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REVIEW

Evolutionary Morphogenesis of Sexual Fruiting Bodies in Basidiomycota: Toward a New Evo-Devo Synthesis

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SUMMARY The development of sexual fruiting bodies is one of the most complex morphogenetic processes in fungi. Mycologists have long been fascinated by the morphological and developmental diversity of fruiting bodies; however, evolutionary developmental biology of fungi still lags significantly behind that of animals or plants. Here, we summarize the current state of knowledge on fruiting bodies of mushroom-forming Basidiomycota, focusing on phylogenetic and developmental biology. Phylogenetic approaches have revealed a complex history of morphological transformations and convergence in fruiting body morphologies. Frequent transformations and convergence is characteristic of fruiting bodies in contrast to animals or plants, where main body plans are highly conserved. At the same time, insights into the genetic bases of fruiting body development have been achieved using forward and reverse genetic approaches in selected model systems. Phylogenetic and developmental studies of fruiting bodies have each yielded major advances, but they have produced largely disjunct bodies of knowledge. An integrative approach, combining

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phylogenetic, developmental, and functional biology, is needed to achieve a true fungal evolutionary developmental biology (evo-devo) synthesis for fungal fruiting bodies.

KEYWORDS Basidiomycota, evo-devo, fruiting body, mating, morphogenesis, phylogenetics

INTRODUCTION

Complex multicellular forms have evolved in just a few eukaryotic clades, including plants, animals, red and brown algae, and fungi. In most of these groups, the complex multicellular entity is a reproducing individual that performs multiple functions, such as acquisition of nutrients (by ingestion, absorption, or photosynthesis), mating, and movement. Complex forms in fungi make up only a part of the individual and have more limited functions. For example, rhizomorphs and hyphal cords act as foraging structures and sclerotia store nutrients. The most spectacular forms in fungi are fruiting bodies (also called sporocarps, basidiomata, ascomata, or mushrooms). Fruiting bodies are probably some of the most emblematic structures fungi produce, but they all have the same function: to produce and disseminate spores (mostly meiospores).

Fruiting bodies manifest an astonishing diversity of phenotypes. Size variation encompasses at least 4 orders of magnitudes ranging from cleistothecia of Ascomycota (on the order of 100 μm), to massive bracket fungi (e.g., *Phellinus ellipsoideus*), to agarics (e.g., *Termitomyces titanicus*), and to giant puffballs (*Langermannia gigantea*), which can measure up to a meter across (see Fig. 1). This large diversity recalls Charles Darwin's poetic vision of "endless forms most beautiful." However, the vast majority of species assume a limited number of canonical forms (e.g., pileate-stipitate forms, crust fungi, puffballs, coral fungi, etc.) and appear to reflect certain functional, phylogenetic, developmental, or genetic principles, which will be the topics of this review.

In contrast to Darwin's vision, variation in fungal fruiting bodies is not endless or continuous. Rather, fruiting body evolution appears to be guided by internal or external constraints that have driven the recurrent evolution of similar forms, that is, yielded a limited array of morphologies (1). Fruiting bodies are anatomically simple and display developmental plasticity, with major morphological transformations resulting from variation in environmental variables such as light and CO_2 concentration. Fruiting body evolution in fungi is marked by extensive convergence and lineages have shifted among a limited number of semidiscrete forms. At the same time, fossil mushrooms in amber show that some forms have been conserved for tens of millions of years (2–4). This apparent paradox—simplicity and plasticity on one hand versus evolutionary conservation and convergence on the other—suggests that natural selection plays a strong role in shaping phenotypes.

One of the grand challenges of mycology is to understand how the diversity of fruiting bodies has evolved. A complete solution to this problem should address four dimensions of fruiting body evolution: (i) historical patterns, (ii) developmental mechanisms, (iii) genetic bases, and (iv) modes of natural selection. Accordingly, mycologists have taken four major approaches to studies of fruiting bodies: (i) phylogenetics; (ii) developmental biology, including morphological and anatomical studies; (iii) genetics and genomics; and (iv) functional biology and biomechanics. Possibly for historical reasons, some of these approaches achieved different levels of progress in different fungal clades. In the Agaricomycotina, tremendous progress has been made toward understanding phylogenetic patterns, whereas scholars focused intensely on the genetics of fruiting body development in the Ascomycota, where well-established laboratory models (e.g., *Aspergillus nidulans* and *Sordaria macrospora*) are available. On their own, none of these approaches are, however, wholly satisfactory; phylogenetic methods suggest general trends in natural selection but do not identify their mechanisms, developmental or genetic studies provide in-depth mechanistic information in single species but are hard to extrapolate to phylogenetic scales, and functional studies in model systems generally do not measure fitness or demonstrate differences in performance across species.

Fungal evolutionary developmental biology (evo-devo) draws on disciplines that proliferated at various points throughout the 20th and 21st centuries. From the mid-20th

FIG 1 Diversity of fruiting body morphologies in the Basidiomycota. Examples of fruiting body morphologies are shown to illustrate the diversity of forms across the Agaricomycetes. (A to C) resupinate fruiting bodies (A, *Cylindrobasidium evolvens*; B, *Xylobolus frustulatus*; C, *Antrodia malicola*); (D and E) pileate-sessile forms (D, *Trametes versicolor*; E, *Piptoporus quercinus*); (F) cyphelloid fruiting body of *Schizophyllum commune*; (G and H) clavarioid/coralloid fruiting bodies (G, *Clavaria rosea*; H, *Clavicorona pyxidata*); (I to K) pileate-stipitate fruiting bodies (I, *Coprinopsis cortinata*; J, *Hygrocybe splendidissima*; K, *Conocybe antracophila*); (L to N) gasteroid fruiting bodies (L, *Scleroderma citrinum*; M, *Geastrum saccatum*; N, *Rhizopogon* sp.). (Photographs by L. G. Nagy.)

century on, fungal morphologists studied fruiting body growth in detail, resulting in a rich terminology for developmental forms (5–7). Some taxonomists formulated explicit hypotheses of fruiting body evolution, such as the secotioid syndrome of Harry Thiers (8) or the "*Clavaria* theory" of E. J. H. Corner (9) (see Glossary), but it was not until the explosion of molecular phylogenetics in the 1990s that tools became available to formally put these hypotheses to the test (reviewed in reference 10). From the first days of molecular phylogenetics, fungal systematists sought to reconstruct historical patterns of fruiting body evolution (see, for example, references 11 and 12). During the last 2 decades, molecular phylogenies revealed transitions between fruiting body morphologies in numerous individual clades. Recently, studies in the Agaricomycotina have used "megaphylogenies" with thousands of species that have documented broad patterns of morphological evolution (13, 14).

During the latter part of the 20th century, several scholars investigated the genetics and cell biology of fruiting body development, focusing on model systems such as

Coprinopsis cinerea, resulting in detailed premolecular views on fruiting body development (see reference 15 for a summary). With the advent of reverse genetics in filamentous fungi, attention shifted toward identifying developmental genes in a number of model systems. Through genetic studies in model systems, progress has been made toward understanding certain developmental phenomena, such as initiation of fruiting body development, the regulation of pileus expansion, or stipe elongation, among others (see, for example, references 16–20). Recently, the proliferation of genomic and transcriptomic data combined with robust phylogenies is beginning to permit comparative phylogenetic analyses of developmental genetic mechanisms (see, for example, references 21–26).

Another approach mycologists have taken to understand how natural selection has shaped fruiting body evolution involves studies of functional morphology. For example, the pioneering mycologists C. T. Ingold and Reginald Buller initiated studies on the biomechanics of spore dispersal (27, 28) which have continued with the application of techniques such as ultrahigh-speed video microscopy (29). Recently, biophysical analyses of forcible spore discharge have suggested a relationship between the size of spores and the "Buller's drop" (see Glossary) and gill spacing (30).

In spite of these advances, fungal evo-devo has yet to elucidate some of the most basic questions, such as the developmental bases and evolutionary origins of fruiting bodies, the mechanisms of morphological patterning, or the causes of cell type differentiation.

Fungal evo-devo is basic science, but it has potential significance for practical applications. Fungal fruiting bodies are increasingly important as sources of sustainable foods (including meat surrogates), and their use as medicinal compounds is gaining momentum. For example, hallucinogenic compounds produced by *Psilocybe* mushrooms show very promising effects in the therapy of major depressive disorders (31), whereas fruiting bodies of *Boletus edulis* have been found to be the richest dietary source of antioxidants (32). Edible and medicinal mushrooms form the basis of a multi-billion-dollar global industry that is growing rapidly. Great advances have been made in the industrial production of key species (33). Nevertheless, the industry has not experienced the sort of advances that characterized crop development during the green revolution or the domestication of animals; cultivated mushrooms remain similar to their wild counterparts. An enhanced understanding of the mechanisms of fruiting body development could have a transformative impact on diverse emerging technologies.

In this review, we assess the state of knowledge regarding genetic and developmental mechanisms, phylogenetic patterns and macroevolutionary trends of fruiting bodies in mushroom-forming fungi. We focus on Agaricomycotina, but we make comparisons to Ascomycota and Mucoromycota where appropriate. We note that research in the developmental genetics of Ascomycota fruiting bodies has made significantly more progress in recent years than that for Basidiomycota fruiting bodies, and we refer the reader to recent reviews on the topic (34, 35). We first place fungal fruiting bodies in the context of research on (complex) multicellularity and then discuss recent progress on understanding phylogenetic patterns and hypotheses gleaned from examining phylogenies across the Agaricomycotina. In the second half of this review, we introduce physiological and genetic factors underlying the process of fruiting body development.

FRUITING BODIES AS COMPLEX MULTICELLULAR STRUCTURES

Fruiting bodies represent the most complex morphological organization to have evolved in fungi. They are recognized as complex multicellular structures (25, 36, 37), unlike the vegetative mycelium, which is better considered a phylogenetic grade of simple multicellular organization, following the widely accepted definitions of Knoll (38). The distinction between simple and complex multicellularity emphasizes a three-dimensional organization in which not all cells are in direct contact with the environment, the presence of a genetically encoded developmental program, and mechanisms for communication between cells. Complex multicellularity evolved in only five lineages, metazoans, green plants, and brown and red algae, as well as fungi (37–39). Of these, fungi show evidence for multiple origins of

complex multicellular structures, in contrast to other lineages, in which complex multicellularity emerged only once (36, 40).

In terms of cellularity levels, the initiation of fruiting bodies on vegetative mycelia represents a transition from a simple to more complex organization, where the main difference lies in mechanisms of hyphal branching, growth, and adhesion patterns. Since fruiting body initiation can be induced under laboratory conditions, it is an ideal model system for experimentally interrogating cellular events of the transition to complex multicellularity. For example, transcriptomic studies can provide real-time readouts of gene expression changes during the transition from simple to complex multicellularity or comparisons of multiple species or multiple structures within the same species (e.g., fruiting bodies and rhizomorphs [41]) can reveal genes key for development.

MULTIPLE ORIGINS OF FRUITING BODIES IN FUNGI

Although different fungal lineages have diverse solutions for fruiting bodies, the broad functions are the same: to produce and protect spores (both sexual or asexual) from unfavorable conditions or biotic impacts (fungivores/mycophages). In this sense, the different types of multicellular, three-dimensional spore-bearing structures are uniformly referred to as fruiting bodies irrespective of their taxonomic affiliation or homology relations. Among fungi, 8 to 11 independent clades (Agaricomycotina, Pezizomycotina, Pucciniomycotina, Ustilagomycotina, Endogonales, *Neolecta*, *Glomus* spp., and *Modicella*) have been identified that contain complex multicellular fruiting bodies (see Figure 1 in reference 36). In some of these clades, fruiting-body-forming species are the minority and are nested within clades which only form hyphae, whereas in other clades (Agaricomycotina and Pezizomycotina) fruiting-body-forming species are dominant. Fruiting bodies in these clades do not show discernible homology at the developmental and morphological levels, which suggests they either evolved independently or have diverged from a common ancestor to such an extent that similarity is no longer detectable. Ancestral state reconstructions are consistent with this notion: the most recent common ancestors of these clades had most likely no fruiting bodies (26, 36), which implies that they evolved convergently in fungi, as has been hypothesized (38, 42–45).

How complex multicellularity appeared independently many times in fungi is an interesting question (37) to which comparative genomic studies provided some clues in the last few years. Whole-genome sequences were published from some crucial fruiting-body-forming species which belong to independent lineages. One of these is the Endogonales (Mucoromycotina), one of the earliest diverging fungal groups in which complex multicellularity appeared (21). However, developmental aspects of these species have not yet been studied. Within Dikarya, the enigmatic genus *Neolecta* also represents an independent fruiting-body-forming lineage that is nested within the mostly yeast-like Taphrinomycotina. Sequencing the genome of *N. irregularis* revealed surprising genomic attributes (22): it has ~5,500 protein-coding genes and very few introns, which makes it more similar to genomes of related yeasts than to those of filamentous or fruiting-body-forming fungi. A significant expansion of a fungus-specific transcription factor family was detected, which could underlie some of the aspects of *Neolecta*'s uniqueness (22). Nevertheless, the limited coding capacity of the *Neolecta* genome suggests that, in terms of gene content, building complex multicellular structures does not require a lot more genetic elements (genes and regulatory interactions) than building simple hyphae or yeast cells (37). This was a surprising observation given that complex multicellularity is considered one of the major transitions in the history of life (38, 46, 47).

The unusual, yeast-like characteristics of the *Neolecta* genome prompted speculation on the potential genetic underpinnings of the independent origins of fruiting body formation (reviewed in detail in references 36 and 37). The proposed scenarios include (i) a single origin and multiple losses of fruiting body formation; (ii) genetic predisposition (e.g., latent homologies), a mechanism that increases the likelihood of convergence (see references 48 and 49); or (iii) genetic settings that could provide the plasticity needed to switch between complexity

levels over hundreds of millions of years (such as simple genetic changes being sufficient for large morphological changes) (37). The predisposition scenario was supported by Merényi et al. (26), who compared developmentally regulated genes in fruiting body transcriptomes of four Agaricomycotina and five Pezizomycotina species. Significant expression dynamics were taken as a proxy for a role in fruiting body development; this approach, although far from a perfect strategy, was found to represent a good compromise in the comparative analysis of multiple species. Similarly, but in the Ascomycota, Trail et al. (23) found that genes whose expression in fruiting bodies diverged fastest between species conferred a phenotype in knockout mutants significantly more frequently than genes selected on other grounds. Phylogenomic analyses of developmentally regulated genes by Merényi et al. showed that more than half of conserved developmental gene families predated the last common ancestor of the Agaricomycotina and Pezizomycotina; thus, the majority of genes were probably coopted for fruiting body development (26). In addition, very similar gene sets were found to be upregulated in Agaricomycotina and Pezizomycotina fruiting bodies, suggesting convergent cooption in these families. These families also showed a high level of parallel gene duplication (82%), which is significantly higher than in other shared, but non-developmentally regulated families (8.3%). The authors of that study interpreted the high prevalence of parallel cooption and subsequent diversification as evidence for genetic predisposition of ancestral species for evolving fruiting bodies. This may mean that ancestral species had certain precursor traits, which easily transformed into fruiting bodies, thereby reducing mutational target size for evolution.

