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Mycofluidics: The Fluid Mechanics of Fungal Adaptation

Marcus Roper[1] and Agnese Seminara[2]

[1]Department of Mathematics and Department of Biomathematics, University of California, Los Angeles, California 90095, USA; email: mroper@math.ucla.edu

[2]Institut de Physique de Nice, CNRS UMR 7010, and Université Côte d'Azur, Nice 06108, France; email: agnese.seminara@unice.fr

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Abstract

Fungi are the dark matter of biology, typically leading cryptic lives, buried in soil or inside of plants or other organisms, and emerging into the light only when they build their elegantly engineered fruiting bodies. Ecological success across so many niches has required that they solve many challenging fluid mechanical problems of growth, dispersal, and transport of fluids across networks. Study of fungal life histories by fluid mechanicians has shown their exquisite capability for engineering and revealed new organizing ideas for understanding fungal diversity.

1. INTRODUCTION

Fruiting body: the sexual structures produced by the fungus to disperse its spores

The ability of mushrooms to spring up after rain, apparently overnight, must have baffled early observers. Pliny the Elder (1634) called the sudden appearance of mushrooms without "any chink or crevasse from which they should issue" one of the greatest "wonders of Nature" (p. 7). He concluded that mushrooms must grow spontaneously out of the "humor of the earth" (p. 132). Yet, as early as the nineteenth century, the physical contexts of fungal growth became known: de Bary (1887), who attempted the first systematic description of the fungal kingdom, realized that the spectacular growth of mushrooms is in fact driven by the hydrostatic expansion of cells. The work of Buller (1909–1950) in the early twentieth century showed the precision with which these fruiting bodies must be sculpted: The spacing between the gills of the mushroom matches closely the distance that the spores liberated from the mushroom travel in their first discharge, and this discharge itself is produced by what is now called a surface tension catapult—by the energy liberated from the coalescence of two osmotically condensed droplets.

Indeed, fungal spore dispersal was, through Buller's pioneering work (Buller 1909–1950), one of the earliest areas of nonmedical biomechanics to bring biology and fluid mechanics together. Yet, as we show, despite a century of activity since Buller, we have only just started to understand the role of fluid mechanics in shaping fungal life. In this review, we describe how fungi have adapted to solve challenging fluid mechanical problems in the course of their growth and dispersal. Our story tracks the fungal life cycle, starting, as the life cycle does, with a single cell that uses turgor pressure to grow into a filamentous cell. In particular, we emphasize how the tuning of intracellular mechanics enables fungi to organize their huge cells. From there, we consider the formation of branched transport networks, through which resources and organelles must continually flow to sustain fungal growth. Finally, we describe adaptations that enable fungi to move between habitats.

Why is understanding the physical lives of fungi a pressing question? Our closest experiences of fungi may be the mushrooms that we see sprout up after rains (or the fuzz we find on fruit or cheese that we leave too long in our refrigerators). But although the number of mushroom-producing species of fungi is large (14,000 species, compared to, for example, an estimated 10,000 species of birds and 5,400 mammals), it is a tiny fraction of the huge diversity of the fungal kingdom [1–5 million species, mostly undescribed (Blackwell 2011)]. Many of these species live cryptic lives, buried in soil or within the tissues of their hosts. Among the fungi are devastating crop pathogens that jeopardize food security (Goyal & Manoharachary 2014), while emerging fungal diseases threaten banana crops, corals, and amphibians (Fisher et al. 2012). Although fungal diseases attract the greatest level of human concern, fungi play a multitude of other ecosystem roles, including as decomposers, symbionts, and parasites (**Figure 1**) (Hawksworth 1991).

Throughout the review, our focus is on the role of fungal adaptations—that is, on the features of the fungus that enable it to manipulate or respond to the physics of its fluid environment (whether this fluid environment is external, such as the wind, or internal, such as the rheological tuning of the fungal cytoplasm). For this reason, we do not discuss at length the problem of long-distance dispersal of fungal spores, since the role of fungal adaptation in influencing the likelihood of successful dispersal is not yet understood. For a discussion of the physics of long-distance dispersal, we refer the interested reader to the excellent recent reviews by Kuparinen (2006) and Nathan et al. (2011) (the latter focuses on seed dispersal but can also be adapted to spore dispersal). Throughout this review, we largely focus on the two most widely studied fungal phyla (see the sidebar titled Fungal Phyla): the Ascomycota, which disperse by ejecting spores from fluid-filled sacs (or asci, singular ascus), and the Basidiomycota, which disperse their spores using Buller's drop mechanism. There are five other known fungal phyla that we do not discuss at length: the Microsporidia, which are single-celled fungi that live on animal hosts; three phyla (Neocallimastigomycota,

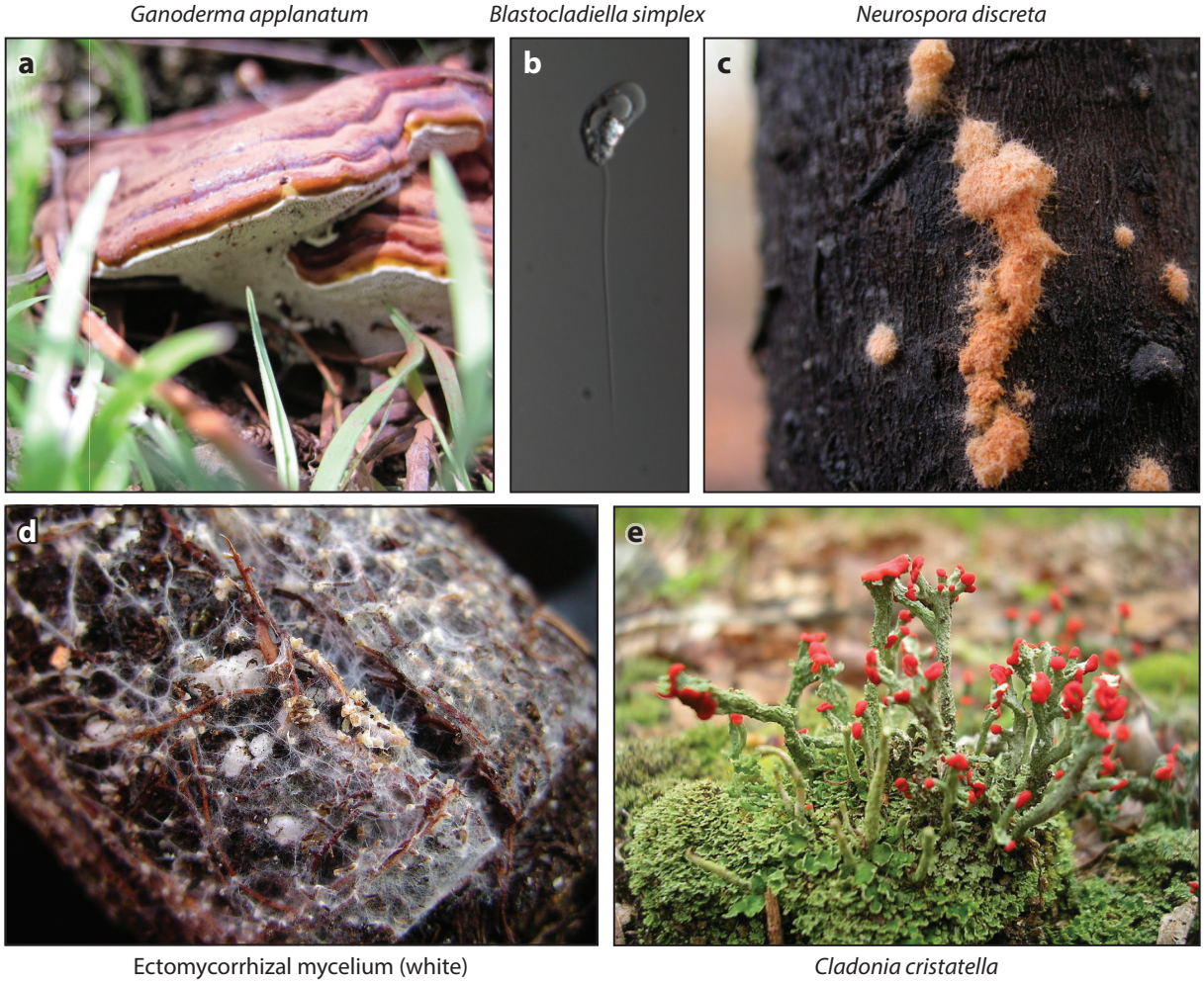


Figure 1

The fungal kingdom is extraordinarily diverse, and its members have varied lifestyles and ecosystem roles, including (*a*) a parasitic mushroom-producing fungus (*Ganoderma applanatum*); (*b*) a parasite with motile, flagellated zoospores (*Blastocladiella simplex*); (*c*) a decomposer that colonizes burned wood (*Neurospora discreta*); (*d*) mutualists that inhabit the roots of the plants (white fibers are the fungal network, and brown fibers are the roots of a spruce seedling); and (*e*) a photosynthetic lichen (*Cladonia cristatella*). Photos provided by (*a*) Marcus Roper, (*b*) Jason Stajich and John Taylor, (*c*) John Taylor, (*d*) André Picard, and (*e*) J.R. Carmichael.

Blastocladiomycota, and Chytridiomycota) that produce flagellated zoospores; and the Glomeromycota, which, similar to many Basidiomycete fungi, form networks of cells that associate with plant roots but, unlike the Basidiomycota, reproduce exclusively by budding off asexual spores. The lack of work on the physical adaptations of these species should not be misread as an indicator of their lack of importance or of the centrality of fluid mechanics to their life cycles. For example, chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis*, threatens to drive thousands of species of amphibians extinct worldwide. Both Chytridomycota and Blastocladiomycota (**Figure 1*b***) produce flagellated zoospores, which spread the disease by swimming from host

FUNGAL PHYLA

In this review, we focus on two phyla of fungi: the Ascomycota and the Basidiomycota. These phyla are distinguished by their different kinds of fruiting body. The Ascomycota include model organisms like brewer's yeast (*Saccharomyces cerevisiae*) and the bread mold *Neurospora crassa*, as well as morels and cup fungi. These species disperse sexual spores from squirt guns or asci. Basidiomycota fungi include all mushroom-forming fungi, as well as stinkhorns and puffballs. In species with forcible ejection, spores are ejected from using Buller's drop (see Section 4.2). Not all species of fungi have sexual spores or forcibly eject these spores. In many cases, assignments of fungi to phyla have to be based on the gene sequence or on features of the fungal mycelium.

to host. Yet, the physics of how zoospores swim to home in on hosts or refuges in potentially fast-moving and turbulent waterways has been little investigated.

