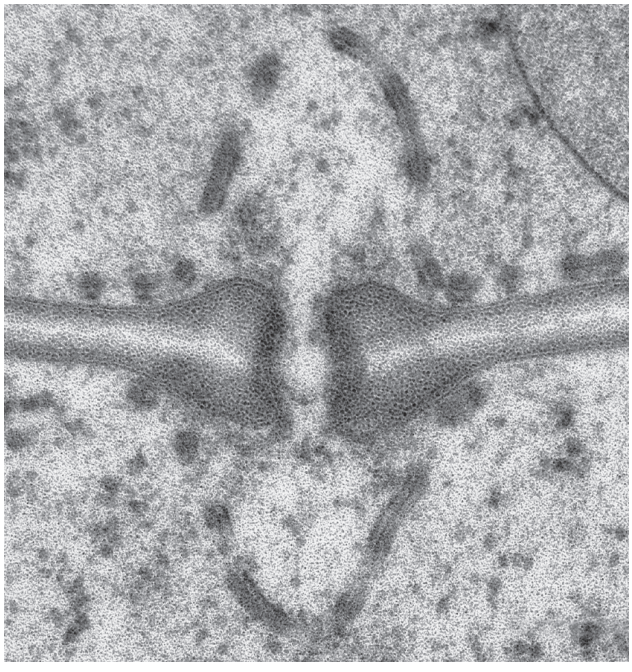
Contents

Dikarya

Dikarya is a subkingdom that unites three phyla, the Ascomycota (molds, yeasts, cup fungi, lichens, etc., also called "ascomycetes"), Basidiomycota (mushrooms, rusts, smuts, etc., "basidiomycetes"), and Entorrhizomycota (see below).

The synapomorphy of Dikarya is the dikaryon, a proliferative phase where mitotically dividing cells have hyphae with exactly two nuclei (dikaryon means "two kernels," referring to the nuclei). However, not every species in the subkingdom forms a dikaryon, and the duration of time or fraction of the life cycle comprising the dikaryon varies from extremely transient, as in many cup fungi, to the majority of the life cycle, as in many gilled mushrooms and polypores. Hyphae of some Dikarya may be multinucleate. The hyphae of Dikarya are divided by crosswalls called septae. However, the septae are perforated by pores that allow cytoplasm and organelles to move along the hyphae. The fine structure of the septal pores and associated organelles, which can be seen with transmission electron microscopy, were used as taxonomic characters to delimit major groups of Dikarya before the advent of molecular characters in fungal taxonomy. Not all Dikarya are filamentous; yeasts have evolved repeatedly in the group.

The vast majority of described species of fungi (97 percent) are in Dikarya, which are the focus of much of the rest of this book; a phylogenetic overview of the major groups of Ascomycota and Basidiomycota is given in Chapter 4 (see pages 114 and 120). However, one group of Dikarya, the Entorrhizomycota, is worthy of note here. *Entorrhiza* forms galls in the roots of sedges and rushes. In some ways it resembles a group of plant pathogenic Basidiomycota called smuts (Ustilaginomycotina), but it has unique aspects of spore germination. *Entorrhiza* used to be classified within Basidiomycota, but molecular data now point to a placement as the sister group of either (1) Basidiomycota, or (2) a lineage containing Ascomycota and Basidiomycota, which supports its recognition (at least for now) as a unique phylum. The unexpected placement of *Entorrhiza* allows a reconsideration of the characteristics of the last common ancestor of Dikarya and reinforces the need for a fungal taxonomy based on molecular characteristics, which is the subject of the rest of this chapter.



→ Roots of the rush *Juncus articulatus* colonized by *Juncorrhiza casparyana* (Entorrhizomycota) can be detected by swollen galls containing spores.

← Transmission electron micrograph of the septal pore apparatus in *Auriscalpium vulgare*, showing the flared "dolipore" septum and membranous septal pore cap.

Decoding fungal relationships

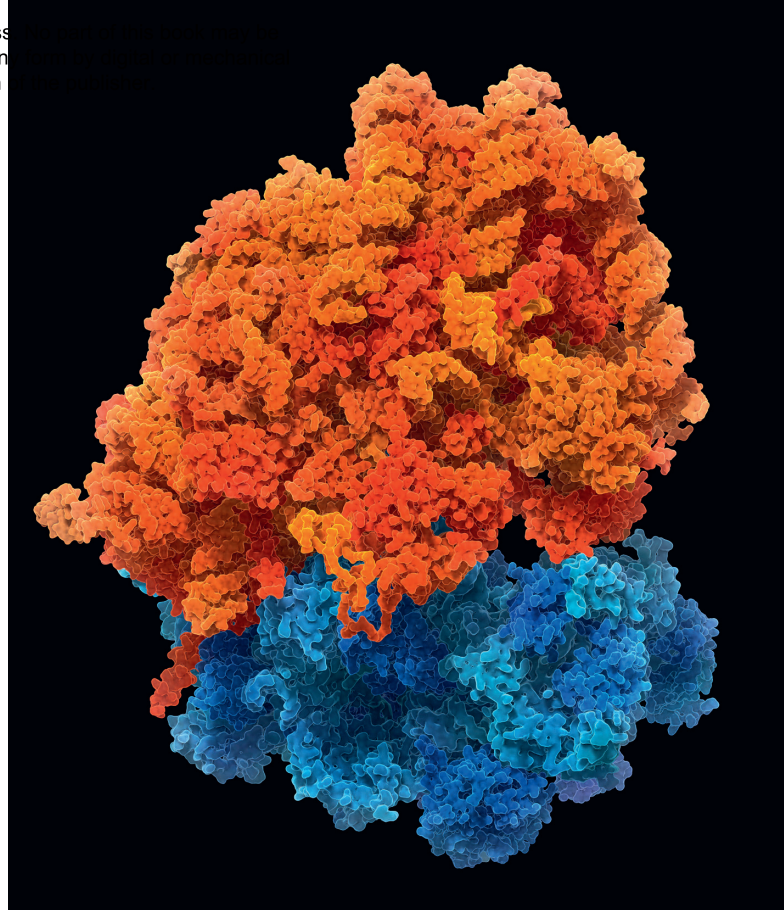
Mycology has been repeatedly transformed by technological advances, such as the development of light and electron microscopes and methods for isolating fungi in pure culture. Since the late twentieth century, a series of innovations in molecular biology have revolutionized the discipline.

Sanger DNA sequencing

In 1977, the British biochemist Frederick Sanger and colleagues developed a method of DNA analysis that made it possible to determine the nucleotide sequence of a gene in a matter of days. This achievement earned Sanger his *second* Nobel prize (the first was for research on insulin). Of course, a DNA sequencing technique is of little use without a gene to sequence, and, at that time, preparation of genetic material for sequencing required extensive bench work to make sufficient copies of a gene. Sanger's method was a disruptive innovation, but it had a delayed impact on mycology.

The PCR revolution

A second disruptive innovation was the invention in the 1980s of the polymerase chain reaction (PCR), which is a "gene amplification" technique that creates many copies of a target gene from a vanishingly small quantity of the total DNA of an organism (PCR is widely used in forensics). DNA fragments amplified with PCR can be easily sequenced using the Sanger method. Prior to PCR, it could take months or years to clone a gene of interest. After PCR, the work could be done in a day.