PHYLOGENETIC PATTERNS IN FRUITING BODY EVOLUTION

One of the major approaches used for understanding the evolution of fruiting bodies involve phylogenetic comparisons, mapping of fruiting body presence/absence on a phylogenetic tree, or inferring trends in the directionality of transformations between different morphologies. Phylogenies can also be used to assess the impact of fruiting body morphologies on diversification by broad comparisons of character-state-dependent speciation and extinction rates. In this section, we discuss information gleaned from the analysis of phylogenetic trees and character states.

An overview of our understanding of Agaricomycetes phylogeny is beyond the scope of this review; however, we note that clarifying the relationships among Basidiomycota subphyla and among classes/orders of the Agaricomycotina has been instrumental to our ability to infer and test evolutionary hypotheses on fruiting body formation. This has been enabled by the revolution in molecular phylogenetics, starting in the 1990s, and more recently in phylogenomics, which refers to the inference of phylogenetic trees from genome-scale data. An important advance has been the clarification of the positions of the earliest-diverging classes Bartheletiomycetes (50) and Wallemiomycetes (51) (including the Geminibasidiales [52]) in the Agaricomycotina, which, nevertheless, due to their apparently reduced nature, represents a limiting factor for inferring the ancestral morphology in the Agaricomycotina.

The Road to Complex Fruiting Bodies

Origin of Agaricomycotina fruiting bodies. Phylogenetic studies suggest independent origin(s) of fruiting body formation in the Agaricomycotina (26, 48), which supports traditional views (43, 45, 53). The ancestral morphology of the Agaricomycetes was robustly resolved as resupinate (crust-like), but this result is based on a broad categorization of fruiting bodies into just five main groups. In these studies, both cushion-like gelatinous and crust-like nongelatinous forms were considered resupinate (there are also fully resupinate forms). Thus, either form might have been plesiomorphic (see Glossary). The simplest resupinate forms are little more than a lawn of naked basidia produced by a loose network of hyphae (i.e., "arachnoid" fruiting body). Support for such a morphology being ancestral is provided by the presence of very simple, resupinate fruiting bodies in early-diverging orders of the Agaricomycetes (e.g., Cantharellales) (Fig. 2 and 3). However, gelatinous, cushion-like fruiting bodies as a plausible ancestral state is supported by the dominance of such fruiting bodies in the two earliest-diverging classes (Tremellomycetes

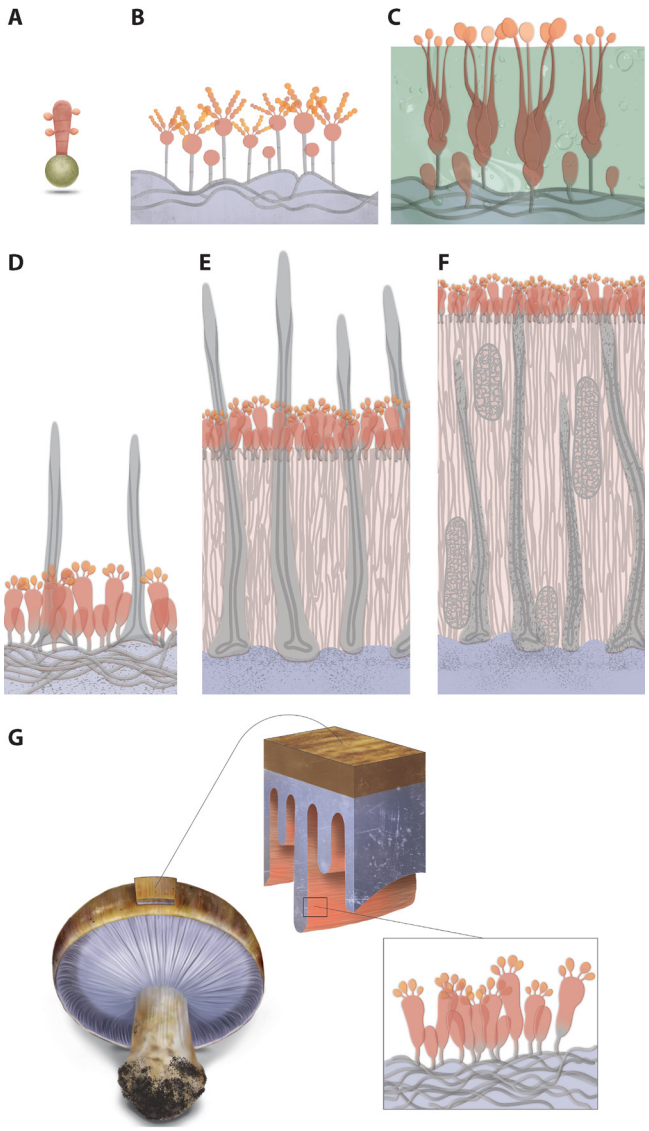

FIG 2 Gradient of complexity levels of fruiting bodies in the Agaricomycotina. The simplest sexual structures are basidia germinating from spores (A) and hymenia composed of lawns of naked basidia (e.g., *Cryptococcus neoformans*) (B). Compact fruiting body structures are represented by gelatinous fruiting bodies of jelly fungi (C), as well as resupinate (D to F) and complex morphologies (G). Note differences in the thickness of the fruiting body and the appearance of sterile cell types (cystidia, gray) in the hymenium. Basidia are indicated in red.

and Dacrymycetes) and the early-diverging Auriculariales and Sebacinales. Gelatinous fruiting bodies also occur in the Pucciniomycotina, and Prasanna et al. argued they represent a shared trait with the Agaricomycotina (54) (although the relationships between subphyla of Basidiomycota are unresolved). Overall, the ancestral morphologies (gelatinous and/or resupinate) and the exact origin of Agaricomycetes fruiting bodies are currently not resolved.

To time the origin(s) of fruiting body formation, inferences can rely on molecular clock estimates for the last common ancestor of Agaricomycetes or Agaricomycotina, since fruiting bodies might have originated in the ancestors of these clades. Wide estimates of early molecular clock studies (300 to 1,000 million years [Myr]) for the Agaricomycetes (55), were narrowed down by more focused studies utilizing more precise calibration points and/or denser sampling. There is a relative consensus among recent estimates of the age of the Agaricomycetes. For the crown age of the class, these are centered around ~300 Myr at the Permian/Carboniferous boundary: ~290 Myr (95% highest posterior density, 222 to 372

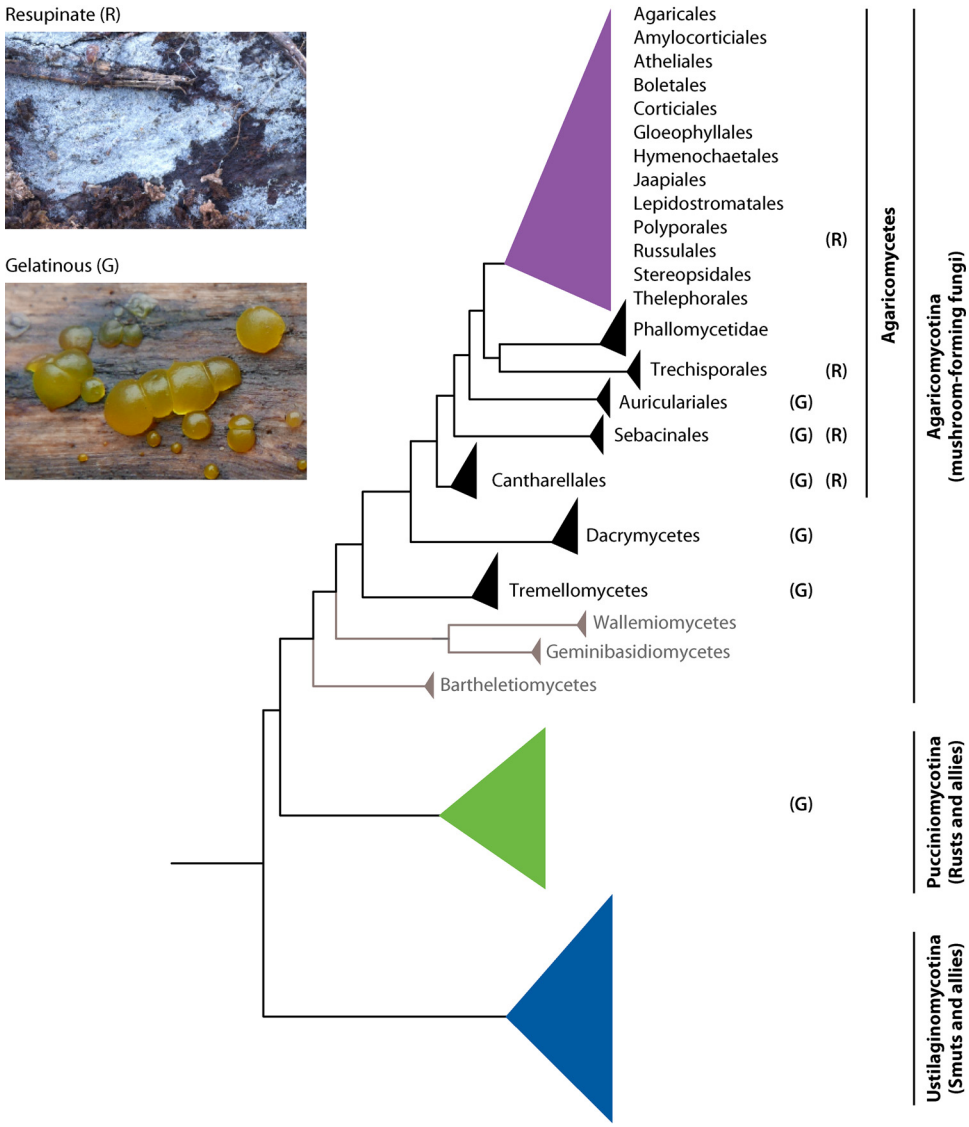

FIG 3 Phylogenetic relationships among major Basidiomycota clades and the distribution of gelatinous [marked by "(G)"] and resupinate [marked by "(R)"] fruiting bodies across basal clades. Orders in the "crown" Agaricomycetes (purple clade) all contain resupinate species in various proportions. The examples shown are *Botryobasidium subcoronatum* (upper image) and *Dacrymyces minutus* (bottom image). The Wallemiomycetes, Geminibasidiomycetes, and Bartheletiomycetes are grayed out, indicating the lack of fruiting bodies in these clades. (Photographs courtesy of Otto Miettinen, reproduced with permission.)

Myr) (56), 294 Myr (57), and ~300 Myr (58), which is consistent with the oldest known clamped hyphae from the Carboniferous (59). However, most of these analyses used very similar approaches to taxon sampling and fossil calibration, so their results are not strictly independent. More recently, Varga et al. inferred slightly older ages, 297 to 387 Myr (mean, 341 Myr) using densely sampled phylogenies and an extended set of fossil calibrations (13). By combining alternative fossil calibration schemes and trees, these authors showed that the difference from previous phylogenomics-based estimates primarily stems from the improved placement of certain key fossils in the tree, highlighting the importance of taxon sampling.

Evolution of complex fruiting bodies. In a bird's eye view of morphology and phylogeny, the evolution of fruiting body types seems to have followed certain trends (60). For example, morphological complexity (e.g., measured as the number of parts) appears to increase as a function of the number of nodes (i.e., number of speciation events) from the root to the tips of the Agaricomycetes tree. However, the existence of general trends in

the evolution of complexity is debated (61, 62), and fruiting body evolution should not be viewed as a steady increase toward more complex forms. Rather, each fruiting body type should be viewed as a result of either evolutionary constraint or ecological adaptation over millions of years of evolution.

The term "complex" as used here is a subjective grouping that includes developmentally integrated forms, such as the pileate-stipitate (Fig. 1i to k), gasteroid (Fig. 1l to n), coralloid (Fig. 1g and h), and pileate-sessile (Fig. 1d and e) fruiting bodies (60). On the other hand, the term "simple forms" mostly refers to resupinate forms in which tissue and cellular differentiation is usually limited (Fig. 2) (also see the Glossary for a detailed description of fruiting body forms). These types do not group into discrete units; there are transitional forms and a continuum of complexity levels within each of the fruiting body types. For example, while resupinate forms likely emerged from lawns of naked basidia and their simplest forms hardly go beyond a few layers of subicular hyphae and basidia, more derived crust fungi can form fruiting bodies that are several millimeters thick with complex hyphal anatomies (Fig. 2).

Several phylogenetic studies have investigated whether there is a general trend toward increasing complexity in Agaricomycete fruiting bodies. These analyses have focused on multiple dimensions of morphological evolution, including (i) historical patterns, (ii) directionality in transformations, and (iii) diversification effects of particular traits. All such analyses are influenced, to various degrees, by taxon sampling and character coding. For example, Hibbett and Binder (63) studied the evolution of resupinate forms using binary character coding (resupinate/nonresupinate) and concluded that resupinate forms are evolutionarily labile, that is, transformations from resupinate to other forms occur more rapidly than transformations in the reverse direction. Based on this result, Hibbett and Binder inferred that there is a trend toward increased complexity in the evolution of Agaricomycetes (63). However, the result was not upheld when the analyses were repeated with the "nonresupinate" state divided into multiple characters (60). A recent large-scale megaphylogeny-based analysis with 8,400 species and multistate coding suggested that (i) the ancestor of Agaricomycetes had a resupinate fruiting body, with many derivations of more complex forms, and reversals to resupinate forms; (ii) resupinate forms do not appear to be particularly labile; and (iii) resupinate forms have reduced diversification rates compared to other forms (14). Thus, conclusions regarding "trends" in the evolution of complex forms in Agaricomycetes will differ depending on whether one emphasizes historical patterns, directionality in transformation rates, or state-dependent diversification.