Although our focus on two fungal phyla reflects the main axis of recent fluid mechanics research, we expect many nonfungal organisms with very similar life cycles to be similarly adapted. For example, plasmodial slime molds also produce complex cellular networks made up of fluid-transporting tubes (Alim et al. 2013), which can be reorganized to minimize transport costs and maximize mixing (Tero et al. 2010, Marbach et al. 2016). Similarly, the water molds (or Oomycota), including the forest disease *Phytophthora ramorum*, which causes sudden oak death syndrome, produce invasive cellular networks within host tissue, have wind- and weather-borne spores, and can also disperse across many miles in waterways via flagellated zoospores (Davidson et al. 2002).

2. HYDROSTATIC SCULPTURE: FUNGAL GROWTH AND FORM

2.1. Hyphal Tip Extension

The fungal life cycle starts from a single spore. Although the nuclei within a spore may remain in a quasi-dormant state for periods ranging from hours to even years, in the right conditions, the spore will start to extrude a filamentous cell or hypha. Hyphal extension is thought to be driven by turgor pressure (but see Money 1997 for an alternate viewpoint). Unlike animal cells (but like plant cells), fungal cells have stiff cell walls surrounding their plasma membranes. These walls are synthesized from chitin polymers interlinked by glucan microfibrils. The fungal cellular interior is maintained at a high turgor pressure, and to hold its shape, the cell wall must be only weakly extensible. Thus, the fungus can grow only at sites where extra material is added to the cell wall, allowing the wall to be expanded by the pressure within the cell. In fungi, a specialized machinery for trafficking cell wall materials and enzymes restricts cell wall growth to the rounded tips of cells (Riquelme & Martínez-Núñez 2016). Constant extrusion of these tips creates the fungus' long filamentous cells. Filamentous growth preserves the surface-to-volume ratio of the cells, which is important since the fungus obtains its nutrients from the surrounding medium, rather than generating them internally.

Mycelium: the vegetative (nonsexual) part of the fungus, made up of a network of thread-like cells (called hyphae)

There is no consensus on what biophysical processes allow the cell wall to expand at hyphal tips, and indeed different fungi may have different strategies for allowing growth. Lew (2011) has argued that the cell wall is strain hardening; so newly created wall can readily expand at the hyphal tip under the hydrostatic pressure within the cell, but then the rate of cell wall expansion slows as material is displaced away from the hyphal tip. Bartnicki-Garcia et al. (1989) proposed that the wall is essentially inextensible, so that growth is purely driven by the distribution of vesicles delivering

new cell wall material to the tip. Boudaoud (2003) proposed a general model for pressurized cell extension (including hyphae and pollen tubes) where an elastic cell wall yields in extension but not in compression. However, in the model fungus *Neurospora crassa*, chitinases (enzymes that break down the chitin in the cell wall) are constantly delivered to the hyphal tip, along with the vesicles that contain new cell wall materials (Riquelme & Martínez-Núñez 2016). Knocking out these enzymes arrests cell growth, suggesting that they play an important role in actively loosening the cell wall to allow it to expand. Thus, Campàs & Mahadevan (2009) adopted a viscoplastic model in which the cell wall yields to the internal pressure of the cell over an actively fluidized region of finite size near the hyphal tip, and then away from this tip the wall is inextensible. Pollen tubes also grow by localized loosening of the cell wall, and Campàs et al. (2012) have also shown that in both pollen tube tips and hyphal tips, the radius of curvature of the hyphal tip is proportional to cell radius, in agreement with a model in which the radius of the zone of fluidity is proportional to cell radius. Importantly, this scaling is not seen in all tip-growing cells. For example, in some water molds, the apical radius is independent of cell size. Indeed, some species of water mold are known not to regulate their turgor pressure and therefore cannot use turgor pressures to expand their cell walls (Money 1997).

Hyphal growth direction is responsive to many different kinds of environmental cues. For example, two hyphae will typically grow away from each other, but two genetically identical hyphae may home in on each other and ultimately fuse (see Fleißner et al. 2008 and the references therein). Some fungi living on plant and animal hosts show thigmotropism (i.e., response to touch). For example, the hyphae of the pathogenic fungus *Colletotrichum trifolii* grow along the valleys between plant epidermal cells as it searches leaf surfaces for possible sites of entry (Almeida & Brand 2017). Thomson et al. (2014) grew *Candida albicans*, which causes yeast infections in humans, on microtextured surfaces to determine how hyphal tips navigate. Because the cell may expand wherever cell wall is added, growth reorientation seems to be driven by changes in the position of a vesicle-trafficking center (called the Spitzenkörper, which means pointed body in German). When *C. albicans* encounters a ridge, the Spitzenkörper shifts to the side of the apical surface closest to the ridge. Generating new cell wall preferentially on the ridge side of the hypha may be necessary since adhesion to the ridge or friction will slow the expansion of the cell wall on that side. It also means that the hypha tends to remain in contact with the wall even if the wall turns through shallow corners, a strategy that may enable the fungus to efficiently search the spaces between cells. An additional factor that has been shown to enable fungi to efficiently search mazes (see Hanson et al. 2006) is that they retain memory of their growth direction: When a hypha encounters an isolated obstacle, it will follow the wall of the obstacle, as noted above, and then resume the direction of its earlier growth (see Thomson et al. 2014). It is unclear how an overall direction of growth is encoded within an indeterminately growing organism that lacks a central organizing system; we suspect that by laying down microfibrils in a single direction (see Section 2.4), the anisotropic elasticity of the cell wall biases hyphal growth.

2.2. Maintaining Hydrostatic Pressure

High turgor pressures need to be maintained at growing hyphal tips: Lew & Nasserifar (2009) used a pressure probe to monitor turgor pressure at the hyphal tip of the model fungus *N. crassa* during osmotic shock (see below). They found under normal growth that turgor pressure was around 0.6 MPa, but growth could occur with tip pressure as low as 0.25 MPa. Growth was completely arrested if the tip pressure was reduced to 0.15 MPa, presumably because there was insufficient hydrostatic pressure to expand the cell wall. The hydrostatic pressure within the hypha is created osmotically: The hyphal cell wall is semipermeable, the cell is rich in osmolytes, and water flows

as the hypha extends, filling the volume created at the hyphal tip. However, as the cell grows, this inflow of water naturally tends to reduce the osmotic pressure. Moreover, fungi growing on real hosts may find themselves challenged by growing through fluids whose osmotic activity varies in both space and time or by needing to compensate for osmotic pressure changes caused by their own metabolic activities. Thus, in fungi [but not all hyphal organisms (Money & Harold 1993)] turgor pressure is actively regulated. Osmotic regulation can involve both input and output of ions via K^+ and H^+ pumps, as well as (on longer timescales) synthesis or breakdown of osmolytes such as glycerol. These linked processes allowed *N. crassa* to recover its turgor pressure in Lew & Nasserifar's (2009) experiments, in which they osmotically shocked the fungus by almost doubling the osmolality of the surrounding fluid. The timescale for recovery depends on the permeability of the cell wall to the osmolyte and ranges from 10 min for NaCl salts (which readily enter the cell) to 60 min for sucrose (which does not enter the cell and so must be compensated by other osmolytes).

2.3. Pressure Attack of Other Cells

In fact, fungi are capable of creating even larger turgor pressures. Rice blast fungi (*Magnaporthe oryzae* and *M. grisea*) invade plant tissues by pushing hyphae directly through the walls of the plant epidermal cells. To penetrate the stiff cuticle that surrounds the plant cell, the fungus creates a specialized round cell (the appressorium) whose walls are reinforced by a thick layer of melanin. Pressures of 8 MPa or higher are generated within the appressorium by glycerol synthesis, high enough to penetrate sheets of Kevlar, yet the cell wall is so stiff that it hardly expands (Howard et al. 1991). Eventually the plant cell wall yields, and a pore opens in the leaf-adhered surface of the appressorium from which a highly pressurized hypha (called a penetration peg) grows through the ruptured cuticle. Although both the mechanics (Howard et al. 1991) and the cell biology of appressorium formation (Wilson & Talbot 2009) have received considerable attention, the impressive feat of adhesion achieved by the appressorium seems to be little noted. If the cell is not securely anchored to the cuticle, then the immense pressure it generates would merely cause it to pop off the plant. Thus, the adhesive between appressorium and cuticle must have a yield strength exceeding 10 MPa (the largest turgor pressure reported by Howard et al. 1991). Loctite™ superglue reports a comparable yield strength of 15–25 MPa (Henkel Corp. 2014), but appressorium glue must achieve this adhesive strength on the leaf's hydrophobic surface.

2.4. Mushroom Growth

Fungal cells are rigidified by their stiff cell walls and by high turgor pressures, allowing fungi to build complex aerial structures without skeletal support. These structures can range from single aerial hyphae to the beautifully diverse mushrooms that show up overnight after rains. De Bary's (1887) careful observations in the nineteenth century showed that the rapid growth of mushrooms is driven by cell expansion rather than by proliferation in the stipe (i.e., stalk; see the sidebar titled Parts of a Mushroom). In these fast-growing structures, cell expansion rates are limited only by how quickly water can be transported through the mushroom and not by the rates of synthesis of new cell wall, hence high growth rates are possible: According to Stiles (1994), the stipe of stinkhorn mushrooms can extend by 5 mm per minute. The mushroom unfurls in an elegant feat of cellular origami from a primordium or mushroom egg (**Figure 2*a***): Presumably, because growth is too fast for cell wall synthesis, the cell wall material for the entire structure is packed in the primordium.

PARTS OF A MUSHROOM

Around 14,000 species of Basidiomycota fungi form mushrooms to disperse their spores. In these species, spores are attached to basidia (club-shaped cells). The basidia are arranged on the sheets of tissue (gills) or inside of pores that line the lower surface of the mushroom cap. The spore-bearing tissue under the cap is called the hymenium. In many species, the cap is supported on top of a stipe (or stalk).