Mycologists were in the vanguard of the PCR revolution. PCR requires "primers," which are short DNA fragments that match DNA sequences flanking the gene of interest. In 1990, the American mycologist John Taylor and colleagues published a set of primer sequences that made it possible to amplify genes encoding ribosomes, which are subcellular molecular structures involved in protein synthesis. Ribosomes are made largely of ribonucleic acid (RNA) molecules, which are transcribed from DNA in one large fragment that is then spliced (cut) before the ribosome is assembled. The parts of the ribosomal RNA (rRNA) genes encoding the mature, functional ribosome are highly conserved across distantly related species (reflecting the importance of protein synthesis to a cell), whereas the "internal transcribed spacer" (ITS) regions that are removed prior to assembly of the ribosome are highly variable and are useful for species identification, as so-called "DNA barcodes." Phylogenetic analyses of PCR-amplified rRNA genes, and later protein-coding genes, established the broad outlines of fungal evolutionary relationships in the early 2000s.

Fungal genomics

A genome is the complete set of genes encoded in the DNA of an organism, embodying the "instructions" for making all the proteins (including enzymes) and RNA molecules of the cell. Genomes provide information about the molecular workings of an organism and they provide rich data for reconstructing evolutionary relationships. Again, mycologists were at the forefront. The first eukaryotic nuclear genome sequenced was that of a fungus, the brewer's and baker's yeast, *Saccharomyces cerevisiae*. Sequenced with Sanger's method, the yeast genome includes about 6,000 protein-coding genes and is just over twelve megabases (twelve million base pairs) in length. When it was completed in 1996, the yeast genome was a major breakthrough, warranting publication in the prestigious journal, *Science*.

The third disruptive innovation affecting mycology was "next-generation" DNA sequencing, which is not one but several technologies that make it possible to generate massive quantities of sequence data in a short time frame. For example, in one 48-hour run the Illumina NovaSeq platform can generate eight terabases of DNA sequences, which is eight trillion base pairs, or about 670 yeast genomes. Since the advent of next-generation DNA sequencing methods, around 2009, thousands of fungal genomes have been produced. With these blueprints in hand, biologists are attempting to make sense out of how the thousands of proteins in a cell work in an orchestrated manner to produce the diversity of fungal phenotypes, the forms an organism or cell presents. With the increase of novel deep-learning algorithms, the potential to predict these phenotypes from the increasingly available genomes is just around the corner. Genomes have also allowed us to reconstruct the fungal tree of life with confidence and revise the relationships inferred with analyses of PCR-amplified "marker" genes. The evolutionary framework used in this text is informed largely by recent genome-based phylogenies.

↗ Three-dimensional model of a mature ribosome. Before this functional structure is assembled, the spacer sequences used for DNA barcoding are removed.

← Even easily observed and collected mushrooms are the source of new species when examined at the DNA level.

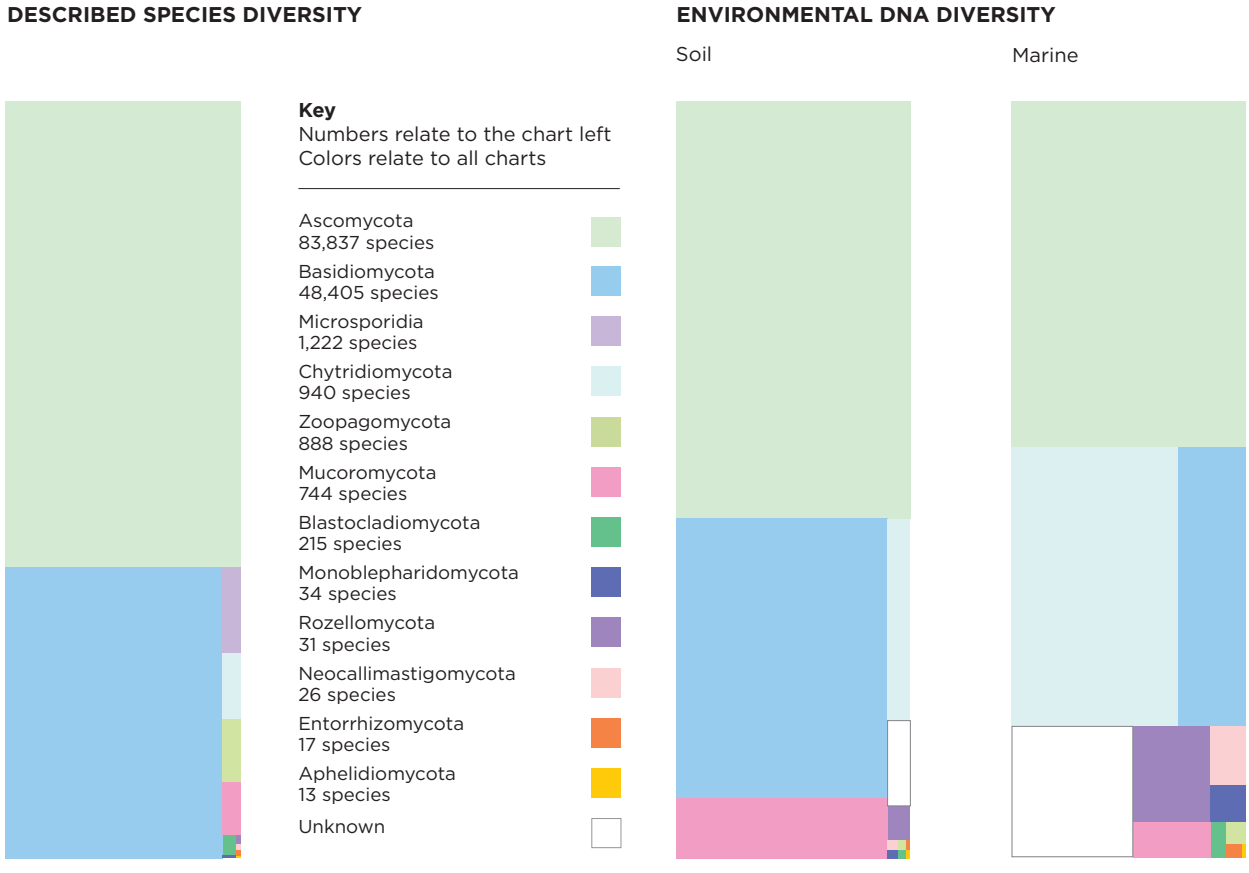
How many species of fungi are there?

The number of species of fungi on Earth is a matter of conjecture, which is a polite, scientific way of saying that we haven't a clue. In 1990, the British mycologist David Hawksworth suggested that there could be 1.5 million species of fungi on Earth. In 2005, the number was revised upward to more than five million species. An estimate published in 2017 speculated that there may even be upward of 166 million species of fungi. This latter inference rested in part on an assumption that every species of animal, including those in hyperdiverse poorly documented groups like mites, harbors at least one species of Microsporidia.

While the actual number of species is uncertain, the number of described species, those that have been formally named by taxonomists, is known with fairly high precision, thanks to nomenclatural databases. Currently, there are about 175,000 described species of fungi. For the last twenty years, taxonomic mycologists have described about 2,500 species per year. If the 2005 estimate of five million species is roughly correct, then at our current pace it will take about 2,000 years to name all species of fungi on Earth. With possibly 97 percent of all species undescribed, mycologists are in the dark about the morphology and function of most of the species. Is there a solution to shedding light on this hidden biodiversity?