The broad categorization of fruiting body types that we have used thus far hardly allows a higher-resolution view to be formulated. A wide range of different forms evolved within each of the fruiting body types, some of which may appear more or less complex than others. In the context of the pileate-stipitate forms, these include veils, various hymenophoral conformations (pores and gills), specialized cell types (cystidia and pseudoparaphyses), or pigmentation patterns to name a few. For example, *Flammulina velutipes* has a simpler, "gymnocarpic" development (see Glossary) in which the young gills are exposed to the environment, whereas *Coprinopsis cinerea* primordia are formed within a nodulus (see Glossary), ensheathed by layers of specialized veil cells. Varga et al. (64) referred to the latter as "enclosed development" and noted an analogy with viviparity in animals, where the most vulnerable structures (primordia or the embryo) are protected by tissue layers during their early development (64). Estimates for the number of cell types in mushroom fruiting bodies are scarce, but Kües and Navarro-Gonzaléz counted at least 30 cell types in fruiting bodies of *C. cinerea* using microscopic observations (65).

At a smaller scale, convergent origins of autodigesting "coprinoid" fruiting bodies (which liquefy themselves at the end of development; see Fig. 1l) in the Psathyrellaceae has been demonstrated, presumably as repeated adaptations to small and labile environments (e.g., dung [66]). A generalization we can derive from these studies is that phenotypic traits that emerged repeatedly and became widespread in their clades provide a fitness benefit to species. Several such traits can be found across Agaricomycetes fruiting bodies, such as the presence of a cap, emergence of a stipe, that of complex hymenophore surfaces, partial or universal veils, chemical characteristics (e.g., toxins), or certain spore characters (67), but only

for some of these has a formal test been carried out (see, for example, references 64 and 67). An overarching message that emerges from phylogenetic relationships, as well as explicit tests of evolutionary trends, is that convergence is a widespread phenomenon in mushroom-forming fungi. This is fundamentally different from animals and plants, where phylogenetic conservation of form has been formalized in the concept of body plans. In the next few sections, we review the patterns of convergence in fruiting body morphologies, highlighting the plasticity of the developmental process in fungi.

Evolutionary Plasticity of Mushroom Morphologies

Convergence in fruiting body morphologies. Convergence was increasingly recognized as physiological and microcharacters started to reveal that the broad macromorphology-based taxonomic classification during the early- to mid-20th century into Hymenomycetes ("fleshy fungi"), Gasteromycetes ("puffballs"), and Aphyllophorales ("polypores" and related fungi) is artificial (12). Later, at the end of the 20th century, during the clarification of fungal taxonomy, gross morphological similarities were shown one after the other to be poor indicators of evolutionary relationships (10). Most of these morphologically counterintuitive relationships derive from the polyphyletic nature of taxonomic groups previously thought to be uniform; these are actually the hallmarks of the convergent evolution of morphological traits.

The approximately 36,000 described species of Agaricomycetes are usually categorized into five main fruiting body types (resupinate, coralloid/clavarioid, pileate-sessile, pileate-stipitate, and gasteroid; Fig. 1), and these forms appear many times in different taxonomic groups (14). Here, we also follow this classification; however, it should be noted that each of these broad categories encompasses a wide range of functionally or morphologically different or evolutionarily independent forms (e.g., cyphelloid taxa under "pileate-sessile"; see Glossary). A finer categorization would be advantageous for increasing the biological realism of the discussion; however, just as in the case of statistical methods (60), this approach would lose power in detecting broad tendencies.

Fossil evidence for fruiting bodies is scarce. However, some of the most spectacular fungal fossils are mushrooms, such as *Archaeomarasmius leggetti* (68) or *Coprinites dominicana* (69), two amber-preserved fossils that reflect the modern agaricoid (see Glossary) morphology (fossils and their potential as calibration points are reviewed in reference 13). This evidence highlights clearly only a very small section of the paleodiversity of mushroom-forming fungi, but it does indicate that the pileate-stipitate morphology has been around and has remained virtually unchanged for at least ~100 Myr (4). While fossils do not allow us to deduce patterns of fruiting body evolution, they can be used to infer ages of major events of evolution using molecular clocks (13, 14). Varga et al. (13) combined comparative analyses of fruiting body morphology with molecular clock estimates and showed that transformations in fruiting body morphology happened convergently and asynchronously. For example, the pileate-stipitate morphology was inferred to have evolved 85 times in clades of different sizes and ages. This suggests that transformations in fruiting body morphologies may not be tied to major abiotic (geologic) or biotic (e.g., radiations of plant groups) events.

As discussed above, resupinate forms appear to be plesiomorphic in the Agaricomycetes (13, 14, 59, 60). Most of the oldest clades of the Agaricomycotina contain resupinate forms (e.g., Trechisporales, Corticiales, and Jaapiales) or morphologies that are lumped in this category (Auriculariales, Tremellomycetes, and Dacrymycetes). The resupinate morphology was probably dominant in the Agaricomycetes from their most recent common ancestor in the Carboniferous to the Jurassic era (13) (Fig. 4). With the emergence of the other forms, the proportional representation of resupinate form began to decline rapidly. Nevertheless, the conservation of resupinate forms suggests that they may be well adapted to certain habitats (e.g., the undersides of logs) or that certain constraints kept them in this form. There have also been many reversals to resupinate fruiting bodies from more complex forms via simplification (13, 14). At least 25 transitions from the pileate-stipitate to resupinate forms were inferred by Sanchez-Garcia et al. (14). Examples include the Agaricales genera *Aphanobasidium* and

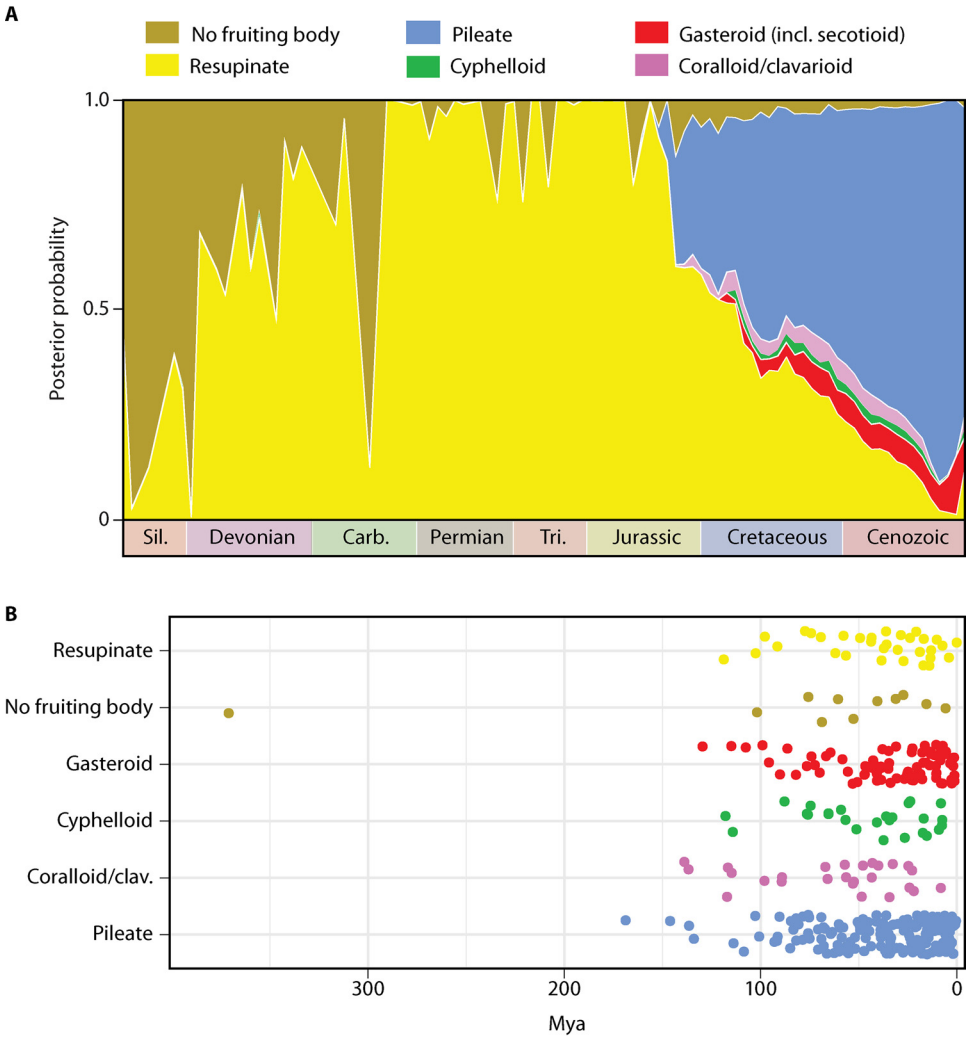

FIG 4 Evolutionary dynamics of the dominance of fruiting body morphologies in the Agaricomycetes. (A) Inferred proportion of a given fruiting body type (*y* axis) through the evolutionary time scale. Pileate-stipitate morphologies started becoming more common and phasing out the resupinate form as dominant fruiting body type around the Jurassic period. (Adapted from reference 13, published under a Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].) (B) Convergent origins of fruiting body types broken down by major morphology. Each dot represents an independent origin of the given fruiting body type. Analysis based on time-calibrated phylogenetic trees from Varga et al. (13).

Brunneocorticium, resupinate taxa that might have evolved from ancestors with more complex fruiting bodies.

Coralloid-clavarioid fruiting bodies are club- or coral-shaped structures with the basidia located on the outer surface (Fig. 1). This morphology is rare among extant mushroom-forming fungi, with its greatest diversity found in the Gomphales (genus *Ramaria* [70]) and the Clavariaceae (71) in the Agaricales. Inferences of evolutionary rates suggested it to be an evolutionarily labile form, which evolved from resupinate ancestors (14, 60) and might represent stepping stones toward pileate forms. Corner postulated that coralloid morphologies are precursors to more complex forms (72), which was Miller's (73) observations of aberrant forms of *Lentinellus cochleatus* being coralloid, similar to those of its close relative *Artomyces pyxidatus*. In the Gomphales, a series of transitional forms between a hypothesized plesiomorphic coralloid/clavarioid (*Clavariadelphus*-like) and putative derived pileate (*Gomphus*-like) form are displayed by clavarioid fruiting bodies with a flattened top (74) (e.g., *Clavariadelphus truncatus*) (Fig. 5). It is also possible that the evolutionary conservation of the ability to switch between pileate-stipitate and coralloid growth forms is related to a stipe elongation and branching program that is likewise widespread among Agaricomycetes.

FIG 5 Transition between coralloid-clavarioid and pileate-stipitate fruiting body morphologies in the Gomphales, illustrating Corner's hypothesis of the emergence of pileate-stipitate forms from coralloid/clavarioid ones. From left to right: *Clavariadelphus pistillaris*, *C. truncatus* convex form, *C. truncatus* concave form, and *Gomphus clavatus*. Note the gradual flattening of the top of the fruiting body into a cap like structure and the transition of the lateral surface (hymenium) of the fruiting body from smooth to wrinkled.

Although coralloid forms appeared many times convergently (28 events) and lost only 14 times, the relatively low abundance of this fruiting body type suggests their low diversification rate compared to others (14). A phylogenetic observation, although not formally quantified, that supports the transient nature of coralloid/clavarioid type is that it is found in several clades, but many cases basally or in early-diverging clades that display high plasticity in morphologies. These include the Clavariaceae in the Agaricales (71, 75), *Pterula*, *Typhula*, and *Macrotyphula* within the Pleurotinae (Agaricales) (76, 77), *Clavaria zollingeri* in the Hymenochaetales (76, 77), or *Clavicorona* in the Russulales (78), among others.

Pileate-sessile forms comprise fungi which have a cap but lack a stipe. This morphology is characteristic of nonresupinate Polyporales (e.g., bracket fungi) and other, mostly wood-associated fungi. Pileate-sessile fruiting bodies evolved from resupinate (91 times) or pileate-stipitate (42 times) forms (14), probably via the curving of the hymenium (see Glossary) off the substrate or via the loss of stipe, respectively. Most of the corky, perennial fungi are found in this category. The shelf-like pilei provide protection from precipitation and possibly other factors, as well as support and increase the spore-producing surface. The number of reversals, from pileate-sessile to resupinate (65) and pileate-stipitate (26), is also considerable. The diversification rate of pileate-sessile groups tends to be greater than that of resupinate, coralloid-clavarioid, or gasteroid forms but lower than that of pileate-stipitate forms.

Gasteroid forms produce spores internally, in a "gleba" structure, and include puffballs, stinkhorns, bird's nest fungi, and secotioid and hypogeous taxa (Fig. 1). Most gasteroid forms are derived from pileate-stipitate lineages (117 times out of 123 events [14]), and most such transformations coincide with the loss of forcible spore discharge (ballistospory; see Glossary) and changes in tramal structure (8, 12, 29, 79). Both are complex features that possibly could not be regained once lost (8); if true, this could underlie the negligible number of apparent reversals inferred (14). For this reason, changes to gasteroid morphologies have been hypothesized to be irreversible (14, 60, 80).

The large number of transitions from pileate-stipitate to gasteroid forms is associated with ecological (animal dispersal) and environmental factors such as aridity or cold as selective forces (11, 81). Hypogeous fungi, a special case of gasteroid forms, were hypothesized to be an endpoint of the fungal body plan, where new selection pressures (e.g., for the production of volatiles) appeared due to the transition to a below-ground growth mode (82, 83). Detailed phylogenetic studies highlighted that even in a short time and at small taxonomic scales, the gasteroid/secotioid forms and hypogeous lifestyle emerged convergently many times from within pileate-stipitate species (84–88). Gasteroid lineages are of various ages and their age correlates with their morphological similarity to plesiomorphic pileate-stipitate forms. For example, the genus *Lycoperdon* is nested within an old, predominantly gasteroid lineage (Lycoderaceae) and shows hardly any similarity to agaricoid mushrooms,

whereas the not too distantly related genus *Endoptychum* is much younger, is nested within *Chlorophyllum* (and is now classified as part of it), and bears a strong resemblance to pileate-stipitate forms (86). There are several examples of multiple origins of secotioid or gasteroid fungi within an individual pileate-stipitate genus. For example, *Cortinarius* (85), *Russula* (84), *Lactarius* (89), or the already-mentioned *Chlorophyllum* (86) all contain multiple secotioid species and phylogenetic trees suggest that these emerged independently in their clades. Further, recent origins of gasteroid taxa were reported in *Laccaria* (originally described as *Hydnangium* [87]), in *Entoloma* (*Rhodogaster* [90]), in *Suillus* (*Gastrosuillus* [91]), and in boletes (*Gastroboletus* [92]), and the list could be continued.