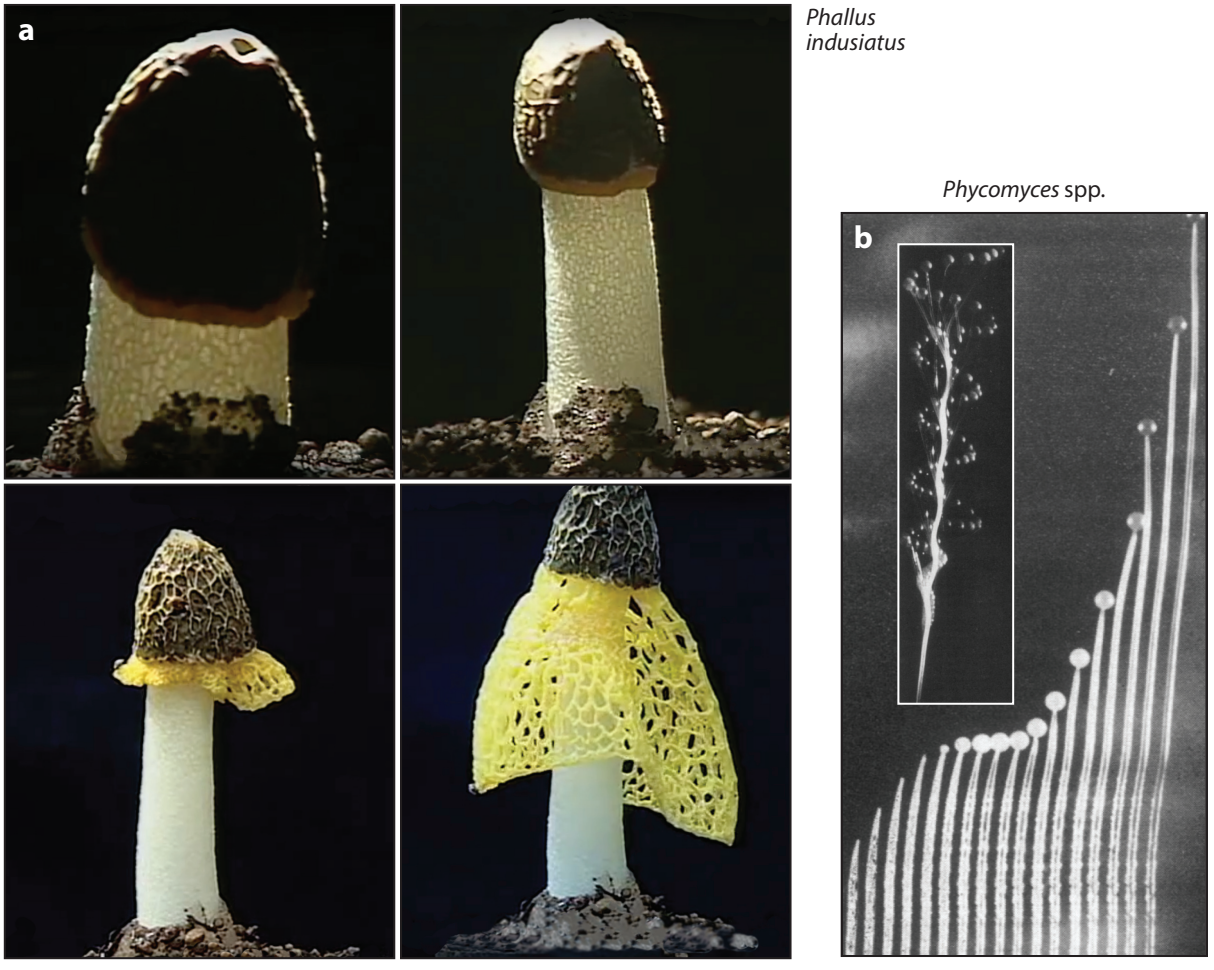


Figure 2

(*a*) Mushrooms unfold from primordia in a feat of reverse origami. Here a veiled stinkhorn (*Phallus indusiatus*) opens from an egg, growing at rates of up to 5 mm per minute. (*b*) *Phycomyces* is a model for fungal morphogenesis. (*Inset*) Helical growth turns spatial variation in light intensity into a time-varying signal, enabling light-sensitive cells to establish the mean light intensity and alter rates of growth between illuminated and shadowed sides of the stalk. Panel *a* stills are captured from a YouTube video (**https://youtu.be/50xqP38f36U**) with permission from The Science Film Museum, Kawagoe, Japan. Panel *b* reprinted with permission from Bergman et al. (1969).

The large turgor pressures within the growing mushroom enable it to push aside branches or other debris: Buller (1909–1950, vol. 4) directly measured the upward pressure exerted by the stipe of *Coprinus* to be about 60 kPa. Stinkhorns exerting a pressure of 1.3 kPa are able to burst through asphalt (Niksic et al. 2004).

The hydrostatic inflation of beautiful and diverse mushroom shapes is a fascinating mechanical problem but, to date, one that has received sparse attention from physical scientists. The most completely understood fungal dispersal organs are the sporangia produced by *Phycomyces* spp. (from the phylum Zygomycota). Each sporangia bears a nearly spherical head, containing asexual spores, on top of a long ($\sim$10 cm) and slender ($\sim$500 μm in diameter) stalk. The direction of stalk growth is determined by both gravity and phototropism: The growth rate increases with light intensity, bending the stalk toward sources of light. However, if growth rate were simply proportional to light intensity then stalks would grow slower in the dark than in bright environments, limiting dispersal potential. To ensure that the mean rate of growth of the stalk is the same in all environments, different parts of their stalk must measure the light they receive relative to the average intensity of light on the entire stalk. But how is the average light intensity measured? *Phycomyces* stalks grow helically, so that if the stalk is unevenly lit, light-sensitive cells that rotate with the top of the stalk will receive light intensities that oscillate in time. Integrating the time-varying light intensity gives these cells the average intensity from all sides of the stalk; local growth can then be accelerated or retarded, depending on whether the light-sensitive cells are on the light or dark sides of the stalk (**Figure 2*b***) (Dennison & Foster 1977). A similar strategy (spinning to compute the average light intensity) has recently been identified as allowing adaptive phototaxis in the green algae *Volvox* (Drescher et al. 2010). Helical growth is common in plant shoots and relies on anisotropic stiffening of the cell wall by fibers (usually cellulose microfibrils). The fibers are laid down in a helix, and when this helix is expanded by an isotropic turgor pressure, the growing shoot twists against the sense of rotation of the helix (Smyth 2016). Goriely & Tabor (2011) showed that the interplay of cell wall stiffening (by glucan microfibrils) and turgor is further enriched if the angle of microfibril deposition changes during growth. They hypothesized that each fiber is laid down in a stress-free orientation, but this orientation alters with the expansion of the stalk: They showed that although the glucan microfibrils always form a right-handed helix, the expanding stalk can twist either clockwise (i.e., against the sense of the helix) or counterclockwise, depending on the length of the growth zone in which new fibers are laid, recapitulating rotation reversals that are observed in *Phycomyces blakesleeanus*.

Even within the presumably clonal mushrooms produced by a single mycelium, features like the length of the stipe, the diameter of the cap, and the cap shape can vary enormously, and it is not clear whether these variations come from genetic or environmental differences or from the imprecision of the unfolding programs that growing mushrooms follow. The existence of such programs was first clearly articulated by de Bary (1887), who, studying *Mycena vulgaris*, showed that the explosive growth of the mushroom from a 3-mm primordium to a 60-mm mushroom was driven by cellular expansion with minimal replication, a result that has been supported by more recent studies (e.g., Bonner et al. 1956, Eilers 1974; but see also Craig et al. 1977). De Bary also discussed how different rates of expansion in different parts of the primordium (he refers to this as "epinasty") might produce the distinctive mushroom cap. How much of the cell wall material in a mushroom is stored in the primordium, and how much is synthesized during growth? By comparing dry weights at different stages of growth, Bonner et al. (1956) showed that cell wall synthesis must be occurring in *Agaricus campestris* mushrooms. However, the meteoric growth of stinkhorns seems too fast for new cell wall to be synthesized, suggesting that it is stored in the primordium, likely in vesicles or cell wall invaginations.

The protoplasm that fills the expanding mushroom cells must flow from the mycelium beneath the mushroom. How is it propelled? Although protoplasmic flow could be driven by the same osmotically created pressure gradients that drive protoplasm through hyphae (see Section 3), might mushrooms, like plants, also use transpiration for fluid transport? Schütte (1956) coated mushroom caps in vaseline and grew them in saturated air to reduce the rate of evaporation. Although fluids were translocated into the mushroom at a slower rate, the mushroom growth rate was negligibly affected. Indeed, transpiration produces flows within plants by lowering the pressure at the top of the xylem; in mushrooms, this would work against the high pressures that are needed to expand the cells. However, in polypores, which grow by adding soft tissue around the edges of a woody mushroom cap, Plunkett (1958) found that increasing the evaporative rate did increase the rate of growth.

Although mechanical constraints are likely to be important in the engineering of mushrooms, nutrition and chemical signaling must also be considered. In plants, growth hormones (auxins) are known to play essential roles in guiding shoot and root growth. However, although surgical removal of the cap or gills does affect mushroom growth (see, e.g., Gruen 1963), no experiment has conclusively shown that any part of the mushroom produces growth hormones. Flows of nutrients from the environment or within an individual may be even more important as determinants of growth (see, e.g., Schütte 1956, Plunkett 1958). Nutrient fluxes have long been hypothesized to shape the growth of lichens, symbioses formed by fungi with photosynthetic microorganisms. Large, flat lichens have shapes close to disks and grow very slowly (at only millimeters per year) by fixing carbon dioxide from the air (Brodo et al. 2001). Most models of lichen growth assume that carbon is depleted largely at the growing edge of the lichen, causing a diffusive flux within the lichen from the center to the boundary (Alpin & Hill 1979, Childress & Keller 1980, Hill 1981). If photosynthesis occurs uniformly across the lichen's surface, these models predict a transition from exponential to linear growth when the surface-to-volume ratio increases and carbon diffusion within the lichen cannot keep up with growth. This transition has indeed been observed experimentally.

If growth is carbon limited, however, photosynthesis cannot occur homogeneously across the lichen. This is because diffusion in the air causes a large flux of carbon dioxide toward the edge of the lichen and a weak flux toward the center, an effect that also produces an enhancement of the electric field at the edge of a charged disk (Jackson 2001) and a deposition of suspended solids at the edge of an evaporating droplet (Deegan et al. 1997). Focusing on carbon dioxide diffusion in the air rather than carbon fluxes within the lichen, Seminara et al. (2018) were able to reproduce the observed exponential-to-linear transition. Diffusion in the air may be key to explaining further observations, e.g., that growth is unaffected when the lichen center is removed (Armstrong 1979) or shaded (Armstrong & Bradwell 2011). Other species of lichens fix carbon dioxide into branching tubular or leafy, rather than disc-like, shapes. Elucidating how lichen shape affects (or is affected by) transport remains an open problem.