Dark fungi

In the late 1980s, fungal ecologists began to use PCR-amplified ITS sequences to identify fungi in mycorrhizae (see Chapter 6, page 180). At first, the



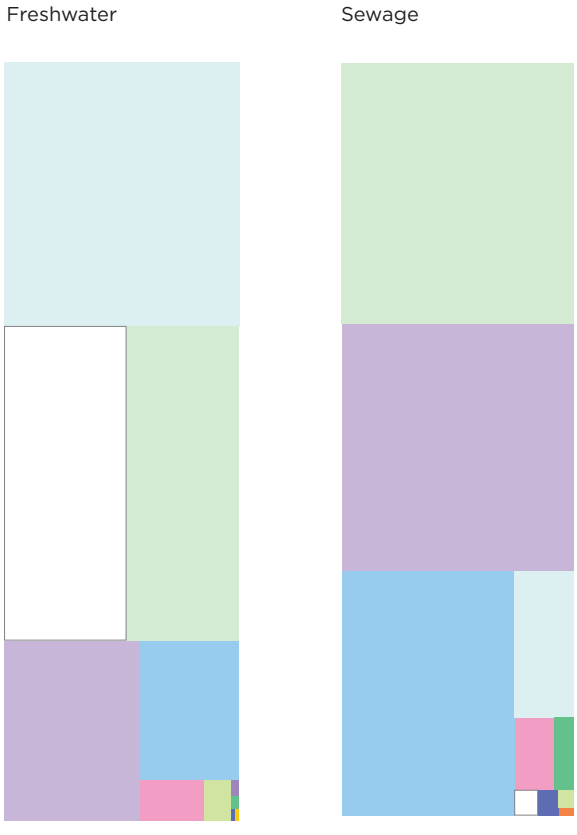
↑ Proportions of formally described species in different phyla. Areas of rectangles reflect numbers of species.

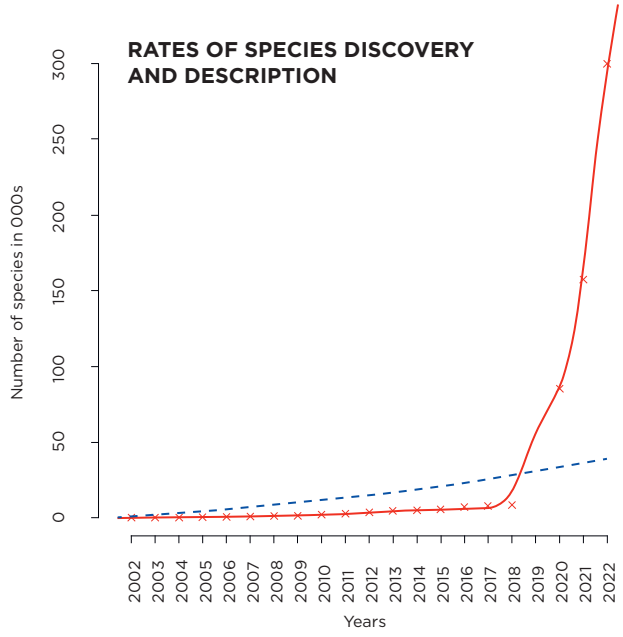
↑ Diversity in soil resembles described diversity; aquatic habitats (including sewage) contain many undescribed Chytridiomycota and Rozellomycota species.

mycorrhizal ITS sequences were identified by matching them with sequences from mushrooms collected at the same sites or in publicly accessible DNA sequence databases. Soon, environmental fungal biologists started to extract DNA directly from plant tissues, soil, or water, and amplify and sequence ITS. The field of fungal metagenomics was born. Most environmental sequences were found to have no match in public databases. These unidentified organisms are the "dark fungi."

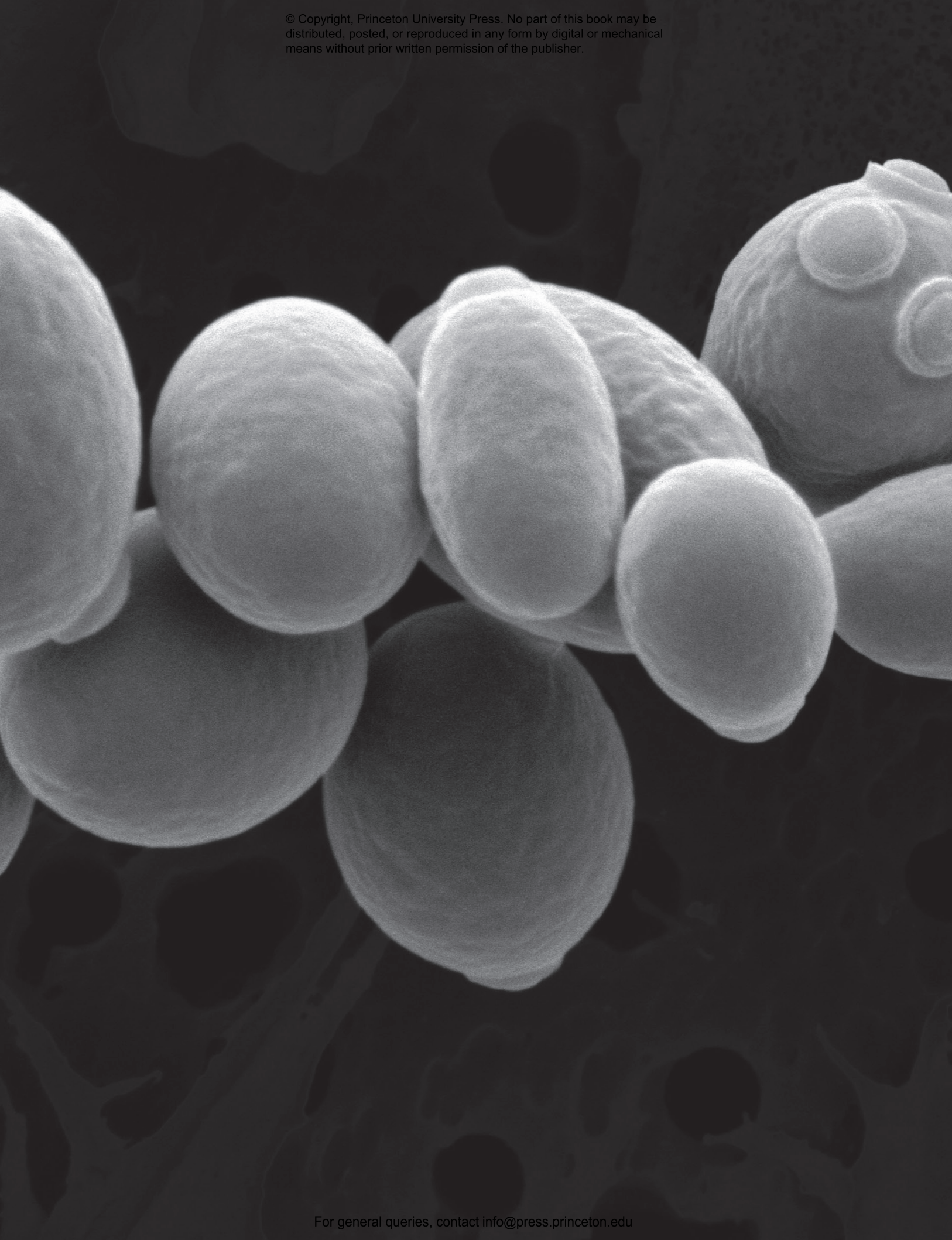
The application of next-generation sequencing methods dramatically increased the rate of discovery of dark fungi. A recent analysis found that more than 300,000 species of dark fungi were detected in just five large metagenomic studies of soil DNA published from 2019 to 2022. Over the same interval, about 12,000 new species were formally named using traditional specimen-based approaches. Some efforts to catalog these dark fungi have been attempted using online platforms such as UNITE and GlobalFungi, that aggregate data to attempt to find the same species across studies. These community resources provide a means of cataloging dark taxa, but they are not sanctioned by the *International Code of Nomenclature for algae, fungi, and plants* (the *Code*), and therefore are not universally used and supported.

Metagenomic studies continue to expand and challenge our ideas about the relative as well as absolute diversity of different groups of fungi, particularly in aquatic habitats. In freshwater and marine environments and in sewage the proportion of chytrids and rozellids is much greater than expected based on described species or metagenomic diversity in terrestrial habitats. At present, the *Code*, which sets the rules for naming fungi, prohibits formal description of species based only on environmental sequences (i.e., without a physical specimen or culture), no matter how many times they have been detected. Consequently, there is a vast and rapidly expanding gap between our current understanding of fungal diversity and the list of formally named species.