Finally, the pileate-stipitate morphology comprises the best-known toadstool forms with cap and stipe. Although fossils indicate that the pileate-stipitate morphology has been around for >100 Myr (4), it emerged quite late in the Agaricomycetes; approximately during the Jurassic (~170 to 180 Myr). As of today, it has reached an ~70% dominance over the other four fruiting body forms (Fig. 4) and could be a key innovation in fungal evolution (13). It evolved in multiple clades, at least 81 times (14) and is present in at least 10 of the 20 orders currently recognized in the Agaricomycetes. Despite the dominance and evolutionary stability of pileate-stipitate forms, 191 transitions from pileate-stipitate types to other morphologies were inferred, the most into pileate-sessile forms (which also includes cyphelloid fungi) (14). Evolutionary success of this morphology can be explained by many features, such as a vertically lifted pileus, protection from biotic and abiotic factors, structured hymenial surfaces that increase the spore-bearing surface, efficient humidification of the microenvironment, and the generation of convective airflows that promote active spore dispersal (66, 93).

Evolutionary transformations at finer morphological scales. The classification of fruiting bodies into five broad morphologies is a crude approximation of their diversity, and focusing on the main types leaves a cornucopia of other interesting trends in fruiting body evolution unexplored. Although these shortcomings will surely be addressed in future studies, a few promising developments are worth mentioning. Convergence was also reported in hymenophore structures, defense mechanisms (partial and universal veil) (67), cyphelloid fruiting bodies (94), autodigesting "coprinoid" fruiting bodies in the Psathyrellaceae, Bolbitiaceae, and Agaricaceae (65, 95–97), among others. In coprinoid fruiting bodies, as in the case of gasteroid/secotioid ones, a general syndrome of morphological change was observed, involving sudden changes to multiple cell types; such correlated evolution in multiple, independent clades of coprinoid mushrooms underlines the intrinsic tendency of mushroom-forming fungi for evolutionary convergence (97).

One of the spectacular traits of mushroom-forming fungi are the highly structured hymenophore surfaces (Fig. 6). The shape of the hymenophore—whether poroid, gilled, toothed, wrinkled, or smooth—is one of the most obvious and easily diagnosable characters of macrofungi. Hymenophore surfaces are subject to fast evolution and, like the main morphologies, extensive convergence. Many transformations between hymenophore types were predicted based on anatomical features (72, 98, 99) and later confirmed with molecular data (10, 100). Recently, Varga et al. (67) showed that the evolution of complex hymenophore surfaces is the preferred direction in evolution, and gills, pores, or teeth emerged in most order-level clades independently. Gills have evolved in at least eight orders of Agaricomycetes (Gomphales, Cantharellales, Hymenochaetales, Gloeophyllales, Polyporales, Russulales, and Agaricales).

Transitions between poroid and gilled or between poroid and toothed hymenophores can be found even in closely related sister taxa, confirming convergent evolution. For example, the Boletales are dominated by mushrooms with a characteristic poroid hymenophore (e.g., *Boletus edulis*), but lamellate hymenophores have evolved repeatedly (101), in *Hygrophoropsis*, *Austropaxillus*, *Paxillus*, the *Gomphidius-Chroogomphus* clade, and *Phylloporus*. Lamellate genera nested in poroid clades are further exemplified by *Lentinus*, *Panus*, and *Lenzites* in the Polyporales (102) or *Neolentinus* and *Heliocybe* in the Gloeophyllales. On the other hand, poroid genera nested within larger gilled clades are represented by *Poromycena*, *Favolaschia*, *Dictyopanus*, or certain species of *Resupinatus* (103) (all in the Agaricales). The genus *Steccherinum* displays both poroid and toothed hymenia (104).

FIG 6 Hymenophore configurations. The diversity of hymenophore configurations in the Agaricomycetes is depicted. From left to right: smooth hymenophore (top, *Auricularia-auricularia-judae*; bottom, *Xylobolus frustulatus*); wrinkled hymenophore (top, *Cantharellus cibarius*; bottom, *Irpex lacteus*); poroid hymenophore (top, *Neoboletus luridiformis*; bottom, *Polyporus tuberaster*); toothed (also called hydnoid) hymenophore (top, *Hydnum* sp.; bottom; *Pseudohydnum* sp.); gilled hymenophore (top, *Hymenopellis radicata*; bottom, *Lepista sordida*). (Photographs by L. G. Nagy, Z. Merényi, and X.-B. Liu.)

A well-studied hymenophore transformation occurs in *Lentinus* (Polyporales), which presents both pores and gills. Pores of *Lentinus* may be angular, broad, and radially elongate (*L. arcularius*, syn. *Polyporus arcularius*) or small and round (*L. brumalis*, syn. *P. brumalis*). Lamellae (see Glossary) may be linear, narrow, and crowded (e.g., *L. crinitus*) or broad, widely spaced, and connected at their bases by tangential "cross bridges" (*L. tigrinus*). Using scanning electron microscopy, Hibbett et al. (102) suggested that the "cross bridges" of *L. tigrinus* may be homologous to the tangential hymenophore elements of *L. arcularius*. If so, the transformation between these forms might be explained in terms of heterochronic shifts in the growth of radial and tangential elements in the hymenophore.

Apparent from these studies is that convergence is characteristic not only of the evolution of the main fruiting body types but also of other, finer-scale traits of fruiting bodies. What underlies this extreme evolutionary plasticity of fruiting body types remains to be understood. Explanations proposed thus far include purely adaptationist views, implying that similarity evolved in response to common selective pressures. Alternative hypotheses involved constraint or neutrality, with small genetic changes being sufficient for dramatic morphological changes (i.e., large mutational target size [11]), the existence of predisposing genetic elements, or developmental potentials (reviewed in references 48 and 49) that are conserved over larger evolutionary distances. Cases are known where mutations in a single locus caused dramatic morphological changes (see, for example, references 65 and 105). One factor that might determine the evolutionary plasticity of fruiting bodies is the plasticity of the developmental programs of fruiting bodies. There are several examples of aberrant or stalled morphologies resembling other morphologies. For example, stipes of certain species grown in the dark elongate or branch in a way that resembles coralloid fruiting bodies (e.g., *Lentinellus* [73]). Pileate-stipitate species often respond to environmental perturbations, or infections with incomplete or stalled cap expansion, reminiscent of secotioid fruiting bodies. These observations suggest that some of the recurring evolutionary transformations (i.e., convergence) might be facilitated by an inherent developmental plasticity encoded in the genomes of mushroom-forming fungi.

Effect of Morphological Innovations on Diversification Rates

Morphological innovations might influence speciation and extinction rates or lead to explosive speciation events, which describe the speed at which new species accumulate in a clade and are collectively known as the diversification rate (speciation minus extinction). Traits could lift constraints on diversification by several mechanisms, such as by optimizing nutritional investment and reproductive efficiency or by allowing the organisms to enter

new, unoccupied niches (106). Whether fruiting body morphologies influenced diversification has been a recurrent question in research on the Agaricomycetes.

The first formally tested hypothesis in this context addressed the impact of the gasteroid morphology on diversification rates in three clades of mushroom-forming fungi (Sclerodermatinae, Lycoperdaceae, and Phallomycetidae [80]). Significant differences in diversification rates of gasteroid versus nongasteroid species were not found, and only under certain, weakly supported models did gasteroid lineages have higher diversification rates. These results are in line with those of a study across the entire Agaricomycetes (14). Model-based predictions suggested that in their clades the equilibrium frequencies of gasteroid forms are higher than their current diversities. Although this prediction is contingent on several parameters, including taxon sampling, it means that, with time, gasteroid forms will become more common in their clades. From this and the observation that the vast majority of gasteroid lineages are very young, the authors derive that the current paucity of gasteroid species in the Agaricomycotina are due to only a few lineages being sufficiently old and successful to accumulate species and that intermediate forms (secotioid species) might be at high extinction risk, whereas, if lineages can pass through this bottleneck, they can diversify at higher rates (13).

Recent large-scale studies showed that pileate-stipitate species have the highest diversification rates, followed by pileate-sessile ones, whereas resupinate species were associated with the lowest diversification rates in the entire Agaricomycetes (13). Such broad analyses have the potential to uncover broad tendencies, but the higher the scale, the more likely interactions between trait- or clade-specific patterns can yield mixed signals (strengthening or quenching). The higher diversification rate of complex morphologies might be explained by their ability to support higher spore-to-biomass ratios, more efficient protection of basidia, or other, as-yet-unknown fitness benefits they confer. Thus, pileate-stipitate morphologies may be key innovations (13), but what property (or properties), from a functional point of view, makes the key difference relative to simpler forms is unknown. To address this question, Varga et al. (67) examined traits that are phylogenetically codistributed with the pileate-stipitate morphology. One such trait is "enclosed development," a developmental strategy in which the young hymenium is ensheathed from the environment by hyphal layers (e.g., by veils). Another trait, one examined by Varga et al. (67), was complex hymenophores (gills, pores, or teeth). Both traits evolved convergently and were associated with higher diversification rates; however, their effects were found to be independent from that of the pileate-stipitate morphology. Thus, although this work highlighted two additional traits that influence diversification rate, they are not the ones that confer evolutionary advantage to the pileate-stipitate morphology. Notably, a distant analogy between enclosed development and viviparity exists, with the remark that in fungi a fruiting body primordium, whereas in animals and plants the embryo itself is enclosed in a protective environment.

An alternative, nonmorphological hypothesis that received significant attention is that lifestyle, in particular, ectomycorrhizal symbiosis (ECM), affects diversification patterns (77, 107–110). Studies of diversification rates in ECM clades yielded mixed support for this hypothesis, often being contingent of taxon sampling ratios or analytical methods. Using a global Agaricomycetes phylogeny, Sánchez-García et al. (14) showed that fruiting body form is overall a stronger driver of diversification rate differences than nutritional mode but that lifestyle might have also spurred diversification at local scales. Several other factors might also influence diversification, such as morphology at smaller scales, life history, dispersal events (110), latitudinal distribution patterns (111), unknown factors, or combinations of factors (77). The first explosive diversification event in fungi was reported in the genus *Coprinellus* (Psathyrellaceae), where streamlining the defense mechanisms for lower nutritional investment was hypothesized as the driver of the adaptive radiation (112). The rich morphological and ecological diversity of mushroom-forming fungi provides a good substrate to test such questions in future studies.

Sources of Developmental Innovation

Development in plants and animals versus fruiting bodies: do mushrooms have a body plan? Animals, plants, and fungi share a unicellular common ancestor which existed at least 1.6 billion years ago (43, 113). They evolved complex multicellular forms independently;

accordingly, few, if any, parallels can be drawn between their developmental programs. A few very broad similarities that animals, plants, and fungi share include a spatially and temporally integrated developmental program or determinate growth in fruiting bodies, animals, and certain plant organs (e.g., inflorescences). However, aside from these superficial traits, development in fungal fruiting bodies progresses along fundamentally different rules from that in animals or plants. For example, animals and plants develop from an embryo, whereas fungal fruiting bodies emerge as hyphal aggregations on the vegetative thallus. As a consequence, many of the concepts and models developed by zoologists or botanists may not be applicable in the context of fungal development. Rather, mycologists might need to develop their own developmental concepts, models, and hypotheses, which should integrate fruiting body morphometrics, evolutionary/phylogenetic inference, and developmental observations (114, 115).

We may also wonder whether body plans exist in fungi. The notion of a body plan was introduced by zoologists (116) (based on the German phrase Bauplan) and eventually adopted also by botanists (117). The term has been of limited use in mycology (see, for example, the study by Liu and Hall [118]); rather, mycologists discussed morphologies in the context of "fruiting body types." A body plan is a group of structural and developmental characteristics that can be used to identify a group of animals, such as a phylum or a higher taxonomic unit (116). Fruiting bodies, on the other hand, show high plasticity even across smaller evolutionary scales, such as orders or families (see below), which makes it difficult to designate a single type for the Agaricomycetes or any of the orders therein. For example, the resupinate genus *Trechispora* was shown to include coralloid *Scytinopogon* (119) species, indicating that transitions in fruiting body morphology can happen within a relatively short time. Similarly, gasteroid species have emerged convergently within numerous agaric genera, such as *Cortinarius* (85) for *Russula* (78). These frequent transformations between fruiting body types just suggest that the basic principles of developmental evolution of Agaricomycetes might be fundamentally different from those of animals, rendering the concept of a body plan not suitable to describe development in mushroom-forming fungi. A body plan concept may apply to fungi at smaller phylogenetic scales, although convergence and secondary simplification are common in many Agaricomycetes families. We note that the concept of a body plan may apply better to the Pezizomycotina, where apothecia, perithecia, or pseudothecia are conserved fruiting body types within their respective classes, providing more ground for discussing body plans as they were defined originally.

Another concept that has been discussed in fungi is the developmental hourglass, an analogy to describe the conservation of embryogenesis during mid-development, with more divergence between species in early and late development (120). This hypothesis experienced renewed popularity recently after an hourglass pattern was discovered in transcriptome data of plant and animal embryogenesis, too (121, 122); however, this hypothesis also received intense criticism (123). An hourglass-shaped transcriptomic signature has been detected in *Coprinopsis cinerea* as well (124), in which the "waist" coincides with the young fruiting body stage when meiosis in the gills prepares nuclei for spore formation (125). Thus, the "waist" region may be interpreted as the effect of the upregulation of highly conserved meiotic genes, which causes the overall conservation of the transcriptome to shift toward more ancient genes (126). Under this interpretation, the observed fungal "hourglass" is generated by fundamentally different mechanisms than it is in animals.