3. CELLULAR FLOW

The fungus *N. crassa* grows so fast that its hyphae can be seen extending in real time. Cytoplasm and organelles must therefore be rapidly supplied to the growing tip. For example, Roper et al. (2013) estimated that a dividing population of 120 nuclei (or 1 cm of linear hypha) is needed to keep the tip filled with nuclei. Many of the organelles needed at the tip can be trafficked by motor proteins [motor protein motion is likely the dominant mechanism for transport in young mycelia or in slow-growing species (Schuchardt et al. 2005, Ruiz-Roldán et al. 2010)], but organelles can also be carried in streams of protoplasm (**Figure 3*a***). Protoplasmic streaming seems to have

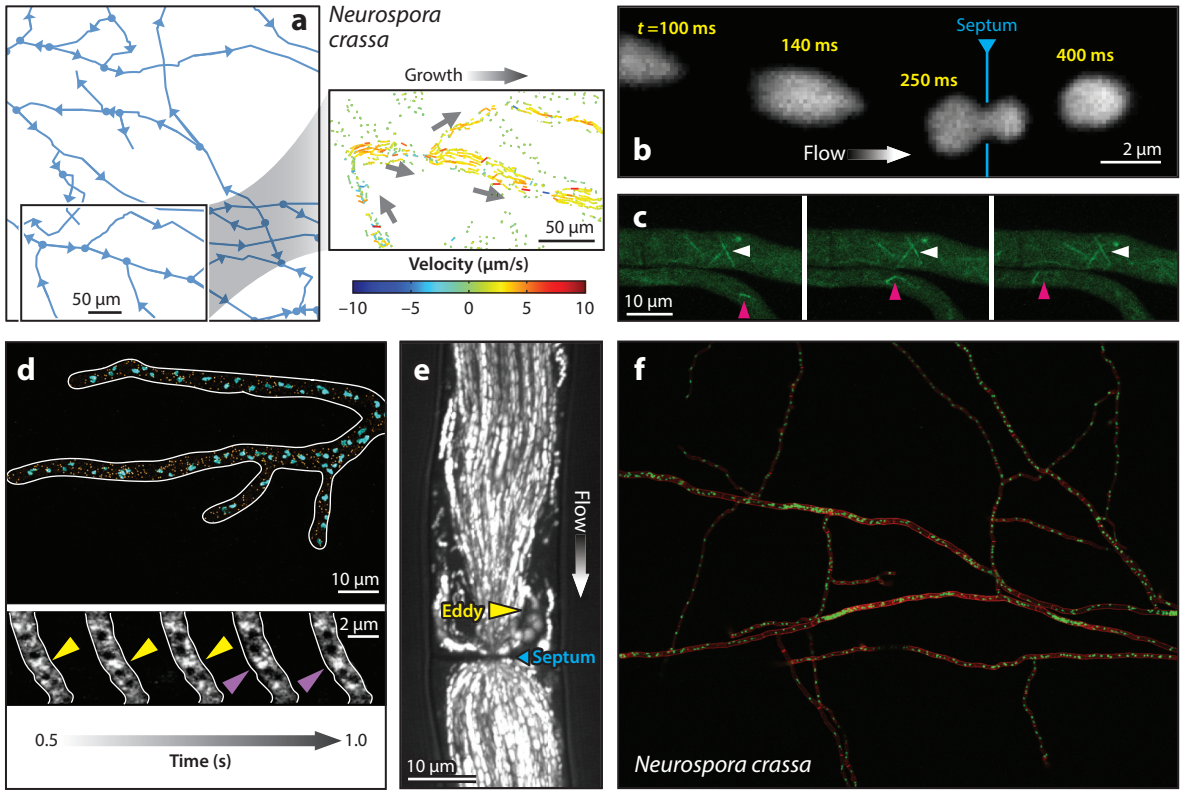


Figure 3

Protoplasmic flow turns fungal hyphae into complex transportation networks and delivers organelles and cytoplasm to growing hyphal tips. (*a*) In *Neurospora crassa*, these flows are complex, multidirectional, and optimized for mixing. The left panel shows a schematic of flows within a small section of the mycelium, and the right panel shows measured velocities within a smaller region of interest. (*b*) Fast protoplasmic flow requires that nuclei rapidly squeeze through septal pores. (*c*) It also leads to continuous disruption of the cytoskeleton. Here, two microtubules are contrasted: In a slow-flowing hypha, microtubules fluctuate slowly (*white arrows*), and in a fast-flowing hypha, they are continuously advected downstream (*pink arrows*). (*d*) Coalescence events show the fluidity of *CLN3*-Whi3 droplets (*bright spots*); two separate coalescences are shown (*yellow and purple arrows*). (*e*) Moffatt eddies forming at septal pores, trapping nuclei (*yellow arrow*). (*f*) Nuclear traffic in *N. crassa* is organized so that higher concentrations of nuclei lead to faster transport velocities. In fast-flowing hyphae, the nuclei assemble spontaneously into rapidly moving antijams. Panels *a*,*c* adapted with permission from (*a*) Roper et al. (2013) and (*c*) Roper et al. (2015). Panels *b* and *d*–*f* provided by (*b*,*e*) Lauren Pieuchot, (*d*, *top*) ChangHwan Lee and Amy Gladfelter, (*d*, *bottom*) Erin Langdon and Amy Gladfelter, and (*f*) Patrick Hickey and Nick Read.

been known in the nineteenth century: Hofmeister's (1867) study of protoplasmic streaming in plants, algae, and slime molds alluded to similar processes in fungal cells, and shortly afterward, Woronin (1870) reported observing motion of vacuoles in the species now called *Cheilymenia pulcherrima*. Yet, as with much of fungal fluid mechanics, the first quantification of the velocities and length scales over which streaming occurs was by Buller (1909–1950, vol. 5). Early studies, which mostly followed visible organelles (vacuoles and endosomes), did not distinguish between different physical methods for driving protoplasmic streaming, and motor protein transport was only conclusively ruled out by Lew (2005), who showed that oil droplets (which are not motor protein affected) travel with the protoplasmic current when injected into *N. crassa* hyphae. Fungal protoplasmic streaming velocities may exceed 50 µm/s, far larger than the typical streaming velocities seen in embryos (nanometers per second) or plant cells (a few micrometers per second)

because they are driven by pressure gradients and not limited to the velocities of motor proteins. Abadeh & Lew (2013) showed the primacy of pressure gradients in protoplasmic streaming in *N. crassa* by reversing the direction of flow within individual hyphae by applying an external osmotic pressure gradient. Roper et al. (2013) used the same method to reverse flows and showed that the relative velocities between each hypha were preserved. Hence, complex and multidirectional protoplasmic flows can be derived from coarse-grained pressure differences between the center and edge of the mycelium. Pressure-driven flow requires only mild pressure differences: For flow of cytoplasm ($\mu = 3 \times 10^{-3}$ Pa·s) at $U = 50$ μm/s through $L = 1$ cm of hyphae of characteristic radius $r = 10$ μm, the Hagen–Poiseuille law predicts that the necessary pressure gradient is $\Delta P = \frac{8\mu UL}{r^2} \approx 100$ Pa, which is a modest fraction of the enormous turgor pressures that hyphae are capable of generating.

Syncytial: of a cell or cell compartment containing more than one nucleus

Although continuous bulk transport allows fungi like *N. crassa* to accommodate their extraordinarily rapid growth, it creates challenges for cellular function since hyphae must perform other cellular functions while transporting protoplasm. But the macromolecules and organelles that coordinate these functions are continually swept downstream by the flow (**Figure 3*c***). As we discuss in the next section, the complex rheology of fungal protoplasm assists the fungus to maintain other cellular functions in the midst of transport.

3.1. Rheology of the Cellular Interior

Rather than being made up of a mass of cells, each containing a single nucleus, fungi are syncytial: Many nuclei may share a single cellular compartment. Specifically, a fungal mycelium is made up of branched and (in many species) reticulated networks of hyphae. In Ascomycota and Basidiomycota, internal cell walls called septa break hyphae into compartments that may contain between 1 and 100 (or more) nuclei (see Roper et al. 2011). These septa prevent the fungus from suffering a catastrophic loss of protoplasm if the cell wall is damaged. In other phyla (notably, Zygomycota and Glomeromycota) there are no septa at all, and all nuclei commingle within a single cytoplasm—an extreme form of syncytism called coenocytism. In wounded cells, the protoplasm presumably gels, similar to the clotting observed in slime molds (Bäuerle et al. 2017). Even within septate hyphae, the need to maintain a mycelium-wide supply chain of organelles and nuclei means that pores in the septa must allow flow of cytoplasm from one compartment to another.

It was long believed that these pores (whose diameters are of the order of 200 nm) are too small to allow large organelles like vacuoles or nuclei (whose diameters are on the order of 1–2 μm) to pass through (Webster & Weber 2007). However, live cell imaging of fluorescently labeled proteins shows that nuclei readily flow through septal pores (Pieuchot et al. 2015). In *N. crassa*, we have tracked nuclei traveling several centimeters and therefore passing through hundreds of septa in an hour (Roper et al. 2011). Yet, since flow is pressure driven, protoplasmic flow in an entire hypha is halted when a nucleus occludes a septal pore. It is likely (although we have seen no published discussion of the matter) that this flow halting results in jerky growth of hyphal tips (Lopez-Franco et al. 1994). To reduce the interruption of the protoplasmic flow, fungal nuclei seem to have adapted to pass easily through septal pores (**Figure 3*b***). Neutrophils must overcome a comparable challenge by constricting through capillaries whose diameter may be half of the diameter of the cell's nucleus. Neutrophil nuclear membranes contain low levels of lamin A, a protein assembled from intermediate filaments that usually stiffens nuclei (Rowat et al. 2013). The flexibility of *N. crassa* nuclei suggests a similar membrane adaptation. Indeed, the membranes of nuclei traveling along cortical microtubules develop long tails (Pieuchot et al. 2015, Roper et al. 2015), similar to the membrane tubules created when motor proteins draw upon soft vesicles (Roux et al. 2002).

How are fungal syncytia physically organized in the absence of cell walls? Since many nuclei share a single cytoplasm, we might expect that free diffusion of macromolecules within the cytoplasm would mean that each nucleus would see the same proteins and RNAs, and therefore, that nuclei would have limited ability to behave autonomously. Yet evidence from fungal heterokarya (genetic mosaic cells that can be created when two different fungal mycelia fuse and pool their different nuclei) has shown that nuclei can show ecosystem-like population dynamics, including competitive exclusion and cyclic dominance (see Maheshwari 2005 and references therein), suggesting that nuclei have a high level of autonomy.