→ Formal species description based on specimens (blue line) has been vastly outpaced by species discovery based on DNA (red line).



CHAPTER 2:
YEASTS, HYPHAE, AND MYCELIA

Fungi produce a bewildering array of forms, both micro- and macroscopic. All are made of cells. In this chapter we examine the two main kinds of cells produced by fungi: yeasts and filamentous hyphae. Yeasts are rounded cells that reproduce by shedding buds from their surfaces or by splitting into two equal halves. Filamentous hyphae are cylindrical cells that are thinner than human hairs, grow at their tips, and branch to form colonies. Colonies of branching hyphae are called mycelia. Yeasts and hyphae feed by absorbing food from the fluid or solid matter in contact with their cell surfaces. Some species of fungi can switch between these types of cells, growing as yeasts when they are submerged in fluids and forming blobs on surfaces before transforming themselves into filamentous hyphae to form mycelia in solid materials.

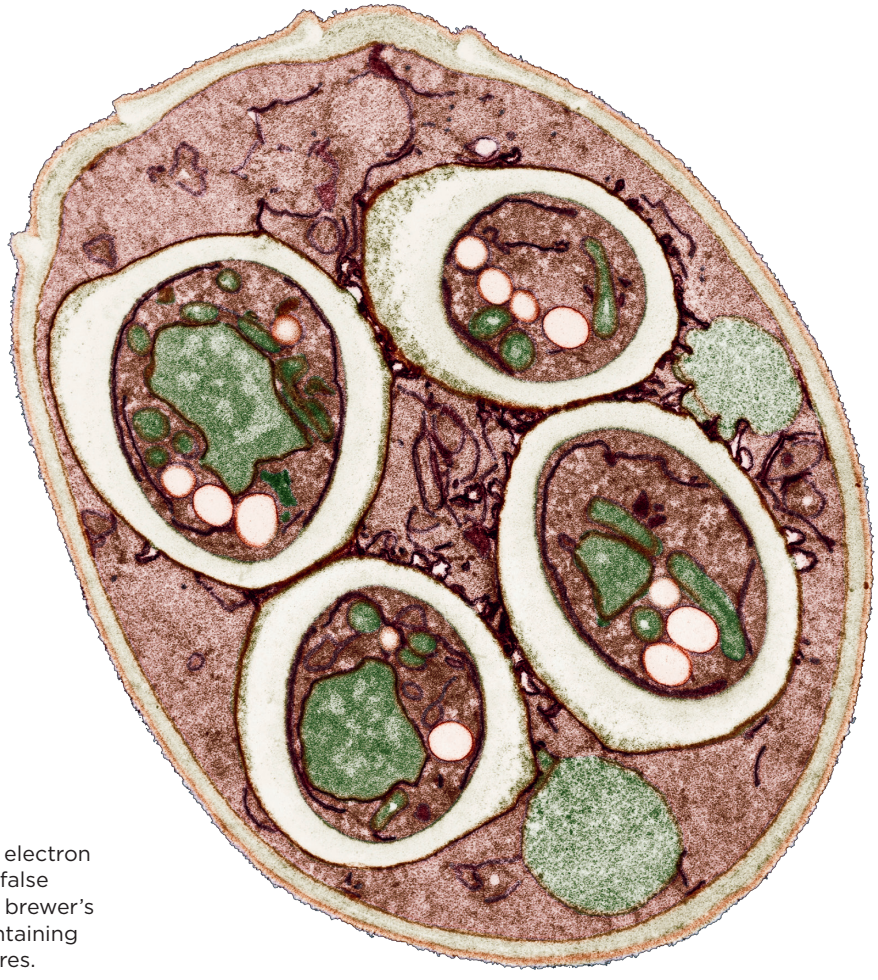
Certain ancient fungal lineages described in the previous chapter—such as aphelids and rozellids—may produce wall-less cells that ingest food by phagocytosis (also called phagotrophy, see page 21). These and the paraphyletic "chytrids" also produce sperm-like cells that can swim through fluids using flagella. Although often unicellular, they are not considered yeasts.

← Scanning electron micrograph of the brewer's yeast, *Saccharomyces cerevisiae*.

Yeasts: Fungi in fluids and on surfaces

Yeast cells are produced by species in all of the fungal phyla, but the greatest number of yeasts belong to the ascomycetes. There are more than 1,500 described species of ascomycete yeasts. The best known of these unicellular fungi is brewer's or baker's yeast, *Saccharomyces cerevisiae*.

Yeast is the microorganism that produces alcohol in beer and wine and releases CO_2 that raises bread dough. It multiplies by forming buds or daughter cells when it is suspended in beer wort and grape must and mixed in bread dough. These buds break from the surface and grow on their own, producing the next generation of yeasts. At its fastest growth rate, a group of one hundred yeasts can grow to a population of hundreds of billions of cells in two days. In addition to its uses in brewing and baking, *Saccharomyces* has been adopted as a model



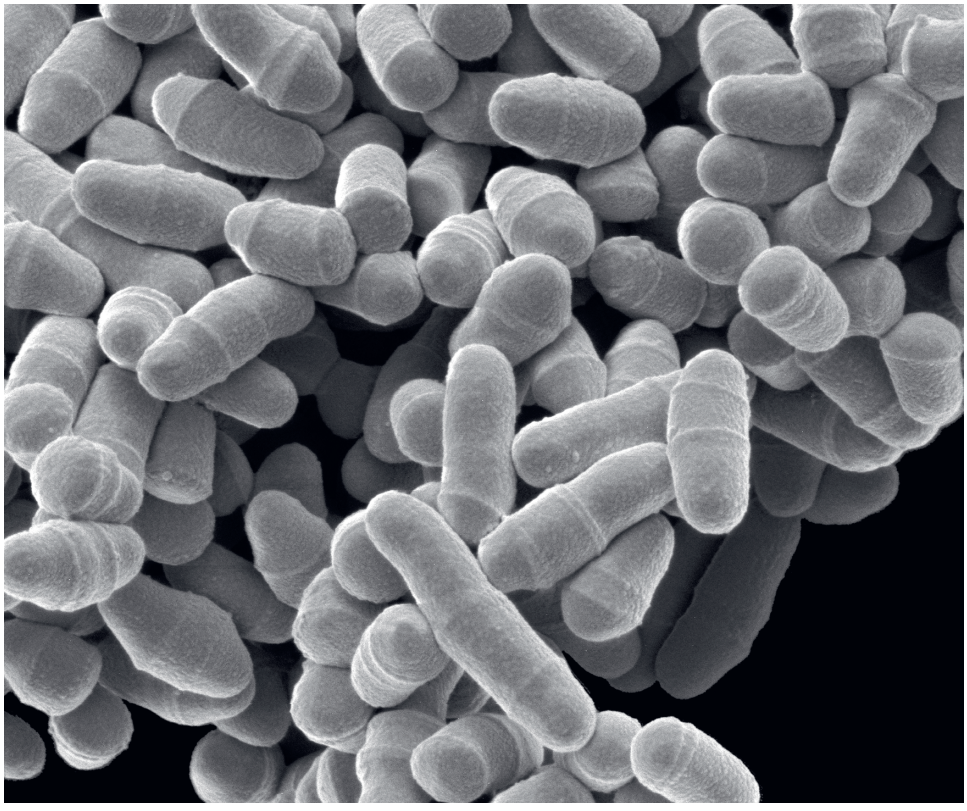
→ Transmission electron micrograph (false colored) of a brewer's yeast cell containing four ascospores.

organism by scientists investigating the fundamental processes of cell biology and genetics. Yeast is a good choice for this research because it grows so swiftly in the lab and can be manipulated using molecular genetic techniques. Yeast is also central to the production of ethanol used in biofuels (see Chapter 5, page 162).

