularity in fungi. We reconstructed 989 genes in the 314 shared developmental families (Fig. 14.4) of the Dikarya ancestor, which showed an enrichment of genes related to several multicellular and developmental functions of fungi (see Merényi et al. 2020), reminiscent of general functions required for fungal development. These include gene regulatory circuits related to sexual reproduction, mating partner recognition, light, nutrient and starvation sensing, fungal cell wall synthesis and modification, cell-to-cell signaling, and morphogenesis. It also includes genetic circuits related to adhesion and cell-differentiation-related genes that are used by non-complex multicellular fungi for pathogenicity or asexual reproduction, although the hypothesis that these were precursors to adhesion and cell differentiation in complex multicellular fruiting bodies remains challenging to test experimentally.

Taken together, the patchy phylogenetic distribution of complex multicellularity makes the fungal kingdom an oddball in the history of the evolution of multicellularity. This patchiness manifests in morphologically diverse fruiting bodies and other structures and led to the formulation of a range of genetic and evolutionary hypotheses. Addressing these from a mechanistic standpoint will require further research and the integration of suitable model systems with genetic, evo-devo and -omics techniques, all of which, fortunately, are at the

disposal of fungal evolutionary biologists and just need to be combined in effective ways to understand one (or many?) of the most spectacular transitions in evolution.

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