For nuclei to behave autonomously, the macromolecules that each produces must be held nearby against both the dispersive effects of flow and diffusion. Taylor dispersion can rapidly smear out solute (see, for example, Saffman 1960). However, Roper et al. (2013) and Abadeh & Lew (2013) made direct measurements of the velocity profiles of nuclei and mitochondria across hyphae. They showed that both organelles have approximately plug (i.e., nonparabolic) flow profiles, often seen in polymeric fluids. By reducing shear gradients within the hypha, the effective diffusivity due to Taylor dispersion is reduced by a factor of ten (Roper et al. 2013).

Rheological tuning seems to occur on even finer scales within the fungal cell: Lee et al. (2013) used an imaging technique called single-molecule fluorescence in situ hybridization (smFISH) to map out the messenger RNAs (mRNAs) that orchestrate nuclear division in *Ashbya gossypii* (**Figure 3*d***). They found that, as would be expected from their autonomous division, each nucleus is surrounded by a corona of mRNAs. In *A. gossypii*, the nuclei themselves are tugged by motor proteins along erratic paths, so coronas can be maintained only if mRNAs are bound to nuclei. This binding may be the result of phase separation due to interactions between mRNAs and other proteins. For example, mRNAs coding for the cyclin *CLN3* form aggregates with a cytoplasmic protein called Whi3. When *CLN3* mRNAs and Whi3 protein are mixed in test tubes, droplets formed from the *CLN3* mRNA-Whi3 protein demix from the cytosol, similar to the demixing of oil droplets from water (Zhang et al. 2015). This phase-separation process is thought to keep *CLN3* mRNAs clustered within living cells. Intriguingly, the affinity of droplets for different mRNAs is highly dependent on their secondary structure, allowing diverse mRNA compartments to potentially be created within the same cell (Langdon et al. 2018). However, the rheological properties of the droplets in real cell extracts are not known; nor is how chemical kinetics and flow interplay to position them at the precise points they are needed within the cell.

3.2. Managing Flow Within the Fungal Network

Although the rheological adaptations highlighted in Section 3.1 enable nuclei and macromolecules to move coherently together through a hypha, many cellular functions require that proteins be trafficked to the cell wall. In eukaryotic cells, this trafficking is generally assumed to be performed by motor proteins, traveling along cytoskeletal filaments, and indeed, fungi use a suite of molecular motors to direct vesicle and nuclear movements near hyphal tips (see Steinberg 2007). However, in hyphae with fast protoplasmic flow, cytoskeletal filaments are carried by the protoplasmic current (Roper et al. 2015). How are macromolecules (and the nuclei that produce them) sent to and held in their right places at the cell wall?

Because Taylor dispersion is weak, protoplasmic mean flow and diffusion control the distributions of cytoplasmic macromolecules. Long-lived macromolecules diffuse over a length scale $\sim\sqrt{D/U}$, where D is the diffusivity of the macromolecule and U is the velocity of cytoplasmic flow. For a small protein such as GFP [green fluorescent protein; $D = 33\ \mu m^2/s$ (Keith & Snipes 1974)], in protoplasm with a velocity of $U = 1\ \mu m/s$, this length scale can be 6 μm or more. Increasing macromolecule size or cytoplasmic viscosity by aggregation (see Section 3.1) decreases

their diffusivity, keeping bodies together in the cytoplasmic stream. But how can macromolecules also be localized to their correct places on the cell wall? We are far from a complete answer to this question, but recent research has revealed adaptations for corralling macromolecules or nuclei from the protoplasmic stream. Septal pores can confine macromolecules to single compartments, and closing pores allows cellular compartments to be partitioned from each other. As noted above, nuclei and even large vacuoles squeeze readily through open septal pores. However, since flow must accelerate to pass through the pore, even in slow-growing fungi, advection becomes strong enough to prevent any upstream diffusion through the pore. Accordingly, septal pores act as one-way barriers to transport, allowing even fast-diffusing macromolecules to be transported toward growing hyphal tips but not in the reverse direction (Roper et al. 2015).

In most fungi, septa are flat cell walls that cross hyphae perpendicular to cytoplasmic currents. Although the cell wall is quite flexible [e.g., it rebounds when large organelles pass through the pore (Pieuchot et al. 2015)], cytoplasmic streamlines must turn sharply to pass through the septal pore. Due to the corner flow (Moffatt 1964), cytoplasmic eddies are created near the septal pore (**Figure 3*e***). Pieuchot et al. (2015) showed that nuclei may be captured within these eddies for several minutes, and have different behaviors from free-flowing nuclei, including cohering together and accumulating proteins involved in septum maintenance. Eddy capture therefore corrals nuclei where they are needed, near septa.

Transport networks, whether they be data or road networks, generally suffer from congestion—a decrease in traffic speed as the density of traffic increases. Congestive slowdown can be catastrophic if the inability of links within the network to carry the traffic assigned to them jams the entire network (Treiber et al. 2000). Hickey et al. (2016) showed that nuclear traffic within the *N. crassa* network is anticongestive—that is, as nuclear density increases, average speed also increases. Nuclear velocity varies because nuclei transition between a free-flowing state and a state in which nuclei are tethered at the cell wall, presumably anchored to cortical microtubules. Cortical tethering and untethering dynamics admit a high degree of tuning (Nazockdast et al. 2017). In particular, in *N. crassa* the transition to the tethered state is cooperative—large numbers of nuclei saturate the finite numbers of tethering sites. Hickey et al. (2016) presented indirect evidence that tethering sites have refractory intervals that prevent them from immediately rebinding to nuclei; thus, when nuclei are concentrated, a larger fraction will be in the free-flowing state. Because concentrated patches of nuclei travel faster than sparse nuclei, they tend to sweep through the hyphae, adding more nuclei as they travel. Hickey et al. (2016) showed that these dynamical ingredients produce solitons: concentrated, coherent patches of fast-moving nuclei, similar to the solitons predicted by water wave models (**Figure 3*f***). How does organizing flow into solitons benefit the fungus? First, anticongestion amplifies the network's ability to efficiently redirect traffic in response to changes in demand (since there is a nonlinear increase in flow rate with nuclear concentration changes). Second, it organizes transport of nuclei within each hypha into alternating phases of high and low traffic, which may be part of the fungus' strategy for remodeling a fast-flowing hypha: By compressing trafficking into short intervals, long intervals are created in which the hypha is mostly cleared of free-flowing nuclei. During these intervals, cortically tethered nuclei can coordinate cellular wall growth.

3.3. Fungi as Transport Networks

Protoplasmic flow allows for long-distance transport in hyphal networks. Although on the scales of centimeters, these flows require only a small modulation of the large pressures generated at hyphal tips; some saprophytic (plant- and animal-decomposing) fungi that live on forest floors can be meters in size. The largest networks may span kilometers, creating the largest organisms by area on

Earth (Smith et al. 1992). Although the energetic costs of transport over long distances are not likely to be large considering the relatively small pressures involved (see Section 2), maintaining pressure gradients constrains cell osmolality, in turn limiting metabolism. So the fungus is incentivized to create networks in which transport costs are low. Unlike engineers designing the best network that connects a set of known sites (e.g., the cities in a highway network), fungi must grow their networks without knowing the location of the sites (food sources) that they will encounter and need to link. The mycelial network must therefore be continuously adapted to efficiently link up the food sources that the fungus encounters, as well as to supply resources to foraging hyphae that grow outward in search of other food sources (Bebber et al. 2007). The broader constraints on fungal networks remain only partly understood. There are trade-offs between building an efficiently connected network (i.e., one that links different food sources with sites of growth at minimal cost) and including enough redundancy to make the network robust to damage, since herbivory and forest floor disturbances continuously break hyphae within the network (Boddy et al. 2010). Further trade-offs emerge from the need to effectively compete with other mycelia for food and territory. Mycelia compete using a combination of steric blocking and chemical signals administered by direct contact, as well as diffusible or volatile molecules (Boddy 2000). Again, the fungus must balance trade-offs between territory defense and efficient transport, since a sparse network may grow faster but do a poorer job than a dense network at defending its territory.

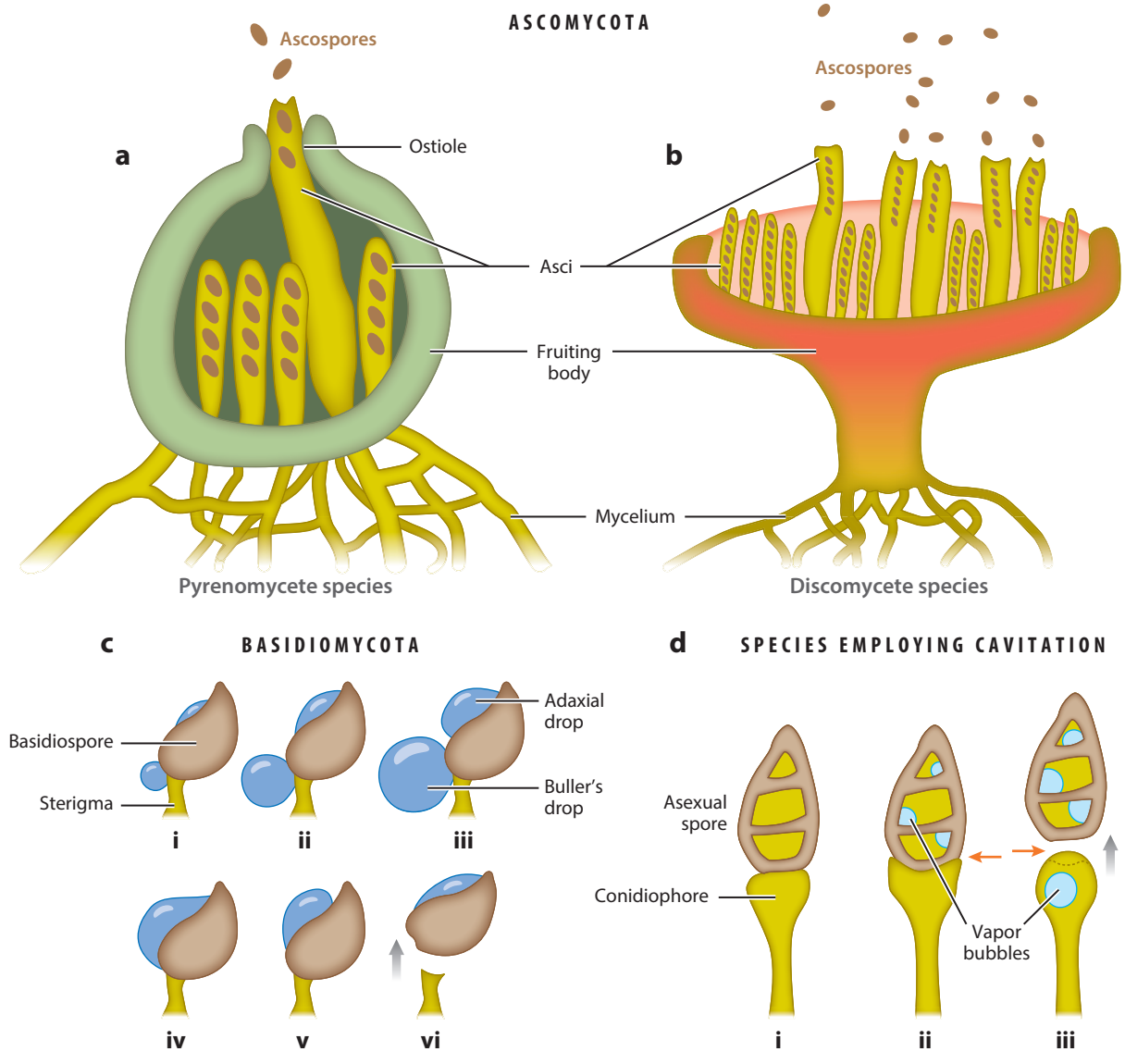
Effective fluid transport constrains fungal networks even at small scales. *N. crassa* is a saprophytic fungus that quickly invades the tissues of plants that have been destroyed by fire. These tissues are likely rich in resources released by the destroyed plant cells, so that *N. crassa* networks do not need to transport nutrients over large distances. However, the fast growth rate of the fungus means that rapid nuclear division and swift internal flows are needed to fill the space created at growing hyphal tips (see Section 2.1). Mutations accumulate continuously as the fungus grows: In a fungus that has grown only a few centimeters, 2–3% of nuclei will have acquired mutations that make them unable to replicate a faithful copy of the original mycelium (Maheshwari 2005). If a group of these mutant nuclei were to arrive at the same hyphal tip, they could impair its growth or even lead to competition between that tip and the genetically different tips that surround it. Roper et al. (2013) showed that *N. crassa* networks are adapted to efficiently mix nuclei during growth, ensuring that when each nucleus divides, its two offspring are delivered to different hyphal tips.