Mechanisms of developmental evolution. Development of plants, animals, and fungi have fundamentally dissimilar anatomical and genetic bases. While we know very little on the mechanistic sources of developmental innovations in fungi, they share some general mechanisms with animals and plants. One of the very few examples is heterochrony (see Glossary), that is, shifts in the relative rate and timing of developmental events. Heterochrony has been invoked in several morphological transformations in fungi (including evolution of the *Lentinus* hymenophore [see above]) or (the genus *Panus* [Polyporales] [102]). The best-known example concerns the evolution of gasteroid species, such as puffballs and false truffles. Gasteroid fruiting bodies represent a naturally replicated example (emerged ~123 times [14]) of developmental innovation and a general "syndrome" of phenotypic changes can be observed in an earlier study (8). Despite initial uncertainties in the directionality of evolution

FIG 7 Series of fruiting body forms that connect pileate-stipitate and gasteroid fruiting bodies in the genus *Cortinarius*. Thiers' hypothesis posits that secotioid and gasteroid fruiting bodies derive from pileate-stipitate ones. (A) Stereotypical pileate fruiting bodies of *Cortinarius glaucopus* with thin, silky partial veil; (B) drought-adapted *C. magnivelatus* with a thick, membranous partial veil that does not break up; (C) *Thaxterogaster porhpyroideus*, a secotioid species with lacunar (not gilled) hymenophore and a globose, closed cap, but slender mushroom-shape; (D) *Thaxterogaster pingue*, another secotioid *Cortinarius* with a shortened stipe; (E) *C. flavopurpureus*, a gasteroid species with clearly visible stipe remnant (columella); (F) *C. infrequens*, a gasteroid species with reduced stipe remnant (columella); (G) *Hymenogaster utriculatus*, with hardly discernible stipe remnants.

between gasteroid and agaricoid forms (mostly stemming from historical taxonomic separation of gasteroid "Gasteromycetes" and agaricoid "Hymenomycetes" [for a review, see reference 10]), it is now clear that gasteroid forms represent a derived state. Thiers observed in the Sierra Nevada, where moisture is mostly supplied by scattered thunderstorms, that fruiting bodies of many fleshy fungi become arrested in development due to the lack of a continuous water supply (8). It has been suggested that evolution of gasteroid forms results from a failure of the pileus to expand, resulting in a permanently enclosed hymenophore (8). If so, then "gasteromycetation" can be considered an example of paedomorphosis (127), a special case of heterochrony, which is the retention of juvenile-like features of an ancestor in a descendant organism.

Gasteromycetation is one of the few evolutionary transformations where a continuum of transitional forms outline a probable sequence of evolutionary events (Fig. 7). From normal pileate-stipitate species, the road seems to lead through agaricoid forms with permanently closed caps and contorted gills to closed (angiocarpic; see Glossary) fruiting bodies in which stipe starts to degenerate and eventually disappear (or remained as a columella) and

hymenial tissue shows transitions to a gleba-like structure (8). Collateral with changes in fruiting body morphology, ballistospory is lost in many lineages (probably made dispensable by internal spore production), and fruiting bodies tend to grow underground and evolve novel aromatic volatiles, which aids animal dispersal. Analogous, closed and/or underground morphologies evolved also in the Ascomycota in truffles and false truffles. The genetic mechanisms of gasteromycetation are not well understood, but its pervasive convergence and some very recent transitions suggest that it may require minor genetic changes or is associated with a large mutational target size (11).

DEVELOPMENTAL BIOLOGY OF FRUITING BODIES

Agaricomycetes, and to a lower degree Ascomycota, produce complex fruiting bodies, with genetically encoded shapes, sizes, and coloration. The ability of mushroom-forming fungi to differentiate and establish cell types within fruiting bodies in a spatially and temporally coordinated manner is truly amazing and is probably unparalleled in the fungal kingdom. How this process works has fascinated mycologists for a long time. Developmental mycologists provided detailed descriptions of the development fruiting bodies in a range of model systems (e.g., *C. cinerea* and *S. commune* [65]). The genetic bases of fruiting body formation began to be elucidated in the late 20th century, and the discovery of gene function was guided mostly by analyses of naturally occurring or laboratory-generated mutants.

There are fundamental differences between the development of fruiting bodies and that of animals or plants. While in the latter, ontogenesis refers to the sculpting of a reproducing individual, fruiting body development refers to the emergence of structures from the vegetative mycelium that enclose and support sexual spore production. Fruiting bodies integrate the apparatus for sexual spore production, including karyogamy, sporangium development (basidia or asci), meiosis, spore morphogenesis, and discharge. However, the genetic processes that fruiting bodies encompass are a lot more complex than just those related to spore production (e.g., meiosis and spore morphogenesis). Several of these represent adaptations to the terrestrial environment (e.g., defense against desiccation or UV radiation) or are related to sculpting species-specific morphologies.

There is a stunning evolutionary plasticity in fruiting body development and class-wide conservation of developmental programs is lacking (e.g., lack of body plans [see above]). Therefore, a general framework that describes all Agaricomycetes probably cannot be discussed, but example or model developmental fates can (65) (see more details below). While generalizations on the developmental program are hard to formulate, the genetic processes that participate in fruiting show some conservation (128). For example, two key events that precede fruiting body development are mating (129–132) and the integration of environmental and internal cues that specify readiness for fruiting across the colony. Another broad and conserved function is pattern formation and cell differentiation; however, cellular and molecular mechanisms and the underlying regulatory and structural genes are mostly unknown. A significant challenge that lies ahead of mycologists is identifying genetic circuits that drive the differentiation of tissue types within fruiting bodies. This will be necessary for the ability to elucidate the evolution of transformations between fruiting body types and gains/losses of various characters within. Below, we review information on the genetics of fruiting body development, both at the level of general processes and at the level of individual regulatory or structural genes.

The Process of Fruiting Body Development

Fruiting body development has been investigated both in model systems (*C. cinerea* and *S. commune*) and in a few edible mushrooms with commercial value, such as *A. bisporus*, *P. ostreatus*, *L. edodes*, and *F. velutipes*. The development of most of these fungi has been reviewed elsewhere recently (15, 65, 133). Therefore, we do not present here the developmental process in great detail; rather, we only briefly discuss the development of *C. cinerea* (Fig. 8), which forms one of the most complex pileate-stipitate fruiting bodies with an estimated 30 different cell types (65). Fruiting body formation starts with the emergence of a loose hyphal aggregate, called a primary hyphal knot, on the dikaryotic mycelium (125, 134). Primary hyphal knots are formed by the differentiation of aerial hyphae that grow toward

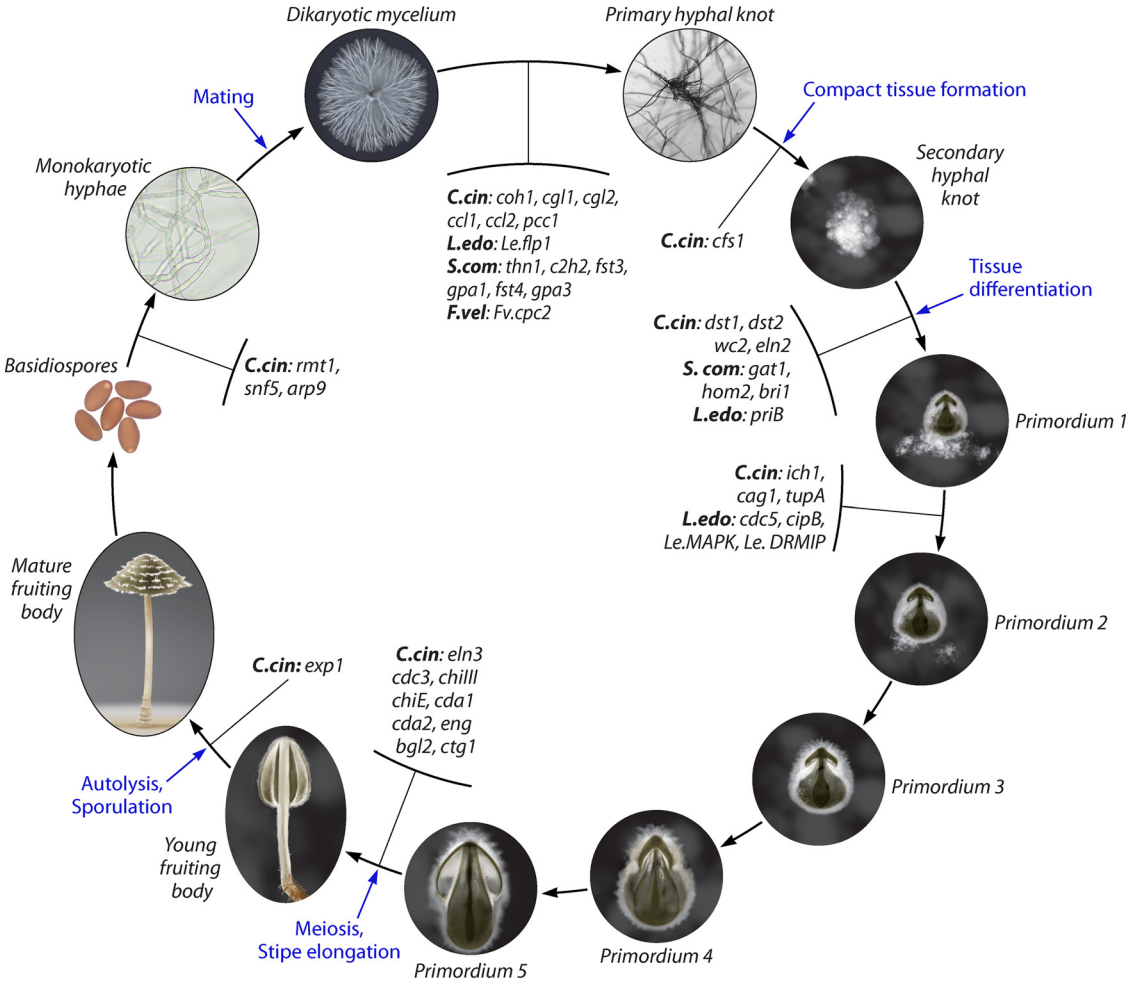

FIG 8 Time scale illustrating the progression of *Coprinopsis cinerea* development. Drawings show key developmental stages. Key genes discussed in the text are shown at developmental transitions. Abbreviations: *C.cin*, *Coprinopsis cinerea*; *S.com*, *Schizophyllum commune*; *L.edo*, *Lentinula edodes*; *F.vel*, *Flammulina velutipes*. Major developmental events (mating, compact tissue formation, tissue differentiation, meiosis, stipe elongation, sporulation, autolysis) are indicated by blue arrowheads.

each other and start to branch extensively with restricted apical growth (125). In secondary hyphal knots, the aggregate is densely packed and a mucilaginous material can be found between the cells that may be involved in hyphal adhesion (134, 135). Anastomosis often occurs between the adjacent hyphae (125). Under appropriate conditions, the hyphal knot develops into a fruiting body primordium, in which the tissue differentiation becomes clearly visible (65). The lower part of the primordium becomes the basal plectenchyma (see Glossary), the middle part gives rise to the stipe, and the upper part differentiates into the pileus (136). The primordia of some fungi, including *C. cinerea*, are surrounded by one or more layers of protective hyphae called the veil (15).

Further development involves the differentiation of the hymenium in a region between the stipe and the cap and continues toward the outer surface of the cap, resulting in dome-shaped rudiments that become the gills (137). The hymenium of *C. cinerea* consists of four differentiated cell types: basidia, cystidia, cystesia, and paraphyses (see Glossary) (138). Karyogamy occurs within the basidia, followed by meiosis (139). Around the end of meiosis, the maturation of the fruiting body starts. In this species, it involves mostly turgor-driven cell expansion and virtually no change in cell numbers and leads to elongation of the stipe, the expansion of the cap, and the production of basidiospores on basidia (65). At maturation, *C. cinerea* releases basidiospores, and the cap autolyzes (140). Hyphal-knot formation, tissue differentiation in the primordium, karyogamy and meiosis in the basidia, growth by cell expansion, and basidiospore production, as well as the regulation of these

by environmental cues (e.g., light), are shared mechanisms among mushroom-forming fungi. However, species-specific departures from this general program are common (e.g., the lack of light regulation of *A. bisporus* [141] or brown film formation in *L. edodes* [142]) but are not the subject of the current review.

Genetic Bases of Fruiting Body Development

Most of our knowledge on the genetics of fruiting body development comes from forward genetic approaches, such as UV- or restriction enzyme-mediated integration (REMI)-generated and spontaneous mutants (e.g., *C. cinerea* [143, 144], *S. commune* [145], and *F. velutipes* [146]). For example, Muraguchi et al. (144) UV mutagenized oidia (see Glossary) of a homokaryotic strain, A43mutB43mut (a self-compatible strain widely used as a model system), of *C. cinerea* and reported 1,018 mutant strains exhibiting fruiting body formation defects. They classified mutants into nine groups: (i) "depress" mutants, where aerial hyphae are reduced and senescent hyphae undergo autolysis (215 strains); (ii) mutants that cannot form hyphal knots (377 strains); (iii) primordium-less mutants in which hyphal knots cannot progress to primordia (89 strains); (iv) maturationless variants, where primordia cannot develop into fruiting bodies (193 strains); (v) elongationless variants possessing mature fruiting bodies with short stipes (*eln* mutants, 19 strains); (vi) extensionless mutants that form mature fruiting bodies but where the cap does not expand (*exp* mutants, 8 strains); (vii) sporeless variants that are not able to form basidiospores (73 strains); (viii) "compound type" variants, which exhibit combinations of the above-mentioned phenotypes (23 strains); and (ix) other mutants, including "dark stipe" or "blind" variants (21 strains). Dark stipe mutants represent an intensely studied group; in these, the pileus and the stipe tissues remain rudimentary, and the basal part of the primordia elongates (19). Beyond targets for follow-up studies, the numbers of mutants in each category from these mutagenesis screens provide information on the mutational target size of each developmental transition. Others generated *C. cinerea* mutants using REMI (143) or utilized spontaneous mutants (141, 143, 147). In addition to mutant collections and other forward genetic approaches, comparative transcriptomic studies are emerging as a valuable source of functional hypotheses on dozens to hundreds of genes. These studies provide information about expression patterns which cannot directly be translated to function; however, they highlight plenty of genes with cell type- or tissue-specific expression, which may eventually be translated into genetic hypotheses.

Within fruiting bodies, cellular processes, such as the generation of fruiting body-specific cell wall architectures (148), storage carbohydrate metabolism and mobilization (125, 149, 150), defense (151), and meiosis and spore morphogenesis (152, 153) are well known. These are orchestrated by molecular and regulatory mechanisms characteristic of eukaryotic cells; of these, transcriptional regulation (see, for example, reference 18), alternative splicing (25), nucleus-specific expression (126, 154), natural antisense transcripts (see, for example, references 126, 139, and 155), RNA editing (though controversial, see reference 126), and small noncoding RNA species (156) received more attention, among others. Fruiting body cells integrate external cues related to environmental factors (e.g., light responses [125]) and communication processes (e.g., through volatiles [157, 158] or unknown hormone-like compounds), which are transmitted through conserved signal transduction pathways. In the following sections, we introduce the progression of fruiting body development through the example of *C. cinerea*, as well as highlight genetic processes for which robust evidence proves a role in fruiting body development.