Budding and fission yeasts

Most yeasts reproduce by forming new cells called buds that break away and continue the reproductive process by forming their own buds. *Saccharomyces cerevisiae* reproduces in this asexual fashion in which a single cell makes copies of itself. Each yeast cell has a single nucleus in which its chromosomes are housed. When a bud forms, the chromosomes are duplicated and the nucleus splits into two during the cell division mechanism called mitosis, with one nucleus remaining in the original cell and one nucleus moving into the bud. The original cell is called the mother cell, and the mother cell divides into a pair of daughter cells. Repeated budding generates a population of cells which are clones, meaning that they have the same genetic makeup. Species of *Schizosaccharomyces* are fission yeasts that use a different division mechanism in which a cross wall or septum develops in the middle of the mother cell to create two daughter cells. *Saccharomyces* and *Schizosaccharomyces* are in two different groups of Ascomycota, called *Saccharomycotina* and *Taphrinomycotina* (see Chapter 4, page 114). It is likely that the yeast form evolved independently in the two groups.

Although some yeasts do not appear to reproduce sexually, sexual reproduction has been studied in many of these fungi (see Chapter 3, page 96). In brewer's yeast, for example, cells come in two mating types that can merge with each other and produce ascospores. When the ascospores germinate they release yeast cells that reproduce asexually by budding.



← Scanning electron micrograph of fission yeast, *Schizosaccharomyces pombe*.

Yeast ecology

Like other fungi, yeasts feed as decomposers, breaking down biological materials and recycling nutrients. They do this everywhere. Some yeasts are generalists, meaning that we find them in many different habitats, whereas others are specialists that grow in a particular environment, such as the gut of an insect.

Yeasts grow in soils, on the surface of plants, in rivers, lakes, and the oceans. Yeasts become trapped in ice and grow in the water flowing from melting glaciers when they are released.

An estimated one sextillion yeasts (10^{21}) bathe in the sea. These marine yeasts grow on tiny food particles and absorb nutrients that are dissolved in the seawater (see Chapter 9, page 308). Their numbers are greatest in the polluted water around coastal cities, where they perform the essential task of breaking down the clouds of human waste. Their numbers decrease in the open ocean, but they have been found in the middle of the Pacific Ocean at a depth of 2½ miles (4 km). Their DNA has also been identified in seawater samples from the deep Atlantic, close to the wreck of the Titanic, and in deep-sea muds.

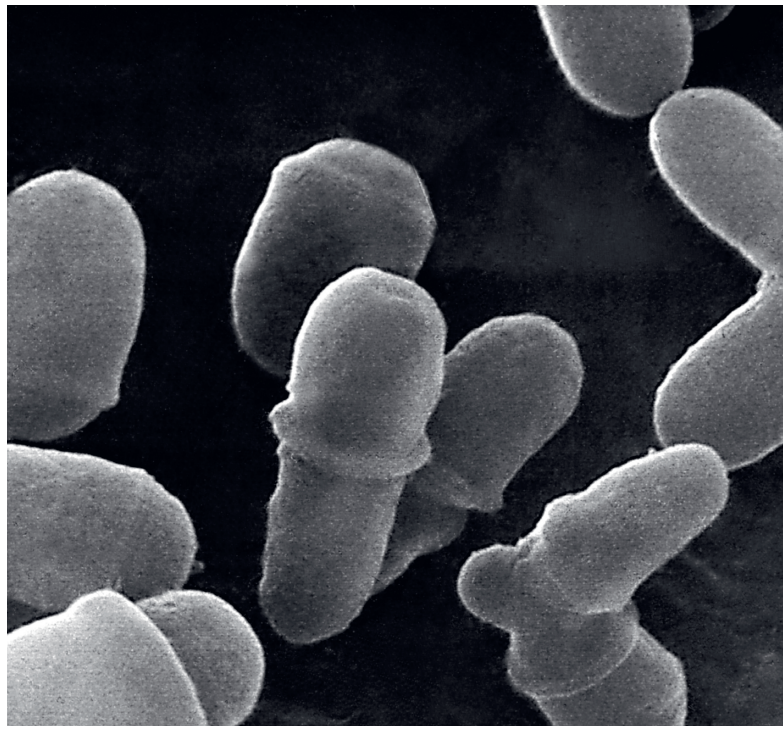
Yeasts in extreme environments

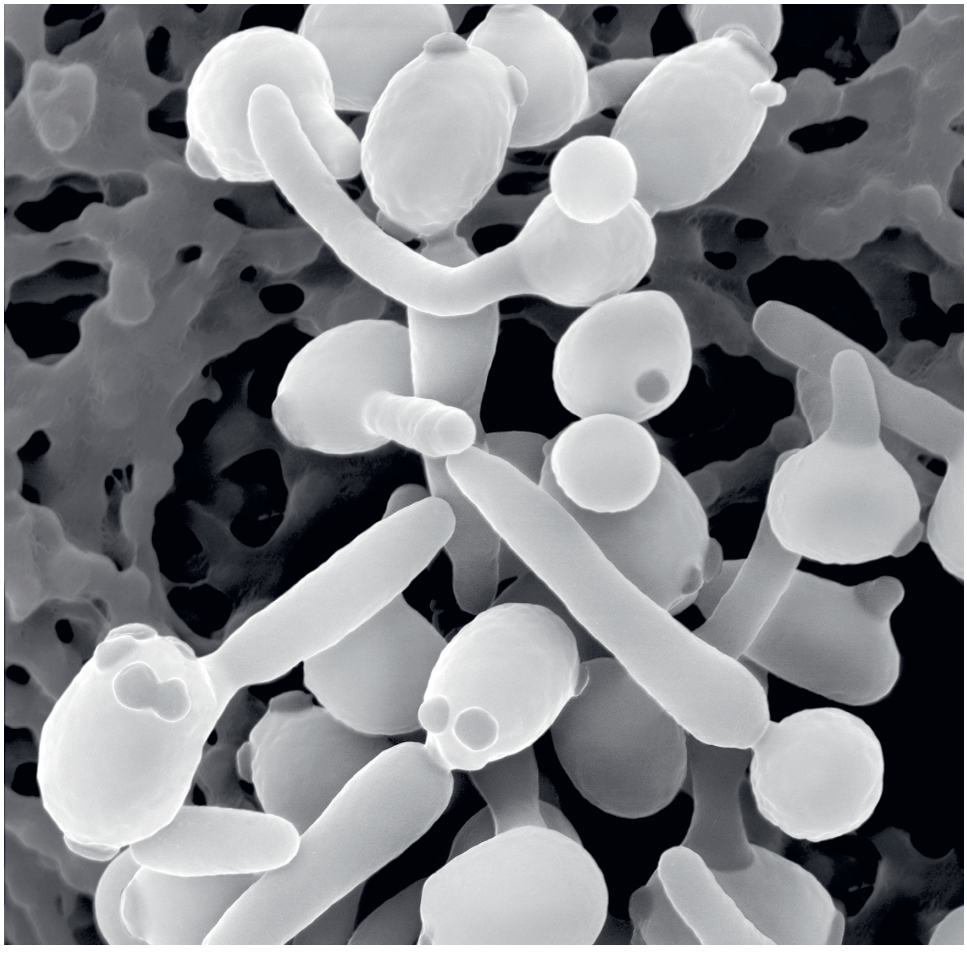
The shape and budding mechanism of yeasts appears to suit them for growth in very challenging habitats where they are exposed to extreme temperatures and chemical conditions. Yeasts grow in acidic soils, alkaline hot springs, salt-saturated brine pools, desert ecosystems, rock surfaces where they have no protection from sunlight, and soils contaminated with heavy metals and radioactive waste. Filamentous fungi are found in some of these locations too, but the yeast cell seems to be particularly resilient to harsh environmental conditions. Earth is a very yeasty planet.