Although a wealth of data supports the idea that fungi are capable of making optimal networks, little is known about the decision strategies that enable fungal networks to thrive in extremely uncertain environments. Statistical decision theory has been successfully used to derive optimal strategies for foraging in the slime mold *Physarum polycephalum*. Although this organism has no central organizing system, it can consistently solve a two-armed bandit problem, i.e., choose between two differently rewarding directions of growth (Reid et al. 2016). Whether this framework is useful for understanding mycelial networks and what is the fungal solution to this ubiquitous dilemma remain fascinating open questions.

4. MECHANICS OF EXPLOSIVE MOVEMENT

Fungi lack legs, fins, or wings for locomotion, but they routinely translocate to new sites and travel globally (Brown & Hovmoller 2002). Their locomotion and survival rely on the dispersal of microscopic propagules: the spores. Sexual spores generally take off abruptly (**Figure 4*a–c***), when they are forcibly discharged from the parent fungus at accelerations of $\sim 10^5 g$, nearly unmatched elsewhere in nature (Money 1998). But despite an impressive initial speed, they decelerate quickly

due to their microscopic size. The Stokes timescale for a spore of size $r = 10$ μm that is about 1,000 times heavier than air, $\beta = 3/2 \times 10^{-3}$, is $\tau = r^2/(3\nu\beta) \sim 2$ ms, where ν is the kinematic viscosity of air, $\nu = 1.5 \times 10^{-5}$ m^2/s. Hence a spore launched at $U = 1$ m/s decelerates to rest after traveling only about $U\tau = 1$ mm. Vogel (2005) likens the challenge of ejecting a spore to throwing a balloon (**Figure 5*b***). Fortunately, spores do not need to be carried any great distance by their discharge—they are small enough to be carried by even weak dispersive winds. However, a spore that travels only 1 mm may not even pass through the boundary layer of nearly still air that clings to the fruiting body from which it is ejected. Accordingly, fungi have multiple adaptations for maximizing the distance traveled by spores during their first explosive movements.



(Caption appears on following page)

Figure 4 (*Figure appears on preceding page*)

Mechanisms of spore ejection. (*a*,*b*) Ascomycota eject sexual spores (ascospores) using squirt guns (asci). Introduction of osmolytes and rapid uptake of water into an ascus causes it to swell. When the ascus reaches a critical size, an apical pore opens and spores are explosively ejected. In Pyrenomycete species (*a*), the fruiting body is flask shaped. Spores must be ejected through a hole (ostiole) at the top of the flask. Only one ascus can discharge at a time. In Discomycete species (*b*), the fruiting body is cup or disk shaped, and asci discharge their spores synchronously. (*c*) Basidiomycota eject sexual spores (basidiospores) from supporting sterigma, using a surface tension catapult. (*c*, *i–iii*) Two droplets (the adaxial drop on the side of the spore and Buller's drop on the base) condense from atmospheric water vapor onto the spore. (*c*, *iv*) At a critical size, these droplets merge. (*c*,*v*) The capillary pressure differences between the drops pull fluid from Buller's drop into the adaxial drop. (*c*, *vi*) Since the adaxial drop is pinned, the moving fluid comes to rest on the spore. Momentum is transferred from the decelerating drop to the spore, propelling it from the sterigma. (*d*) Some species use cavitation to eject asexual spores. (*d*, *i*) Spores are supported on stalks called conidiophores. (*d*, *ii*) Evaporation reduces the volume of cytosol within the spore and conidiophore, causing the wall between the spore and conidiophore to collapse (*orange arrow*). At the same time, negative pressure within the cytosol causes vapor bubbles to form (*blue*). (*d*, *iii*) Rapid growth of a vapor bubble within the conidiophore causes the wall to explosively snap back to its original shape (*orange arrow*), ejecting the spore. Panel *d* is based on drawings in Meredith (1961).

4.1. Turgor Guns

In Section 2, we discussed how enormous turgor pressures must be generated at hyphal tips. When the cell wall is ruptured, these pressures explosively discharge protoplasm, until septal pores are closed. The same turgor pressures are used by fungi in the phylum Ascomycota to eject their spores. In these species, a specialized cell called an ascus contains the spores and swells enormously before bursting open, releasing the spores in a stream of cell sap (**Figures 4*a*,*b*** and **5*a***); the pressure within an ascus just prior to ejection is comparable to tip growth pressures (∼1 MPa) and is similarly maintained by ion influx and by the reprocessing of glycogen as sugars (Ingold 1939, 1971; Fischer et al. 2004; Trail et al. 2005).

Fungal pressure guns are optimized to minimize dissipation during discharge. Many species eject spores through an elastic ring at the tip of the ascus that strongly deforms as the spore exits (Fritz et al. 2013). Here the thickness, h, of the lubricating layer of ascus sap that separates the ejecting spore from the ring must be carefully tuned. If h is too small, then viscous friction between the spore and the ring will slow spores down. If h is too large, then ascus sap will be lost along with the spores, causing the pressure head to decrease. There is an intermediate value, h^*, that optimizes the spore ejection velocity. The morphology of the apical ring and of the spore must be finely tuned to achieve this layer thickness. Real morphologies of 43 species widely distributed across the fungal tree of life are within 2% of the optimum. Optimal apical rings evolved at least twice independently and may have any number of shapes (thin/fat and flat/round) so long as a particular combination of shape parameters lies close to the optimal manifold. Conversely, nonfunctional apical rings produced by fungi that do not forcibly eject their spores have drifted far from the optimal manifold. Apical rings therefore provide a model system for studying how forces of selection find biomechanical optima.

Spores are discharged when the internal pressure reaches a given fraction of the elastic modulus of the ring, $p/E \sim 0.2$. Hence, since ejection must be coordinated between different asci, the build up of turgor pressure in different asci must be tightly regulated. In Section 2.1, we discussed how hyphal tip pressures are regulated. But expansion of the ascus cell wall requires even finer attention to turgor and cell wall elasticity since, unlike hyphal walls, the ascus cell wall is not inextensible, and because its expansion is much more rapid than hyphal growth. It has been proposed that in some species, the ascus wall material remains only weakly extensible but that extra wall material forms invaginations that flatten out during expansion (Reynolds 1981, Parguey-Leduc & Janex-Favre 1982, Minter & Cannon 1984), but not all species have these invaginations