Alternative splicing. Alternative splicing (AS) is a posttranscriptional modification of mRNA that leads to the emergence of multiple transcript isoforms with potentially diverse functions (159). AS can expand the organism's protein repertoire, which has been correlated with an increased complexity of multicellular organisms (154, 160–162), or could regulate transcript levels posttranscriptionally via the nonsense-mediated RNA decay (NMD) pathway. Based on available evidence, the latter seems more likely as a function of AS in fungi (25). In fungi, intron retention is the most abundant splicing event, followed by the alternative 3′ splice site and the alternative 5′ splice site, whereas exon skipping

amounts to <2 to 3% of all splicing events (25, 161). In contrast, exon skipping is the most prevalent AS form in animals and is the major source of the expansion of protein-coding repertoires (163). Intron retention is mostly viewed as a mechanism to destine transcripts to the NMD pathway that degrades them and thus regulates transcript levels posttranscriptionally (163–165). Nevertheless, the Agaricomycotina possess more exons and introns than other fungi (166, 167), and AS occurs in a significantly higher proportion of genes (36 to 46%) than in other fungi (1 to 8%) (25). Several genes have developmentally regulated transcript isoforms which, whether translated to proteins or destined to decay, expand the space of developmentally regulated transcripts (25). In *S. commune*, AS occurs in diverse genes (e.g., transcription factors [TFs] and carbohydrate-active enzymes [CAZymes]), and alternative transcript expression highly increased in aggregates and fruiting bodies compared to vegetative mycelium (168), whereas in another study splicing prevalence did not change significantly with developmental stages (25).

Transcriptional regulation. Recent studies revealed that transcriptional rewiring may be key to the transition from vegetative mycelium to fruiting body development (128, 169). Perhaps somewhat unsurprisingly, transitions between developmental stages are associated with changes in the transcriptional regulation of genes. However, the most remarkable transcriptomic changes in the life cycle of the fungus (e.g., measured in the numbers of differentially regulated genes) were encountered at the transition from vegetative mycelium to fruiting body initials (25, 41). This aligns with the transition from simple to complex multicellular growth and the onset of several processes not characteristic of vegetative mycelia (adhesion, differentiation, and possibly intense communication [36]). Within fruiting bodies, cap development seems to be associated with more transcriptomic changes, whereas stipe development seems to be associated with fewer transcriptomic changes (25, 26, 41). It is currently not known whether this transcriptional rewiring is achieved through the activation of certain ("master") regulatory cascades or via more general changes to chromatin structure (e.g., promoter accessibility), or both.

Mating. A prerequisite of sexual fruiting body development is the formation of a fertile dikaryon by compatible monokaryotic hyphae, which is governed by mating and the mating loci. The basics of basidiomycete mating systems were known a hundred years ago (170). Basidiomycota are usually heterothallic, which means the haploid individual is self-sterile and for sexual reproduction has to find a mating partner that carries different alleles at the mating-type (*MAT*) loci. Among fungi, only the basidiomycetes possess a tetrapolar or bifactorial mating system, which requires two different alleles at two physically unlinked loci, called *HD* and *P/R*, that segregate and generate different mating types (171). It is hypothesized that the ancestral state was tetrapolar, and unifactorial or bipolar mating systems emerged convergently, by the loss of function in determining mating specificity of the *P/R* locus or by fusion of the *MAT* loci, resulting in physically linked loci that act as a single locus (172–176). Tetrapolar species (e.g., *C. cinerea* and *S. commune*) can have numerous (up to hundreds) alleles in both *MAT* loci, resulting in thousands of different mating types (177), that promote outbreeding (129, 178–180).

The *HD* mating-type locus encodes two different homeodomain (HD) transcription factors (HD1 and HD2) that regulate the initial steps of clamp formation and the coupling of haploid nuclei (181). In dikaryotic mycelium, the HD1/HD2 heterodimer regulates the expression of genes involved in establishing and maintaining the dikaryon (182). Hitherto, no direct targets of the HD1/HD2 TF-complex have been identified (183). In bipolar species HD proteins are solely able to promote sexual development (176). On the other hand, the *P/R* locus encodes a pheromone/receptor system that controls reciprocal nuclear exchange and nuclear migration (184). The pheromones of basidiomycetes are not essential for plasmogamy but determine mate compatibility (182). They are small prenylated lipopeptides, which are ligands of a pheromone-sensing G-protein-coupled receptor (GPCR) (171). The recognition of a pheromone by the target GPCR activates a full line of signal transduction pathways, including Ras, cAMP-dependent signaling, MAP kinase cascade, and Cdc24 (185–187). However, it is important to note that because there is a large temporal separation between mating and fruiting body formation, mating is a necessary but not sufficient event in fruiting.

Triggers of fruiting body development. The induction of fruiting body development is a result of complex interactions between internal and external cues, such as nutrient (C/N) availability, temperature, light, CO_2 concentration, or nutritional status of the mycelium, among others (188). Several similarities exist between triggers of fruiting body initiation (as a morphogenetic process) and that of sexual reproduction in model fungi or in nonfungal eukaryotes, although the genetic circuits governing these responses became significantly more complex during the evolution of mushroom-forming fungi. While for simpler species (e.g., yeasts) nutrient depletion is often necessary and sufficient to initiate sexual reproduction, the corresponding networks in mushroom-forming fungi integrate multiple internal and external cues into a developmental decision for sexual reproduction. This section summarizes some generalities on the induction of fruiting body formation.

Nutrient accessibility is one of the major factors governing sexual reproduction in microbes. Starvation triggers sporulation not only in fungi but also in slime molds (189), suggesting that this response is ancient. The mechanisms behind starvation sensing in mushroom-forming fungi are not well explored; however, the conservation of the response suggests that it may be possible to extrapolate information from simpler organisms to mushroom-forming fungi. Starvation-induced pathways are well studied in *Schizosaccharomyces pombe* (190), *Aspergillus* spp. (191), *S. cerevisiae* (192), and *Candida albicans* (193), in which GPCRs, Ras, cAMP-dependent pathways, and MAPK, as well as carbon/nitrogen sensing pathways, play key roles (reviewed in reference 194). cAMP-dependent pathways are involved in nutrient sensing and chemotactic cell aggregation also in *Dictyostelium* (189). In *S. cerevisiae* and *S. pombe*, the addition of glucose to starved cells increases cAMP levels (via activation of adenylate cyclase) and causes cAMP to bind cAMP-dependent protein kinases (PKAs), leading to the inhibition of mating and sporulation. On the other hand, under starvation, reduced cAMP and PKA activity induces sexual development. Fruiting body development responds to nutrient availability putatively through similar mechanisms. Constitutively active versions of the *S. commune* G-protein α-subunits resulted in a 160 to 200% increase in intracellular cAMP levels in dikaryons and suppressed fruiting, indicating a role for cAMP signaling in mushroom formation (195). In *C. cinerea*, cAMP levels rise during hyphal knot and primordium formation and then decline during fruiting body maturation (196). The addition of exogenous cAMP induced fruiting in *C. cinerea* (197) but not in *S. commune* (198).

Mushroom-forming fungi have specific temperature requirements to initiate and sustain fruiting body development. In many species, fruiting body development is triggered by dropping temperature, which might be a response to the onset of favorable conditions (e.g., autumn). For example, *Pleurotus* spp., *Lentinula* spp., *Flammulina* spp., or *Armillaria* spp. require a 5 to 10°C temperature drop compared to what is optimal for vegetative growth (41, 199, 200), whereas other fungi (e.g., *C. cinerea*) are able to fruit without a temperature downshift (125). Molecular aspects of the response to temperature changes are, to our best knowledge, unknown in mushroom-forming fungi.

Light influences diverse aspects of fungal physiology, including fruiting body morphogenesis (125, 201, 202). Vegetative hyphae can grow in complete darkness, whereas light is required for fruiting in most species (one exception being *A. bisporus* [141]). The active wavelength required for fruiting lies between the blue-light and near-UV range (400 to 520 nm and 320 to 400 nm, respectively), corresponding to the excitability of blue light receptors (white collar complexes and cryptochromes) (16, 202). Several aspects of the light response are species specific. For example, primordium induction requires light in most but not all species (201), whereas several developmental processes and checkpoints (e.g., cap formation and meiosis) require multiple alternating blue-light illumination/dark periods (125) in a species-specific manner. For example, under low light or in darkness, *F. velutipes* produces long stipes and underdeveloped caps, which is taken advantage of in industrial production of "enoki" mushrooms (201, 203).

There are several other factors, such as CO_2 concentration, aeration, humidity, salinity, or pH, that affect fruiting body development and that are often taken into consideration during commercial mushroom cultivation. High CO_2 concentrations tend to promote mycelial growth and suppress fruiting, whereas low CO_2 concentrations lead to malformed fruiting

bodies with longer stipes and reduced pilei (e.g., *F. velutipes* and *P. eryngii*) (199, 204, 205). In several species, higher CO_2 concentrations inhibit, whereas lower CO_2 concentrations promote pileus expansion in a number of species. CO_2 is sensed via the cAMP signaling pathway, and its concentration may be relayed by first converting it to bicarbonate by carbonic anhydrases, which then modulates the activity of soluble adenylyl cyclase so that cAMP is synthesized (206). In *S. commune*, the repression of fruiting by exogenous cAMP may mimic high CO_2 conditions and may act through similar pathways (207).

How the above-mentioned signals are sensed and what gene regulatory networks converge on the induction or repression of fruiting body initiation is poorly known in most cases. Our knowledge is too patchy at the moment to infer evolutionary patterns, which may also be complicated by the fast evolution of responses, which probably evolve hand in hand with habitat preferences. There is no ubiquitous change that leads to fructification in all fungi; instead, fruiting is a result of potentially additive effects of cascades of cellular and genetic events triggered by the environment, which are not necessarily the same across the fungal diversity. To understand these, it will first be crucial to identify key regulators and their regulatory interactions with genes, in particular those that are conserved across species.

Early developmental events. Fruiting body development starts with the formation of loose hyphal assemblages called hyphal knots or aggregates that emerge on dikaryotic mycelia. This is formed by neighboring hyphae forming a tiny round shape, called the primary hyphal knot (~0.1 mm) (65, 125). Upon light induction, in *C. cinerea*, the primary hyphal knot further develops into a tightly interwoven structure, the secondary hyphal knot (~0.2 mm) (65, 125, 204), whereas in continuous darkness, it develops into sclerotia, globose resting structures (123, 127, 146, 204, 208). Secondary hyphal knots and sclerotia very likely share the same initiation pathway (149, 209) and have similar transcriptomic profiles (149). Hyphal knots are three-dimensional structures, whereas in the vegetative mycelium hyphae run in a loose arrangement (following fractal-like dimensions). Accordingly, a number of multicellular processes, such as adhesion, cell wall remodeling, and possibly new pathways of cell-to-cell communication are activated in hyphal knots. Many of these are quite poorly known currently; however, we have some information on cell surface proteins, which may be related to adhesion, as well as some structural and regulatory genes.

A group of cell surface proteins, hydrophobins, are known to play crucial roles in the aerial growth of hyphae. For example, SC3 of *S. commune* has been reported to be present in the cell wall of monokaryotic and dikaryotic hyphae and fruiting bodies as well (210) and was thought to be crucial for hyphal-knot formation. SC3 homologs have been described in multiple species (ABH3 of *A. bisporus* [211], CoH1 of *C. cinerea* [212], POH1 of *P. ostreatus* [212], FVH1 of *F. velutipes* [213], and LeHYD2 of *L. edodes* [214]). Added to the culture medium, purified SC3 could complement the aerial hypha defect of the ABH3 mutant of *A. bisporus*, suggesting conserved roles for these hydrophobins during fruiting body initiation (211). Some hydrophobins were reported to line air channels within fruiting bodies (e.g., SC4 of *S. commune* [215] and ABH1 of *A. bisporus*) that prevent water entering into these channels. Other cell surface proteins, the galectins CGL1 and CGL2 of *C. cinerea* may be involved in defense during fruiting body formation (147, 216, 217). *Cgl2* expression is induced in hyphal knots, and its expression is maintained throughout fruiting body formation, whereas *cgl1* is expressed in primordia and mature fruiting bodies (218). The promoter sequence of *cgl1* and *cgl2* of *C. cinerea* contains a CRE motif, which suggests that their expression is regulated by cAMP (218). CGL1 and CGL2 bind to some as-yet-unknown β-galactoside-containing lipids (219). Other lectins were also shown to be upregulated in hyphal knots (128), such as the jacalin-related lectin in *F. velutipes* (220) and the AAL galectin of *A. aegerita* (221). Some lectins (e.g., CCL1 and CCL2 of *C. cinerea*) play a defense role against predators (222). GPI-anchored and fasciclin-like proteins may also be involved in cell adhesion (41). A fasciclin-like molecule encoded by *Le.fl p1* was identified as a fruiting body-specific gene in *L. edodes* (223); it is localized in the gills, especially at the boundary between the subhymenium and the trama, where the differentiation of the basidia takes place, as well as at the cortical regions of the primordium, the pileus, and the stipe (223). Transcriptomic studies suggest

that further GPI-anchored and fasciclin-like proteins are upregulated in hyphal knots (25), suggesting an important role of secreted proteins in early development.

The regulation of hyphal-knot formation involves G-protein-activated MAP kinase and cAMP/PKA signaling (224). The spontaneous *thn1* mutation of *S. commune* suppresses aerial hyphae and subsequent fruiting body formation (145, 225). *thn1* encodes a GTPase activating protein that negatively regulates G-protein signaling (226). Its deletion in *S. commune* revealed that *thn1* is involved in multiple processes, such as the temporal coordination of clamp cell (see Glossary) formation, the production of certain metabolites, and the induction several hydrophobins (e.g., Sc3 [224]). The role of G-protein signaling in aerial hypha formation and fruiting was further evidenced by the lack of aerial hypha and fruiting body formation in mutants with constitutively active α-subunits, *Gpa1* and *Gpa3*, of *S. commune* (195). This resembled the phenotype of the *thn1* mutant (195). The mutation elevated intracellular cAMP level in *S. commune* (227), which might mechanistically explain the block in fruiting. An interesting G-protein β-subunit-like protein with seven WD40 domains, *cpc2* was also reported to regulate intracellular cAMP level in *F. velutipes* (228). The knockdown of *Fv.cpc2* completely impaired fruiting body formation in *F. velutipes*. CPC2 is a widely conserved protein among fungi, and *Fv.cpc2* was able to complement the sexual developmental defects caused by *cpc2* deletion in *N. crassa* (228). Its *C. neoformans* ortholog, *Gib2*, was reported to function as an alternative G-protein β-subunit (229).