← Thermotolerant yeasts have been discovered in the geothermally heated hot springs of Hakone, Japan.

Yeasts in medicine

Many of the fungi that grow on the human body are yeasts. These include species of *Malassezia* (Basidiomycota) that grow on the scalp and billions of *Candida* (Ascomycota) yeasts that grow alongside the trillions of bacteria in the gut. Although the yeasts are less numerous than the bacteria, they are much larger cells and provide a huge collective surface area for interactions with the immune system. In patients with weakened immune systems, *Candida* can switch from its budding form to filamentous hyphae and penetrate the epithelial tissue barriers and the endothelial cells lining blood vessels. This is a prime example of dimorphism, and we refer to *Candida* as a dimorphic fungus. In this way, the fungus that develops as a pathogen can spread through the body producing a serious disseminated infection or mycosis.





↑ *Malassezia* yeast cells.

← *Candida* yeast cells forming hyphae.

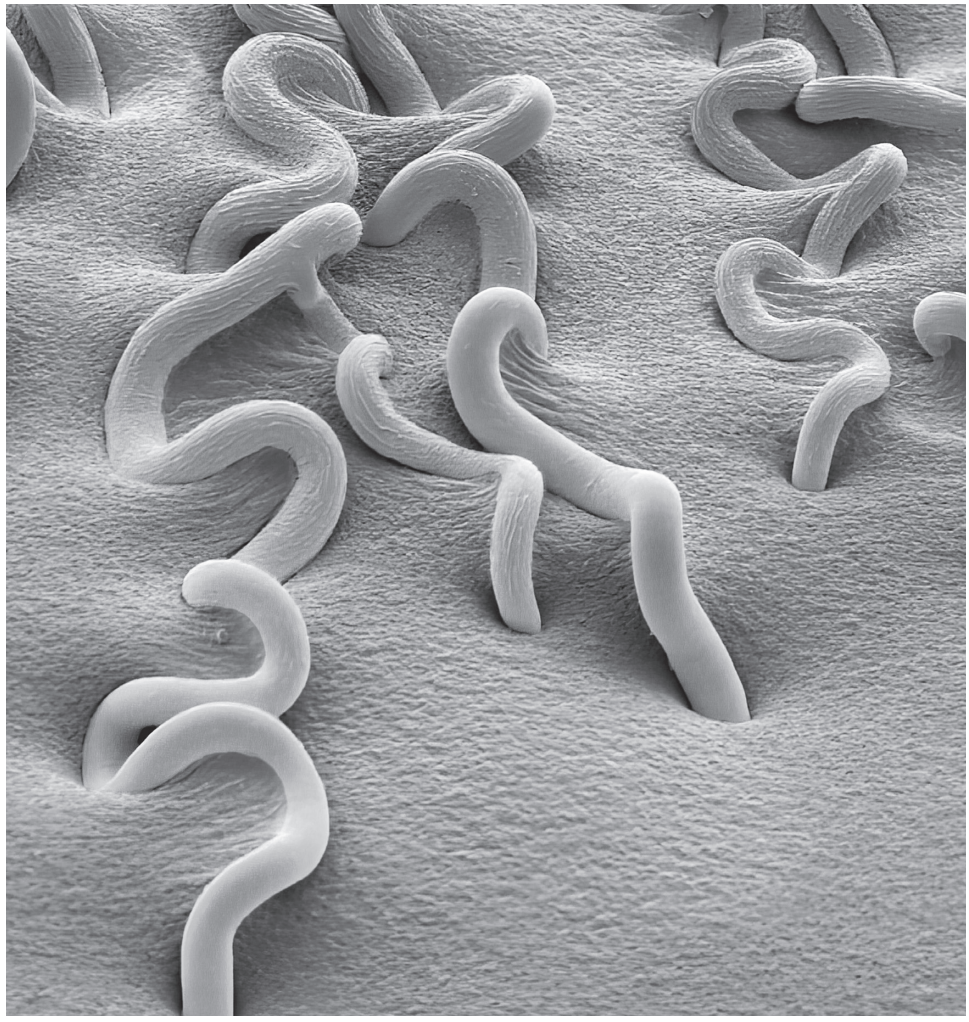
Hyphae and invasive growth

While yeasts tend to grow in fluids and on surfaces, hyphae grow in three dimensions by penetrating solid materials. This developmental mechanism is called invasive growth.

Hyphae allow filamentous fungi to grow as saprotrophs by penetrating soils and decomposing the tissues of dead plants and animals. Filamentous fungi that grow as pathogens use the same invasive process to feed on the tissues of living plant and animal hosts. The efficiency with which filamentous fungi proliferate in solid sources of food suggests that the hypha evolved as an invasive device.

↑ Single fungal hypha with branches.

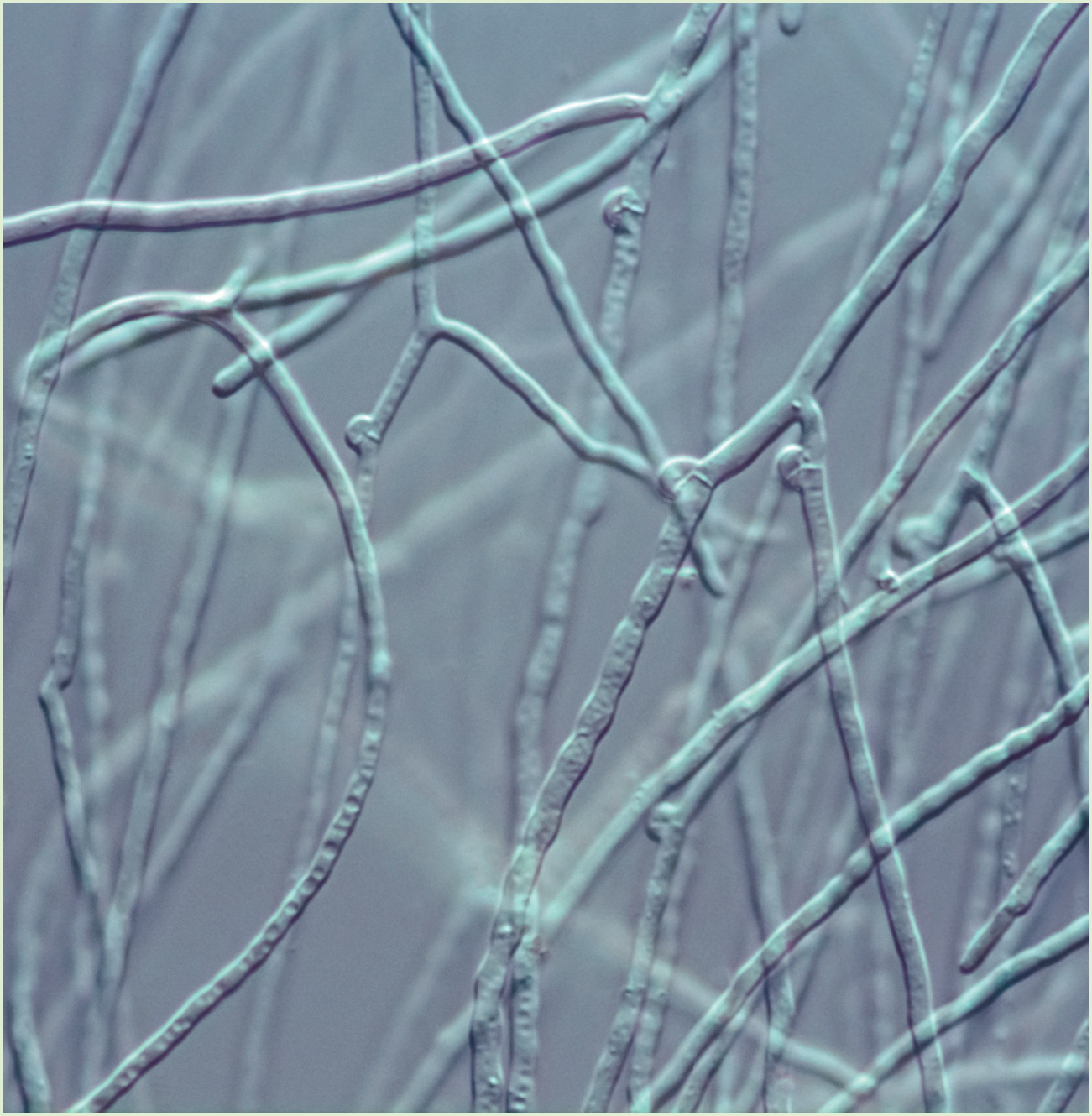
→ Hyphae of a drug-resistant strain of the fungal pathogen *Aspergillus fumigatus* on agar media.