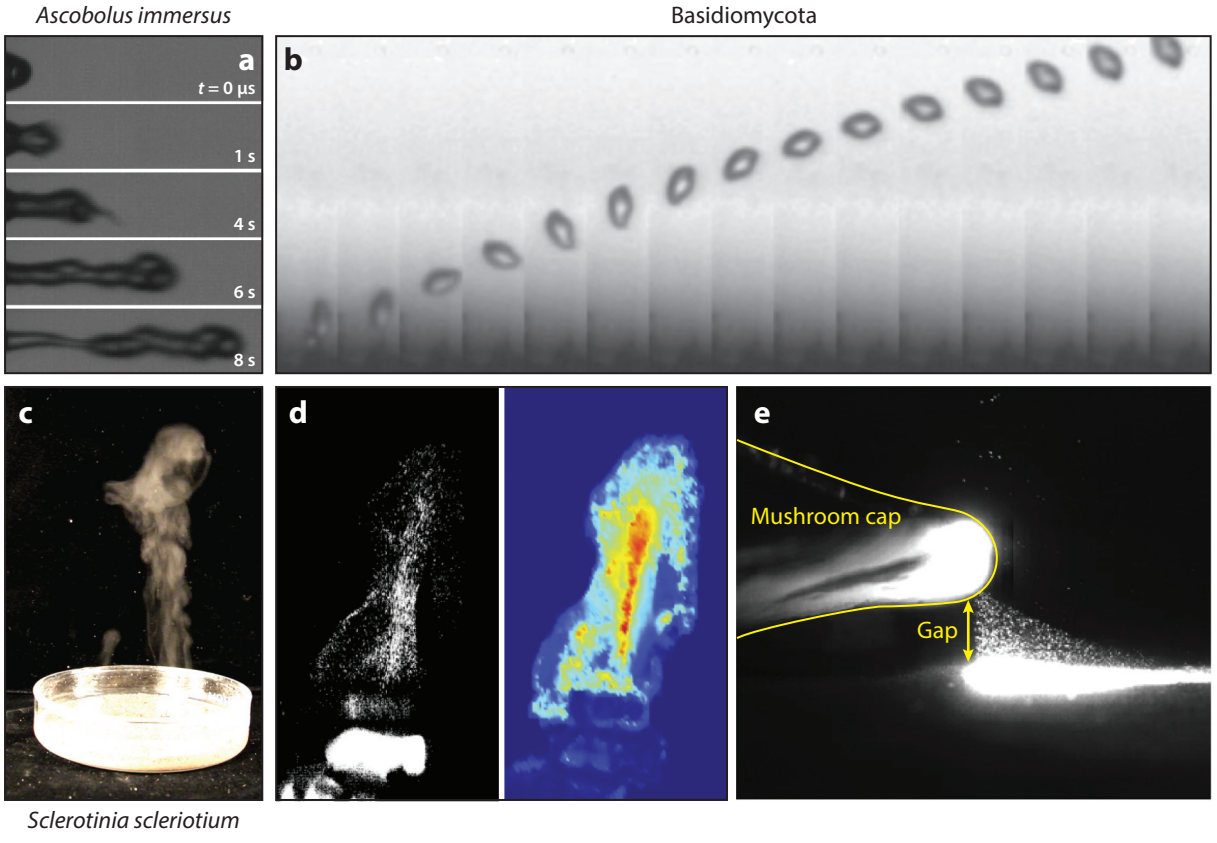


Figure 5

(*a*,*b*) High-speed videography has exposed the explosive mechanics of spore ejection. (*a*) In Ascomycota, spores are ejected in a squirt of ascus fluid. Spore shape and ascus pores are precisely adapted to maximize range. (*b*) Basidiomycota eject their spores using a surface tension catapult. Despite an initial speed of ejection on the order of meters per second, the spores are quickly brought to rest by air drag. (*c*–*e*) To increase the likelihood of spores reaching dispersive airflows, fungi have ingenious adaptations for sculpting nearby airflows. (*c*) *Sclerotinia scleriotium* ejects $\sim 10^5$ spores synchronously. These spores mobilize the air above the fungus to create a coherent jet. (*d*) Spore ejection proceeds in a wave across the fruiting body. High-speed particle image velocimetry imaging shows that the jet is sheet-like. The hydrodynamics of the sheet forces each spore to eject to optimally contribute to the airflow above the fungus. (*e*) High rates of water loss cool the air around a shiitake mushroom, creating a gravity current that disperses spores from under the mushroom cap. Panels adapted with permission from (*a*) Yafetto et al. (2008), (*b*) Noblin et al. (2009), (*c*) Roper et al. (2010), and (*e*) Dressaire et al. (2016). Panel *d* provided by Marcus Roper, Agnese Seminara, and Mahesh Bandi.

(Read & Beckett 1996). How turgor is regulated is mysterious since all of the DNA within the ascus is packaged within spores, making mRNA transcription impossible (Trail & Seminara 2014). Moreover, it is unlikely that asci simply translate existing mRNA into proteins, as transmission electron microscopy of mature *Fusarium graminearum* asci show only very few ribosomes (F. Trail, personal communication). In *F. graminearum*, stretch-gated channels and voltage-gated channels regulate calcium levels and thereby turgor pressure (Hallen & Trail 2008, Cavinder et al. 2011). Stretch-gating means that turgor pressure may be directly regulated with the ascus wall tension, allowing asci to orchestrate their swelling and discharge reliably without protein synthesis.

To escape its parent and reach new habitats, a spore must penetrate through the boundary layer of nearly still air surrounding the fruiting body. The spore's inertial range, $Z = U\tau$, depends on

the launch speed U and on the Stokes timescale, $\tau = m/\zeta$, where m and ζ are the mass and drag coefficient of the spore, respectively. The larger the range Z, the thicker the boundary layer a spore can escape from. Spore launch apparatuses maximize the ejection velocity U; is there any mechanism to minimize the drag coefficient? Forcibly ejected spores across the Ascomycota tree of life have drag-minimizing shapes, computed assuming an ejection speed between 1 and 3.5 m/s. A total of 73% of the species lie within 1% of optimality, and the remaining species may in fact be optimized for a different ejection speed. Drag-minimizing shapes emerged despite a comparatively modest gain of 5 to 10% on the inertial range, suggesting strong selective forces on range maximization (Roper et al. 2008). As Ingold (1928, 1933) noted, fungi may manipulate projectile mass, m, to increase their Stokes timescale by, e.g., launching several spores tethered together by mucus. Pointy fruiting bodies decrease the thickness of the boundary layer itself (Pringle et al. 2017).

4.2. Surface Tension Catapults

Surface tension is a powerful means for generating forces at small scales, as the relative magnitude of surface tension to inertia is given by the inverse Weber number, $1/We = \gamma/\rho U^2 R$, which is inversely proportional to size, where γ, ρ, U, and R are surface tension, density, speed, and size, respectively. The fungi have found a way to effectively convert surface energy into spore acceleration, and the vast majority of fungi in the Basidiomycota discharge spores through this surface tension catapult (Buller 1909–1950, Ingold 1939). A fluid drop (Buller's drop) condenses out of water vapor near the base of the spore due to a secretion of hygroscopic substances by the spore itself (Webster et al. 1984, 1995). Similarly, a second drop, the adaxial drop, forms at the surface of the spore, and as soon as the two drops coalesce, the resulting shift in mass distribution detaches the spore from its substrate (**Figure 4*c***). Low-speed (Buller 1909–1950, Turner & Webster 1991) and high-speed (Pringle et al. 2005b, Noblin et al. 2009, Stolze-Rybczynski et al. 2009) video imaging show that spores are discharged at about 1 m/s and decelerate to rest in about 1 mm. To understand directionality, one must analyze momentum transfer from the liquid to the spore. The surface tension catapult functions in two stages: First, Buller's drop is mobilized and coalesces with the adaxial drop, and second, the acquired momentum is transferred to the spore (Webster et al. 1984, Pringle et al. 2005b, Noblin et al. 2009, Liu et al. 2017). A simplified model (Liu et al. 2017) further exposed the fluid mechanics of this two-stage process. In the first stage, Buller's drop drains onto the adaxial drop owing to its higher Laplace pressure, and the liquid accelerates toward the tip of the spore. If the contact line is pinned, the liquid eventually slows down, and the momentum of the decelerating droplet is transferred to the spore. The spore is loosely attached at its base and is ejected clear of the fungus.

Several hundred thousand surface tension catapults are deployed within a single mushroom. Catapults decorate the surface of gills (or the vertical tubes) underneath the mushroom cap. The gills are arranged radially, facing each other; the spores are shot horizontally and then fall freely out of the mushroom. Spore ballistic range must be smaller than the inter-gill distance to avoid impact with the next gill. If mushrooms are designed so as to maximally pack spores with a minimum amount of biomass, then the inter-gill distance should be exactly twice the inertial range of the spores, with possibly an additional margin of safety (Buller 1909–1950, Ingold 1992). Gill separation is inhomogeneous across the cap, as gills are radially distributed. In fact, many species grow secondary gills, suggesting that packing a large number of spores in a small space is a key constraint underlying mushroom forms (Fischer & Money 2010).

A developmental process generating optimally packed gills would depend critically on a spore's horizontal range, $U\tau$, where U is the speed of ejection and τ is the Stokes timescale—the Reynolds number here is of order 1. But what sets the ejection speed U? Balancing the surface energy of

Buller's drop with the kinetic energy of the spore–liquid complex yields $U = \sqrt{2\gamma R_D^2/m}$, where γ and R_D are the surface tension and radius of Buller's drop, respectively, and m is the mass of the spore–liquid complex. This energy balance entirely ignores dissipation as well as the geometry of the adaxial drop and spore, yet it correctly reproduces the experimentally observed ejection speeds in both live ballistospory and biomimetic replicas (Ingold 1971, Webster et al. 1984, Noblin et al. 2009, Stolze-Rybczynski et al. 2009, Liu et al. 2017). Dissipation significantly reduces the ejection velocities of droplets in the related phenomenon of coalescence and jumping of drops on superhydrophobic surfaces (Boreyko & Chen 2009, Liu et al. 2014). The high efficiency of fungal surface tension catapults results from their operating in a low–Ohnesorge number regime, where inertial-capillary effects caused by the drop dominate and viscous dissipation is weak (Liu et al. 2017). Interestingly, experiments show that U varies only 5% to 15% within a single species (Stolze-Rybczynski et al. 2009, Fischer et al. 2010). Robustness is critical here, considering that spores that travel too far (or not far enough) may not leave the narrow gill space. This robustness may result from the fact that in the capillary-inertial regime, the adaxial drop provides directionality, but the ejection velocity is insensitive to its precise geometry (Liu et al. 2017). Still, this small variation is surprising, considering that the entire process occurs extracellularly and is thus greatly affected by external conditions. The extent to which fungi are capable of controlling Buller's drop radius and thus the range of discharge of their spores remains to be elucidated.

4.3. Discharge by Cavitation

A number of fungi use cavitation to explosively launch their spores. The banana pathogen *Deightoniella torulosa* was the first fungus discovered to forcibly eject its conidia through cavitation (Meredith 1961), although many other examples are now known (Ingold 1956; Meredith 1962, 1963, 1965), as reviewed by Money & Fischer (2009). All of these cavitation-based designs share some basic features. Microscopic fluid-filled chambers are delimited by thick walls (**Figure 4*d***). Evaporation through the chamber walls causes fluid loss and deformation of the weakest portion of the cell wall, resulting in an accumulation of elastic energy. The wall opposes mechanical resistance to deformation, which causes pressure in the chamber to decrease enormously and become negative. At sufficiently large tension, the fluid within the chamber suddenly breaks and a vapor bubble appears. The deformed wall then snaps back to its original position and releases the elastic energy slowly accumulated during evaporation. In *D. torulosa*, the deforming wall is directly adjacent to the spore, which is kicked into the air when the wall snaps back into its original position (**Figure 4*d***) (Meredith 1961). In *Zygophiala jamaicensis*, evaporation causes the stalk that supports the spore to shrink and coil. The spore is kicked off when the formation of a vapor bubble within the stalk causes it to spring back to its original shape (Meredith 1963). Similar physics of cavitation and snapback is used to disperse spores by mosses (Whitaker & Edwards 2010) and ferns (King 1944, Noblin et al. 2012).

5. SPORE DISPERSAL

Despite a violent departure, the inertia of the spore may be insufficient to carry it across the boundary layer surrounding the fruiting body from which it was ejected. For a fruiting body of size $L = 1$ cm, the steady boundary layer thickness is $\delta \sim \sqrt{L\nu/V} \sim 2$ mm, with an air speed close to the ground of $V \sim 5$ cm/s. Fascinating adaptations increase the likelihood of spores crossing the boundary layer.