Transcriptional regulation of hyphal-knot initiation is also known to some extent. In *S. commune*, seven functionally characterized transcription factors (*bri1*, *c2h2*, *fst3*, *fst4*, *hom1*, *hom2*, and *gat1*) are significantly upregulated during fruiting body formation (18), of which three were proven to be involved in aggregate (i.e., hyphal-knot) formation. Of these, *hom2* and *bri1* affect vegetative colony morphology: deletion mutants have symmetrical colonies and cannot form aggregates (18). Another transcription factor, *fst4*, does not affect vegetative colony morphology but regulates aggregate formation and is hypothesized to act downstream of *hom2* and *bri1*. These transcription factors, as well as their expression patterns, are conserved among mushroom-forming fungi (169). Deletion mutants of the transcription factor *c2h2* of *S. commune* form aggregates, but their development cannot progress further. *c2h2* probably functions downstream of fst4 and seems to be conserved among Agaricomycetes (18). Its expression was found to be developmentally regulated in several species (25, 169, 230), and its overexpression accelerated fruiting body formation in *A. bisporus* (198). In *L. edodes*, *priB*, a transcriptional regulator with a Zn (II)2Cys6 zinc cluster and a bZIP domain, was found to be upregulated in the primordium and the fruiting body of *L. edodes* (231). Its binding site was determined (232), and three genes (*priB*, *mfbC*, and *uck1*) were reported to be *priB* dependent (233). Its homologs are also developmentally regulated in *S. commune* and *Auriculariopsis ampla* (169). Although *priB* was originally reported to be most abundantly expressed in primordia of *L. edodes* (231), transcriptomic data showed that it is already upregulated at the initiation of fruiting body development in *L. edodes* (142). In *F. velutipes*, an HMG-box protein, PDD1, was also reported to be involved in fruiting body initiation. Knockdown of *Fv.pdd1* resulted in slower vegetative growth and the lack of primordium formation, whereas its overexpression resulted in higher yields (234). However, further investigations are needed to elucidate its exact function. Another interesting HMG-box transcription factor that impacts the initiation of fruiting is *pcc1* (235). In *C. cinerea*, mutation of the *pcc1* gene leads to pseudoclamp formation and precocious fruiting in homokaryons without mating. *pcc1* is suggested to be a transcriptional repressor (207, 236). In addition, the pH-responsive transcription factor Rim101/PacC family govern pH-dependent morphological transition and virulence in many fungal pathogens (237–241). Interestingly, the homologous gene in *Ganoderma lucidum*, GlPacC, was developmentally regulated during fruiting body formation, and the GlPacC-silenced strain was defective in primordium formation (242); its cell wall was 25 to 30% thinner and contained ~20% less β-1,3-glucan than that of the wild-type strain (243).

Less is known about the chromatin-level regulation of hyphal-knot development. Investigation of REMI-generated mutants of *C. cinerea* uncovered a putative arginine methyltransferase, *Cc.rmt1*, which seems to be involved in multiple processes during

growth and fruiting body development (244). The *rmt1* mutants showed a reduced growth rate on media containing glucose as a carbon source and altered differentiation of aerial hyphae and failed to form normal hyphal knots and oidiophores. Interestingly, on media containing sucrose as a carbon source, the vegetative growth defect was not observed, but the cultures still failed to develop hyphal knots and oidia (244). *Cc.rmt1* is homologous (244) to *A. nidulans RmtA* (245), an epigenetic developmental regulator that is reported to methylate histone H4 (245) and control conidium and sclerotium differentiation.

REMI-generated mutants of *C. cinerea* comprised another strain with a hyphal-knot defect, in which the underlying gene was identified as a homolog of the yeast *snf5* protein (246), a well-studied component of the *S. cerevisiae* SWI/SNF complex (247). *C. cinerea* snf5 contains two repeats (rp1 and rp2), but unlike its yeast homolog (247), it also contains a C-terminal GATA Zn-finger domain. Further analysis of the *Cc.snf5* gene showed that the complete disruption of the gene affects dikaryon formation, while the disruption of the Zn-finger domain alone affects only hyphal-knot formation (244). *snf5* was reported to play a role in the sexual differentiation of *C. neoformans* (248) and to interact with transcriptional regulators in *S. cerevisiae* (249). *Cc.snf5* may act similarly, with the additional GATA Zn-finger domain extending its function (244).

During hyphal-knot formation, hyphae branch extensively, which requires fast mitotic division. The genetic background of this process is poorly understood; nevertheless, two mitosis-related genes were reported to affect hyphal-knot formation (250, 251). The first is the *Cc.arp9* gene (251), a homolog of *S. pombe arp9*, which is an actin-related protein and reported to be a part of the SWI/SNF and the RSC chromatin remodeling complex (252). The deletion of the last 33 C-terminal amino acid residues of *Cc.arp9* caused defects in vegetative growth, hyphal-knot initiation, and oidiophore development (251). This suggests an indirect role for this gene during fruiting body formation, possibly via its role in sister chromatid cohesion, as was reported for its fission yeast homolog (251). Via analyzing temperature-sensitive strains with fruiting body formation defects, Muraguchi et al. (250) identified *Cc.smc1*, which plays a role in metaphase to anaphase transition during the cell cycle of *C. cinerea* and, interestingly, affects hyphal-knot differentiation. The *Cc.smc1* mutants carried a 14-amino-acid insertion in the highly conserved C-terminal region of the protein (250). The homologs of *smc1* are cohesin subunits of the SMC (structural maintenance of chromosomes) family proteins (253) and are involved in mitotic sister chromatid cohesion. The authors hypothesized that rapid cell division in the compact core of the hyphal knot of *C. cinerea* might be impaired by the altered kinetics of the cohesin subunit (250). Although both *Cc.arp9* and *Cc.smc1* were reported to play a role in mitotic sister chromatid cohesion and are linked to hyphal-knot defects, they seem to act in different phases of hyphal-knot formation. However, the available information on these mutants is not sufficient to decide whether or not both genes are involved in the same process during the intensive branching within the hyphal knot. The differentiation of the hyphal knot into fruiting body also depends on correct dikaryotic cell cycle that ensures the synchronous division of the two parental nuclei within the dikaryotic cells. In *C. cinerea*, the knockdown of the expression of two conserved kinases of the DNA damage response pathway, Cc.Atr1 and Cc.Chk1, resulted in high level of abnormal mitosis and the silenced strains were arrested in the hyphal-knot stage (254).

Interestingly, a UV-generated mutant of *C. cinerea* is also arrested in the transition between the hyphal-knot and primordium stages, and the gene responsible for the mutant phenotype was reported to encode a cyclopropane fatty acid synthase, *cfs1* (255). Mutant *cfs1* carries a mutation in a domain suggested to be involved in the catalytic function of the enzyme (255). It was upregulated during the hyphal knot to primordium transition (222). However, its exact function remains to be elucidated.

Tissue differentiation and growth. (i) Regulation of differentiation. Under appropriate conditions (e.g., light induction [see details on light regulation below]), the hyphal knot differentiates into primordia with clearly distinguishable stipe, cap, and gill tissues (65) (Fig. 8). This "pattern formation" is barely understood in mushroom-forming fungi. The process needs, among others, a blue-light stimulus (125). Under constant darkness, *C. cinerea* produces "dark stipes" in which the stipe and the pileus remain rudimentary

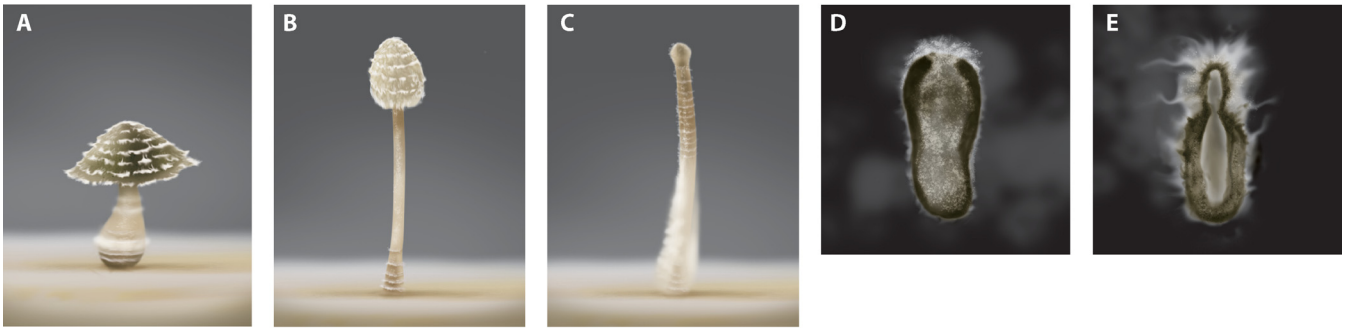

FIG 9 Phenotypes of five developmental mutants of *C. cinerea*. (Drawings are based on photographs in references 17, 147, 267, 282, and 289.) (A) *eln3-1* (*elongationless3*) mutant. The stipe of the mutant does not elongate due to a mutation in the *eln3* gene that encodes a membrane-bound glycosyltransferase (289). (B) *exp1-2* (*expansionless1*) mutant. The mutant is defective in pileus expansion and autolysis; therefore, its cap never expands and remains pale due to a mutation in the transcription factor gene *exp1* (282). (C) *dst1-1 dst2-1* (dark stipe1,2) mutants. In these mutants, the cap and the stipe remain rudimentary, and the basal plectenchyma elongates due to a mutation in the ortholog of the conserved blue light photoreceptor *wc-1* (*dst1*) and a putative novel photoreceptor (*dst2*) (17). (D) *ich1-1* (*ichijiku*) mutant. The mutant is unable to develop a differentiated pileus and cannot produce basidiospores. The differentiation of the veil cells of the *ich1-1* mutant is restricted to a small area at the top of the primordium (267). (E) *cag1-1* mutant. The mutant is unable to develop gills due to a mutation in the *cag1* gene encoding a conserved transcriptional repressor (147).

and the basal plectenchyma elongates (19, 125) (Fig. 9C). Two mutant strains of *C. cinerea*, *C. cinerea dst1-1* (19) and *C. cinerea dst2-1*, showed the same phenotype even under fruiting inducing conditions. The genes underlying the mutations are *dst1* and *dst2*, respectively. *dst1* was reported to be a homolog of *N. crassa wc-1*, which encodes a phototropin-like receptor (WC-1) that interacts with WC-2, forming the white-collar complex (WCC) that regulates the expression of light-responsive genes (16). Like its homolog, *C. cinerea dst1* was also suggested to function as a blue-light photoreceptor (19). *dst2* encodes a protein with a FAD-binding 4 and a berberin-like domain and was suggested to act as a novel photoreceptor, though its exact function remains to be elucidated (17). The genome of *C. cinerea* harbors a homolog of *N. crassa* WC-2, the other component of the WCC. The disruption of *Cc.wc2* resulted in a dark stipe phenotype, confirming an identical role for its *N. crassa* homolog (256). In *L. edodes*, the orthologs of WC-1 and WC-2, PHRA and PHRB, respectively, were reported to interact with each other via a PAS domain, and the light-dependent regulatory role of PHRB was confirmed (257). In *S. commune*, inactivation of either *wc-1* or *wc-2* also resulted in a blind phenotype (258): instead of asymmetrical colonies, the dikaryon formed symmetrical ones, and mushroom formation was abolished (230, 258). Gene expression studies on WCC deletion strains identified several downregulated genes known to be involved in fruiting body development of *S. commune*, including *c2h2*, *hom1*, and multiple hydrophobin genes (230).

Though blue light (wavelengths 400 to 525 nm) is known to be the most important for the initiation of fruiting body differentiation, other photoreceptors were suggested to play a role in fruiting in several species. Flavin-related molecules might be photoreceptors in *S. commune* (259) and *C. congregatus* (260). In *P. ostreatus*, a cryptochrome-like photoreceptor system was suggested to regulate hyphal aggregation (261), whereas putative far-red sensing photoreceptors were reported in *Armillaria ostoyae* (41). (For a detailed review of photoperception and light-regulated gene transcription, see references 202 and 262.)

The velvet family contains fungal-specific transcriptional regulators, which play a key role in coordinating sexual and asexual development and secondary metabolism (263, 264). Their function is mostly studied in Ascomycetes; however, velvet proteins appear to have morphogenetic roles in the Basidiomycota as well (e.g., *Umv1*, *Umv2*, and *Umv3* in *Ustilago maydis* [265]). RNA-seq studies in mushroom-forming fungi revealed that velvet family members were developmentally regulated and show conserved expression patterns in *L. bicolor*, *S. commune*, *C. cinerea*, and *L. edodes*, etc. (26, 222, 266).

(ii) Differentiation and maturation of the cap. In *C. cinerea*, after the hyphal knot receives a light stimulus, the differentiation and later the maturation of the cap and the stipe happen more or less in parallel. Limited information is available for the differentiation of the cap and its tissues. The *ichijiku* mutant of *C. cinerea* is a result of a spontaneous

mutation that produces an odd-shaped primordium in which the pileus does not differentiate and the mutant is unable to produce basidiospores (267) (Fig. 9D). The *ich1* gene encodes a protein with a methyltransferase domain and a winged helix-like DNA-binding domain, but its exact function is unknown (267). Another cap-related mutant is the UV-generated *cag1-1* mutant of *C. cinerea*, in which the stipe and the pileus start to differentiate, but gills do not develop (147) (Fig. 9E). *cag1* is a homolog of *S. cerevisiae tup1* (268), a conserved transcriptional repressor (269) involved in several cellular processes such as sexual and asexual development and developmental switching (270, 271). *C. cinerea* has a paralogue of *cag1*, named *Cc.tupA*. Using reciprocal tagging, their location was visualized, and both genes were found to be highly expressed in the developing gills (147). *cag1* and *Cc.tupA* might be members of a conserved regulatory network involved in differentiation processes in fungi.

Interestingly, in *L. edodes*, a MAP kinase, Le.MAPK, and its interacting partner (Le.DRMIP) were shown to be highly expressed in the developing gills (272), especially in hymenophores. The hymenophore contains gill trama hyphae which at this time start to differentiate into probasidia, in a topology similar to the localization of *tupA* of *C. cinerea* (147, 272). Cdc5 is a putative transcription factor known originally from *L. edodes* and is hypothesized to affect the differentiation of the cap (273). Immunohistochemistry showed that *Le.cdc5* is expressed in similar levels in the primordia and the immature fruiting body, but the primordial prehymenophore (the region between the pileus and the stipe, where the hymenophore develops) contained larger amounts of its transcript (274). In *L. edodes* the abundance of *Le.cdc5* transcripts was observed in the hymenium, where basidia and basidiospores are formed. Cdc5 contains a Myb-type DNA-binding domain and several putative nuclear localization signals and is reported to bind a 7-bp consensus sequence (5′-CAACAC/T/G-3′) (274). However, orthologs of *cdc5* from *S. cerevisiae* (275), *S. pombe* (275), and the human *Cdc5L* (276) were found to be essential for pre-mRNA splicing. No such role was reported for *Le.cdc5*, while Nakazawa et al. (277) proved that the protein is able to interact with another DNA-binding protein, Le.CIPB, and together they regulate the expression of the gene *ctg1*. The same regulatory circuit was found in *C. cinerea*, where *ctg1* was proven to be exclusively expressed in vegetative mycelia and in the hymenium (278). *ctg1* overexpression resulted in a more rapid stipe elongation in *C. cinerea* and *L. edodes* (278). This suggests the existence of an interesting regulatory process where a cap-specific gene regulates the elongation of the stipe.