Osmotrophy

Yeasts and hyphae feed by absorption. Small molecules can be absorbed without chemical modification, whereas fungi use secreted enzymes to break down larger molecules (polymers) into smaller molecules (monomers) for transport through their cell membrane. This feeding mechanism has been called osmotrophy. The term osmotrophy must not be confused with osmosis, which refers to the movement of water across a membrane. (Osmosis allows cells to absorb water; it is not a feeding mechanism.)

↓ Branching hyphae of a basidiomycete fungus with clamp connections (see page 94).



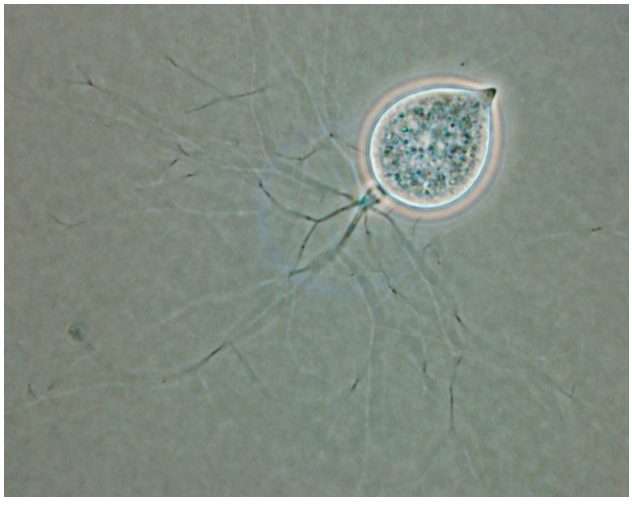
Origins of filamentous growth

The evolution of filamentous growth allowed fungi to penetrate solid foods. Chytrid fungi that produce zoospores in aquatic environments use thin cell extensions called rhizoids to anchor their thalli (microscopic bodies) to surfaces.

Chytrids evolved in aquatic habitats before fungi colonized terrestrial habitats. It is possible that tip-growing hyphae evolved from the rhizoids of chytrids.

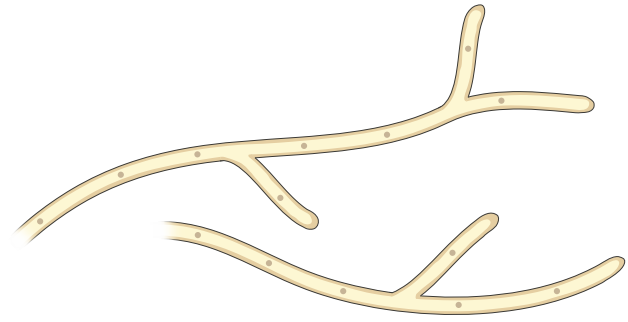
Tip-growing hyphae are also produced by water molds (Oomycetes), which are more closely related to brown algae and diatoms than they are to *Fungi* (see Chapter 1, page 30). This is an example of convergent evolution, which demonstrates the effectiveness of filamentous growth for invasive feeding.

Most zygomycetes (Mucoromycota and Zoopagomycota) display filamentous growth. Molecular phylogenetic studies suggest that zygomycetes are more ancient lineages of fungi than the Dikarya, which contains the Ascomycota and Basidiomycota. Hyphae that form the mycelia of most zygomycetes are aseptate, meaning that they are not organized into compartments separated by cross walls or septa. This is consistent with the idea that fungi with septate hyphae evolved from aseptate ancestors.

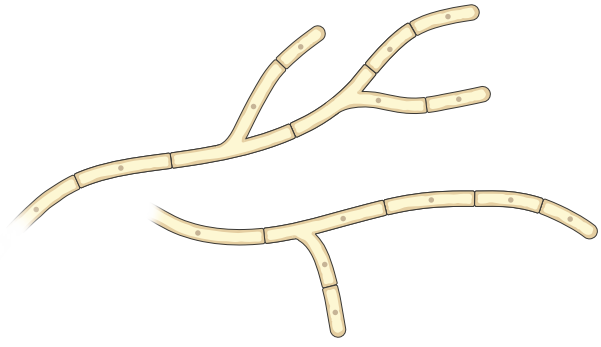


↑ Chytrid thallus with fine rhizoids extending from the base.