5.1. Passively Liberated Spores

Although fungi have various apparatuses (described in Section 4) for forcibly ejecting sexual spores, many rely on passive wind dispersal to liberate asexual spores, a process called abscission. Abscission of spores from the parent fungus by wind is often thought to be an inefficient process: in experiments on spores produced on moldy hay, Gregory & Lacey (1963) showed that wind speeds of 4.9 m/s liberated only 15% of spores. Yet, under some estimates, passively liberated spores account for the majority of airborne spores (Lacey 1996). This paradox has since been resolved by scientists studying wind-dispersed plant seeds, such as the achenes of dandelions. Here, the average velocities of wind flow in the grass canopy where the dandelions are found are typically too small to liberate achenes from the flower, but intermittent gusts of wind may yet be fast enough to do so. Indeed, spores may increase their likelihood of being dispersed significant distances by only abscissing in the strongest winds seen by the fungus (Greene 2005).

In *N. crassa*, asexual spores are typically borne at the tips of slender aerial hyphae that grow upward from whatever substrate the fungus grows on. Presumably, the function of these hyphae is to grow up through the boundary layer, exposing spores to faster wind flows that are more likely to liberate them from the fungus. Typically we would expect that spores on longer aerial hyphae would be exposed to faster winds, but longer aerial hyphae are also prone to collapse under their own weight. Optimally, we therefore expect the length of aerial hyphae to be regulated along with boundary layer thickness—longer when the boundary layer is thick, and shorter when the boundary layer is thin. However, boundary layer thickness depends on both the wind speed and the size of the substrate on which the fungus grows, and it is not clear how the hyphae, which are only a few microns across, can sense these macroscopic variables. Recently, Y. Lin, J. Torres, S. Foshe, M. Tomasek, E. Dressaire & M. Roper (manuscript under review) showed that aerial hypha length in *N. crassa* grows in proportion to boundary layer thickness. They showed that the evaporation rate allows the fungus to monitor boundary layer thickness. Specifically, assuming a given relative humidity C_0, we expect the rate of evaporation from the fungus to scale as $(1 - C_0)\sqrt{DV/L}$, where D is the diffusivity of water vapor. Thus, if the length of an aerial hypha is inversely proportional to the evaporation rate, its length will correctly track the boundary layer thickness both as a function of V or of L. Now, the total rate of evaporation from a hypha is not proportional to its length [similarly to how the evaporation rate from bodies of water is not proportional to their area (Shahidzadeh-Bonn et al. 2006)], so how fungi measure evaporation rate is not known. But given their precise control of turgor and thus water content, it is plausible that they have a mechanism to measure evaporation rates.

5.2. Wind Manipulation

Different groups of fungi have evolved strategies to actively manipulate their fluid environment and create airflows to carry spores clear of the fungus. In Ascomycota, asci do not usually discharge independently but are packed together, along with turgid filaments called paraphyses. In cup-shaped fruiting bodies, hundreds to hundreds of thousands of spores are discharged in a single puff lasting a fraction of a second (**Figure 5*c***) [the earliest account of this phenomenon is by Micheli (1729)]. Although a single spore, decelerating to rest, adds negligibly to the momentum of the surrounding air, as first intuited by Buller (1909–1950), hundreds of thousands of coejected spores mobilize the air in a millimeter-thick layer close to the fungus, creating a spore-laden jet that can travel for tens of centimeters (**Figure 5*c***). The spore jet attains most of its gravity-limited range (Roper et al. 2010), i.e., it overturns the conventional wisdom that the ranges of small projectiles are

necessarily limited by air resistance rather than gravity (Vogel 2005). Mature asci coordinate their spore ejection through a wave that crosses the fruiting body (Roper et al. 2010). The numerical simulations of Roper et al. (2010) showed how unequally the benefits of coordination are shared among spores, with the first spores to be ejected being sacrificed to create the flows of air that disperse their brethren. Thus, since spores are not genetically identical to each other, they are incentivized to delay their ejection until late in the puff. The puff would be disrupted if all spores delayed their ejection. Hence, spatial coordination of spores is biologically important because spores must measure the moment of their ejection against the arrival of the wave of discharges, which produces a sheet-like jet (**Figure 5*d***). Since a spore achieves its optimal range by ejecting at the time of the wave's arrival, each spore's optimal strategy leads it to contribute optimally to the creation of the jet. Thus, the hydrodynamics of the jet self-polices the selfish spores that create it. The wave of ejections is likely caused by the release of elastic stresses within the turgid cup, but the dynamics of wave propagation remain unexplained. Mechanics within fruiting bodies plays an important role in the timing of spore ejection in flask-shaped fruiting bodies as well, since here only one ascus can swell to its full size at once, meaning that asci must time their ejections to avoid their neighbors, rather than synchronize with them (Trail & Common 2000, Sikhakolli et al. 2012).

In Basidiomycota, spores are released from underneath the mushroom at heights that can range from centimeters (for stalked mushrooms) to meters above the ground (for mushrooms that form on the trunks of trees). Spores released high enough from the ground will simply sediment into dispersive airflows. However, mushrooms also grow in Mediterranean climates, where humidity is low between winter rains. In these climates, mushrooms are often found close to the ground, or even under a layer of leaf litter. How then do spores find dispersive winds? High rates of transpiration (see Section 2.4) cool the mushroom cap by ~2–4°C below the ambient temperature (Husher et al. 1999). Cooling the air around the mushroom creates a gravity current of cooled air that spreads outward from the mushroom (**Figure 5*e***) (Dressaire et al. 2016). However, this gravity current can only carry spores with it if temperature gradients or the cap shape create an asymmetry, allowing air to flow into the gap beneath the cap on one side and out on another. Unintuitively, this method of wind-creating is enhanced by crowding, since outward-moving currents can climb up nearby obstacles, carrying the spores up as well as outward from the cap. Dressaire et al. (2016) showed that mushrooms sustain water loss rates higher than plants and even simple hydrogels. These data suggest that mushrooms do not simply lack adaptations to conserve water but are adapted to maximize their rate of water loss. Xu et al. (2017) have shown that the efficiency with which mushrooms use solar energy to create water vapor exceeds human-built solar steam generators.

The shape of the mushroom cap may also assist in the creation of dispersive flows. Smoke visualizations of the airflows around mushrooms in wind tunnels suggest that flows around the dome may be adapted to prevent freshly ejected spores from being blown back to the mushroom (Deering et al. 2001).

5.3. Strategies for Spore Liberation

The body of work reviewed above shows that the microscopic mechanisms of spore discharge are exquisitely optimized. But once a spore reaches dispersive atmospheric flows, its fate is dictated by a series of stochastic events unknown to the fungus. This fundamental mismatch makes spore dispersal wasteful compared to the coordinated migration of mammals or birds (Nagarajan & Singh 1990). Do fungi surrender to this high uncertainty simply by producing a large number of spores? Different lines of evidence suggest that this is only part of the story. First, simply

producing a large number of spores does not guarantee dispersal. Indeed, dispersal still limits the occurrence of fungi (Norros et al. 2012), and genetic data show that truly cosmopolitan species are rare (see, e.g., Koufopanou et al. 1997, Pringle et al. 2005a, James et al. 2006, Peay et al. 2007). Second, specific characteristics of the fungi influence dispersal: Fungal traits, especially spore shape, have been found to be good statistical predictors of invasive potential (Philibert et al. 2011). One potential explanation is that spore geometry controls the efficiency of their deposition onto vegetation, since larger particles with longer Stokes timescales are more likely to leave air flows and impact on obstacles (Hussein et al. 2013, Norros et al. 2014). Third, the timing of spore liberation may allow fungi to influence dispersal; for example, spores released in highly convective conditions are likely to disperse further (see, e.g., Aylor 1999). The time of day is an important determinant of atmospheric conditions, as stably stratified conditions and weak turbulence commonly occur at night, whereas solar radiation causes convection that powers turbulence during the day. This daily alternation between weak and strong turbulence is widespread, but the reliability and strength of this cycle vary widely with geography and season: Releasing spores at a specific time of the day may yield reproducible dispersal in Mexico, but not in Canada (D. Lagomarsino, A. Pringle, A. Mazzino & A. Seminara, manuscript in preparation). Interestingly, experimental data show that different species release spores with a variety of specific patterns (see, e.g., Rossi et al. 2001, Mondal et al. 2003, Guo & Fernando 2005). Some species appear to release spores at specific times of the day, whereas others show an extremely intermittent pattern with no clear daily periodicity (Savage et al. 2013). Whether and how spore liberation is shaped by atmospheric transport, as well as how strategies for spore liberation may vary with geography, season, and global change, remain fascinating open questions.

SUMMARY POINTS

1. Turgor pressure enables fungi to create cellular networks, sculpt complex fruiting bodies, and invade host tissues.
2. The rheology of the flowing protoplasm is adapted to facilitate the coherent motion of macromolecules and the nuclei they originated from, enabling nuclei to behave autonomously.
3. Cellular functions in a continually flowing cytoplasm require special adaptations: (*a*) Septa prevent upstream diffusion and behave as one-way doors, (*b*) corner flow at septa generates eddies that can trap nuclei for several minutes, and (*c*) antijamming by nuclei traveling in high-density packs allows for long stretches of traffic-free networks, where nuclei tethered to the cell wall quietly organize cell wall functions.
4. Sexual spores are ejected through optimized microscopic mechanisms at accelerations nearly unmatched elsewhere in nature. (*a*) A cell inflated osmotically explodes and forcibly discharges its ascospores with minimum dissipation during launch. (*b*) Coalescence of extracellular drops powers surface tension catapults that eject basidiospores with minimum viscous dissipation. Regulation of extracellular coalescence and of ascus elongation in the absence of transcription/translation remain to be elucidated.
5. Spores are easily carried by the wind, but they need special adaptations to reach dispersive airflows. For example, synchronous ejection of many spores mobilizes jets of air, while cooling-driven gravity currents transport spores even in crowded environments.

FUTURE ISSUES

1. The unfurling of a mushroom is a feat of reverse origami. However, the programs used to create mushrooms of diverse and elegant shapes by expanding a primordium are not yet known.
2. Fungal multicellularity is radically different from that of plants and animals; we understand little about the general processes by which cellular functions can be orchestrated in syncytia and in a continually flowing protoplasm.
3. Fungal transport networks may be optimized for many different physical goals. Can we use the underlying physical optima as part of a method for organizing and understanding diversity?
4. Spore discharge is beautifully optimized, but spore trajectories in the atmosphere are highly unpredictable. Do fungi surrender to uncertainty, or can they strategize to exert some control over the fate of their progeny?
5. The fungal kingdom remains woefully underexplored, and there are many fungal lifestyles that we have not discussed. For example, how do flagellated zoospores of fungi and oomycetes navigate turbulence and chemical cues to find their hosts?

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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