Limited information is available about the differentiation of the basidium. Some information on basidium development is available in *C neoformans* (279). *pum1* of *C. neoformans* is an RNA-binding protein that is known to play a crucial role in basidium differentiation and sporulation (279). Its deletion causes severe defects in basidium morphogenesis and was shown to induce the expression of *csa2*, which temporally coordinates meiosis and basidium maturation (280). In *F. velutipes*, five *pum1* homologs were identified, among which two were significantly upregulated in young fruiting body cap and fruiting body cap, where basidium differentiation happens (281).

The cap of *C. cinerea* expands 3 h before the onset of autolysis. Muraguchi et al. (144) found two strains defective in pileus expansion and autolysis. A single gene, named *exp1* (expansionless 1), proved to be responsible for this phenotype in both mutants (282) (Fig. 9B). *Exp1* encodes an HMG1/2-like protein with two HMG-boxes at its C terminus. The expression level of *exp1* is low in the primordium and reaches the highest level in the pileus 3 h before its expansion (282). *exp1* was also identified in *L. edodes*, found to be expressed postharvest, and speculated to control cap senescence (283). The main effectors behind the autolysis of fruiting body cells are cell wall-degrading enzymes (283). These results suggest that *exp1* in fruiting-body-forming fungi participates in the regulation of the expression of genes responsible for cell wall degradation.

(iii) Differentiation and elongation of the stipe. The stipe differentiates parallel with the cap to support and position it for efficient spore dispersal. The stipe elongates via the lateral extension of stipe cells with no or limited contribution of cell division to the elongation process (284). In the secondary hyphal knot, hyphae line up in parallel to give rise to the stipe, emerging from the primordial shaft (125). The *elongationless2* mutant (*eln2-1*) of *C. cinerea* produces a "flat" primordium and later develops a short

stipe in the mature fruiting body (285). This is the consequence of a mutation in the *eln2* gene, which encodes a cytochrome p450 enzyme with an as-yet-unknown function. In plants, cytochrome p450 enzymes are reported to regulate development via the production of phytohormones (286, 287). No such cytochrome p450 produced compounds were reported to be involved in fungal development, but it should be noted that a hormone-like substance, 10-oxo-*trans*-8-decanoic acid, was reported to influence stipe elongation of *A. bisporus* (288); therefore, the existence of such substances cannot be ruled out (285). Another mutant, *elongationless3*, produces a fruiting body with a short stipe (289) (Fig. 9A). As mentioned above, in wild-type *C. cinerea* stipe cells line up in parallel and form side-by-side contacts with each other. In the mutant, stipe cells were not arranged in a parallel fashion and did not elongate properly and, unlike in a normal stipe, there was a space between the cells (289). The *eln3* gene encodes a glycosyltransferase located in the plasma membrane and was found to be expressed in the rapidly elongating stipes, but no further details are available about its function (289). It is noteworthy that homologs of *eln3* were found to be developmentally regulated in *F. velutipes* (281), *V. volvacea* (290), *Rickenella mellea*, *Lentinus tigrinus*, and *A. ostoyae*, all with a well-developed stipe but not in the stipeless cyphelloid *S. commune* and the resupinate *Phanerochaete chrysosporium* (25, 41). The UV-generated mutant collection produced by Muraguchi et al. (144) contained one more elongationless mutant for which the underlying gene was elucidated and designated as *eln8-1* (291). The mutation was rescued by a septin gene homologous to *S. cerevisiae cdc3*. In the mutant, stipe cells fail to elongate; instead, they inflate. The mutation also affects the veil cells that take a lemon shape. It was concluded that *Cc.cdc3* is responsible for the maintenance of the cylindrical shape of stipe and veil cells. Shioya et al. (291) labeled *Cc.cdc3* using enhanced green fluorescent protein to observe its cellular localization and found that before the elongation of the stipe, *Cc.cdc3* can be found in patches, while in elongating stipe and veil cells *Cc.cdc3* arranges in abundant thin filaments around the cortex parallel to the longitudinal axis of the cells (291).

During stipe elongation, the lateral extension of stipe cells is limited to the apical region of the stipe in most fruiting-body-forming fungi (292–295). The molecular background of stipe elongation has been reviewed thoroughly recently (148); therefore, we only briefly discuss here the genes involved in the process. In the elongating stipe, the rigid fungal cell wall must be loosened by the concerted action of several cell wall-modifying enzymes (e.g., chitinases, β-1,3-glucanases, and β-1,6-glucanases [148]) to provide plasticity for the wall to be able to expand following increasing turgor pressure during stipe elongation (148). Several proteins belonging to the above groups were proved to be developmentally regulated during fruiting body formation (25). Chitinases are reported to play a key role in this process. In *C. cinerea*, two chitinases were proven to be able to reconstitute heat-inactivated stipe elongation: ChiE1 (296) and ChiII (297). Double knockdown of these two enzymes resulted in a defect of stipe elongation in *C. cinerea* (298). Besides chitinases, β-1,3-glucanases are also known to be involved in cell wall extension during stipe elongation, including ENG, BGL2, or EXG of *C. cinerea* (for a detailed description, see reference 290). Another exo-β-1,3-glucanase, EXG2, belonging to glycoside hydrolase family 55, participates in stipe elongation, cap expansion, and senescence of *V. volvacea* and *L. edodes* (299). Chitin deacetylases, such as *cda1* and *cda2*, are also involved in stipe elongation of *C. cinerea* (300).

APPLIED ASPECTS OF FRUITING BODY DEVELOPMENT

Fruiting body development has important and dynamically expanding biotechnological applications. Mushrooms represent healthy and sustainable food products, a source of promising medicinal (antioxidant, antimicrobial, and immunomodulating) compounds and, most recently, are becoming industrial workhorses in diverse applications. The global edible mushroom market is valued over US$54 billion in 2020 and is expected to expand, as the per-capita consumption of the ~350 edible mushroom species rises (33, 301). The pharmaceutical uses of mushroom fruiting bodies are outstandingly versatile, and the medicinal application of mushrooms has a long history and new applications in modern health care (302, 303). For example, lentinan, a polysaccharide isolated from *Lentinula edodes* (Shiitake),

is one of the most widely used β-glucan due to its immunostimulatory activity (304). Clinical trials showed that psilocybin, a compound isolated from fruiting bodies of *Psilocybe* spp., is very promising in treating depression, anxiety, and certain addictions (31, 305).

The improvement of commercial mushroom strains is still mostly based on conventional breeding approaches, such as selection and hybridization (306, 307). Industrially important traits have been targets of breeding programs, such as senescence (shelf-life), shorter life cycle, resistance to pathogens, or spore production. A non-browning button mushroom (*A. bisporus*) strain constructed using the CRISPR-Cas9 technology became the first organism confirmed unregulated by the USDA (U.S. Department of Agriculture). This non-browning strain is defective in one of the polyphenol oxidase enzyme-encoding genes (308). A sporeless strain, deficient in the *msh4* gene, has been reported in *P. ostreatus*, which overcomes the problems caused by intense sporulation of this species (309, 310) (e.g., allergic reactions, spread of diseases, and depletion of genetic diversity in natural populations). Spent mushroom compost is rich in both lignocellulosic plant materials and fungal compounds, such as the antimicrobial polysaccharide chitosan, and is thus particularly apt for circular economy (311). Beyond fruiting body production, mycelia of Agaricomycetes have promising applications as insulating or building materials or as animal-free leather alternatives (312). Leveraging evo-devo knowledge for improving the biotechnological application of mushroom-forming fungi is becoming feasible as we gain more and more information on how fruiting bodies develop. With the application of "omics" and genome-editing techniques, we can gain insight into the molecular mechanisms and regulatory networks of fruiting body morphogenesis, including industrially exploitable target genes for improving environmentally sustainable manufacturing (313–315).

CONCLUDING REMARKS: TOWARDS MUSHROOM EVO-DEVO

Fruiting bodies are the result of a fascinating developmental process and of a possibly even more fascinating series of evolutionary transformations. The field of "evo-devo" studies how developmental programs change during evolution; as such, it is a marriage between evolutionary and developmental biology. Research on fruiting bodies has made tremendous progress in both fields. Phylogenetic studies uncovered trends in how morphologies evolved, while developmental and genetic studies revealed some key genes underlying fruiting body development. Here, we reviewed advances in both fields and showed that these are painting an ever more refined picture on the biology of fruiting bodies.

One of the big questions mycologists will need to answer is what drives convergence in fruiting body morphologies. Is it driven purely by ecological adaptation, or are there genomic factors that promote the repeated emergence of the same morphologies? Genetic mechanisms that constrain evolvability or that promote convergence are starting to emerge from studies of fungal and nonfungal organisms (48, 316). From an ecological perspective, we currently have little information on how external factors could drive the convergent evolution of fruiting bodies. While we suspect that gasteroid morphologies represent adaptations to dry habitats, no such clear hypothesis exists for others and species with various morphologies (e.g., pileate-stipitate and coralloid) can coexist in what appears to be the same habitat.

On the other hand, genetic information on fruiting body development has been accumulating at a steady pace, thus far mostly based on the analyses of spontaneously occurring mutants of ones selected from mutagenesis screens. A promising new source of functional hypotheses are genomic and transcriptomic data sets, especially comparative ones, which can pinpoint dozens to hundreds of genes that show a consistent association with fruiting body development across species. The community has just started to develop and embrace routine genome engineering techniques (CRISPR/Cas9) (317–323), which will allow us to test functional hypotheses on genes in a systematic and large-scale manner. Population genomics might prove to be another rich source of hypotheses on genotype-phenotype mapping, especially in economically interesting species.

As of today, however, insights from phylogenetics and developmental biology remained largely disjunct, which is a major caveat that should be remedied for mushroom evo-devo to really come to fruition. The multitude of approaches available for mycologists today should allow a new synthesis of phylogenetic and developmental/genetic

information into a coherent picture on fruiting body evolution. This will answer, or at least outline strategies for solving, many of the biggest questions of the field, such as what genetic circuits (e.g., master regulators) underlie the induction of fruiting body development, tissue differentiation (e.g., into cap and stipe), and cell fate within fruiting bodies, as well as, from an evolutionary perspective, the origins of fruiting bodies, transformations between fruiting body types, or convergence. Although these topics represent a subjective listing of what we perceive as some of the grand questions in fruiting body biology, if solved successfully, such research should allow mushroom evo-devo to emerge and mature as a field. Solving pieces of this puzzle will also allow improving applied aspects of mushroom biology as well, such as the development of better mushroom crops and biotechnology.

APPENDIX

GLOSSARY

agaricoid A type of fruiting body with stipe, cap, and gills.

angiocarpic/hemiangiocarpic/gymnocarpic development These terms describe the position of the hymenium relative to the environment during fruiting body development. The hymenium can be exposed completely during development (gymnocarpic) and can be enclosed first and exposed during spore production and maturation (hemiangiocarpic) or enclosed during fruiting body development and ruptured only after spore production (angiocarpic).

basal plectenchyma A tissue at the bottom of the differentiating primordium consisting of undifferentiated hyphae. It stores glycogen that is translocated into the fruiting body during development.

ballistospory The forcible spore discharge mechanism of Basidiomycota.

Buller's drop A drop of liquid that appears at the apex of the basidiospore. Rapid movement of the liquid drop is essential for spore discharge.

clamp cell (=clamp connection) Hook-like structures formed by terminal hyphal cells that ensure the proper distribution of genetically distinct nuclei during mitotic cell divisions. Clamp cells are unique to the Basidiomycota.

***Clavaria* theory** A hypothesis put forth by E. J. H. Corner, which posits that pileate-stipitate morphologies evolved via coralloid/clavarioid intermediates.

coralloid A fruiting body type that resembles a club or branched coral, without a cap and stipe.

cyphelloid Derived, cup- to barrel-shaped, pendant fruiting body type with a size usually less than 2 mm in length and diameter.

cystesium (plural: cystesia) A specialized adhesive cell type in the hymenium of several Agaricomycetes that makes adhesive contact with a cystidium.

cystidium (plural: cystidia) A specialized cell type in the hymenium of several Agaricomycetes. They are believed to act as spacer cells.

gasteroid A fruiting body type in which spores are produced internally. Their hymenium evolved into an enclosed structure called gleba.

heterochrony Shifts in the relative rate and timing of developmental events.

hymenium Spore-bearing tissue layer on the hymenophore of Agaricomycete fruiting bodies where hyphae develop into basidia.

hymenophore The hymenium-bearing structure of the fruiting bodies.

lamellae Gills of a mushroom.

oidium (plural: oidia) A thin-walled fungal spore, produced asexually by certain fungi.

paraphysis Specialized, vacuolated, sterile spacer cells, considered to be the major structural components of the lamellae of certain mushroom-forming fungi. They arise as secondary branches of subbasidial cells.

pileate-sessile A fruiting body type with a cap, but no stipe.

pileate-stipitate A fruiting body type comprising the classic toadstool morphology with a cap, a stipe, and complex hymenophore (gills, pores, and teeth).

pileus The cap of a mushroom.
plesiomorphic An extant property that reflects an ancestral character state.
resupinate A fruiting body morphology that usually consist of a flat layer of fertile hyphae with additional supporting hyphal layers in some species. Resupinate fruiting bodies follow the morphology of the substrate (see Fig. 1A).
secotioid A fruiting body morphology that bears resemblance to both gasteroid and pileate-stipitate types. They resemble a pileate-stipitate mushroom in which the cap fails to open. Secotioid species typically retain ballistospory (Fig. 1A to C).
secotioid syndrome A series of morphological traits (closed pileus, shortened stipe, thickened veil) that cooccur in secotioid fruiting bodies. This "syndrome" was recognized and forms the basis of a hypothesis put forth by Harry Thiers on the origins and adaptive value of secotioid fruiting bodies.

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