ASEPTATE (COENOCYTIC) HYPHAE



SEPTATE HYPHAE



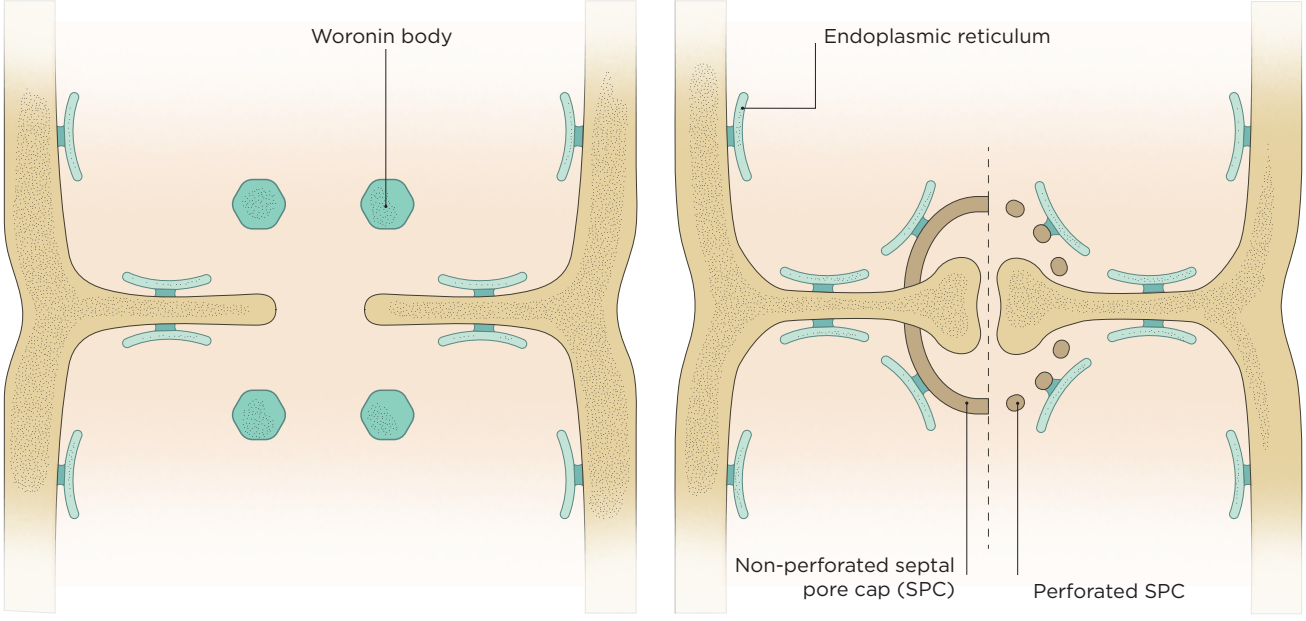
Aseptate hyphae

Colonies of large aseptate hyphae produced by species of *Mucor*, *Rhizopus*, and related fungi in the Mucoromycota are fascinating to look at under the microscope. The hyphae of these colonies, or mycelia, become wider as they grow and form branches, with the largest hyphae swelling to a few tenths of one millimeter in diameter. Even at low magnification, the streaming of cytoplasm within these cells can be seen. This fluid flow pulses, speeding up, then slowing down, and reversing direction every few minutes. Particles within the cytoplasm move within the stream, along with large fluid-filled vacuoles. Streaming connects the largest hyphae to their branches and onward to the extending tips of the colonies. Nuclei move freely in the fluid too, so that a colony operates as a single cell filled with multiple nuclei. As the fungus expands, some of the main branches become separated from other parts of the colony by cross walls but there does not seem to be a fixed pattern to their formation. If the colony is injured, proteins form jelly-like clumps to prevent the cytoplasm from leaking out of the hypha.

Septate hyphae

The hyphae of the Ascomycota and Basidiomycota tend to be thinner than those of the Mucoromycota, on the order of one hundredth of one millimeter. These hyphae are divided into compartments of varying length by septa, and the septa have central holes through which the cytoplasm flows. Damage to hyphae with septa is repaired by closing the septa so that the bleeding of the cytoplasm is limited to a single compartment. Pre-formed crystalline bodies are stationed on either side of the septa of ascomycetes. These plug the central holes of the septa when damage occurs. The septa of basidiomycete hyphae have barrel-shaped swellings around their central holes. The septal swellings are capped by membranes perforated with tiny holes that act like sieves. These holes limit the movement of larger organelles between compartments and can be closed when the hypha is damaged. Even though septa and associated structures control the movement of cytoplasm through ascomycete and basidiomycete hyphae, fluid streams through the colonies and even the nuclei squeeze between compartments.

HYPHAL SEPTA OF AN ASCOMYCETE (LEFT) AND BASIDIOMYCETE (RIGHT)



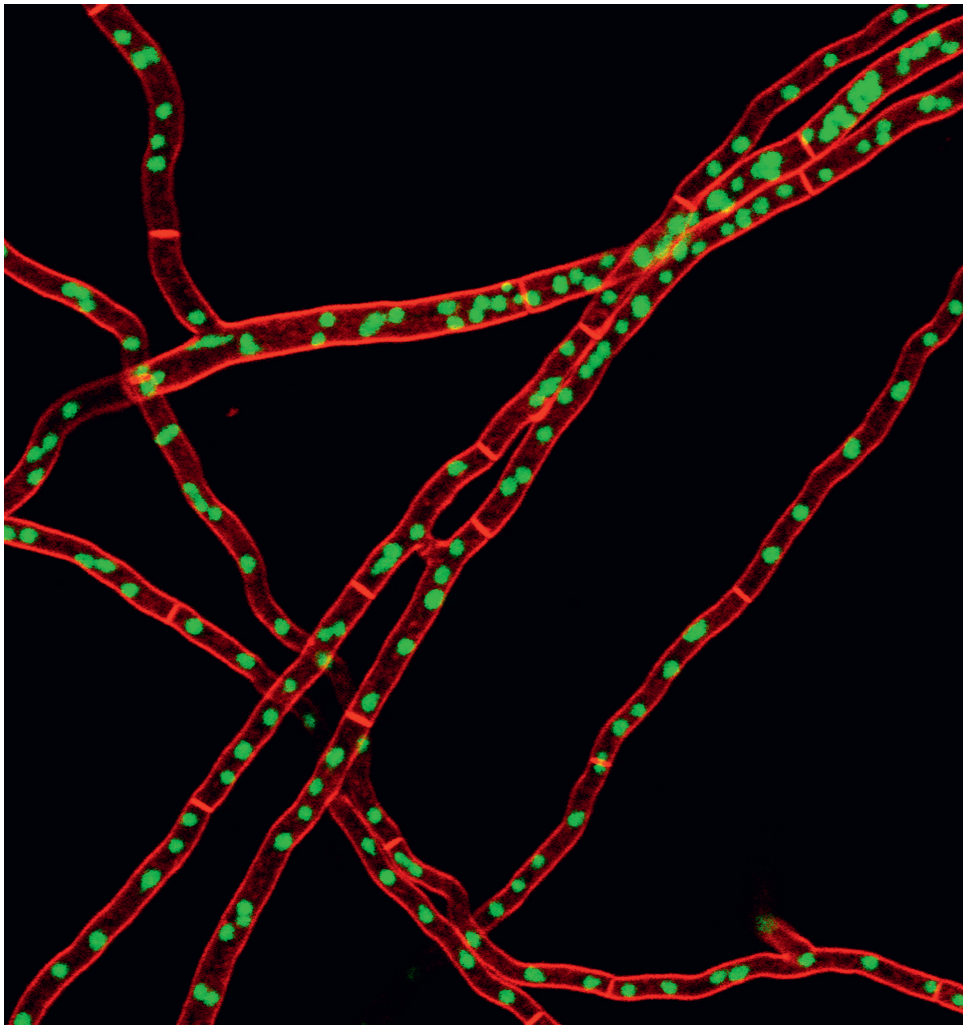
Hyphae as multinucleate cells

Hyphae are fungal cells that contain multiple nuclei. Nuclei are distributed throughout aseptate hyphae and are carried with the cytoplasm as it flows into branches and throughout the mycelium.

The distribution of nuclei is more complicated in septate hyphae because the individual compartments separated by septa contain single nuclei in some species and multiple nuclei in others. The arrangement of nuclei also changes during the life cycles of fungi as the mycelia of different mating types fuse as part of the process of sexual reproduction (see Chapter 3, page 88).

Vesicles and tip extension

To understand how hyphae grow we need to look at the tips of these filamentous cells where they elongate at top speeds of 1 millimeter per hour. Hyphal tips are packed with vesicles, which are tiny pockets of fluid surrounded by membranes. These vesicles merge with the cell membrane at the hyphal tip, adding new membrane

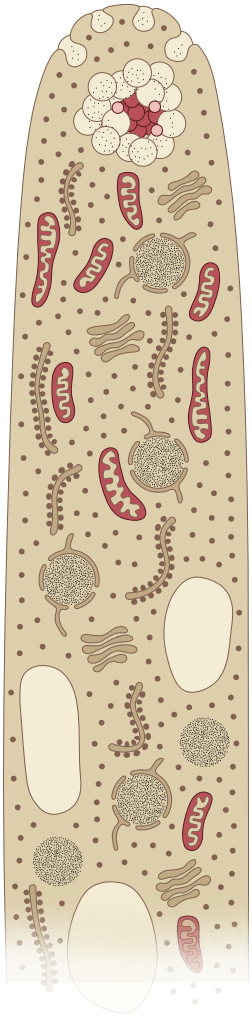


← Multinucleate hyphae of *Neurospora crassa*. Nuclei are stained green with a fluorescent dye. The red dye is associated with membranes, including those around the septa.

to the expanding surface through the process of exocytosis. The vesicles also supply the structural molecules (building blocks) and enzymes to assemble the expanding cell wall on the surface of the membrane.

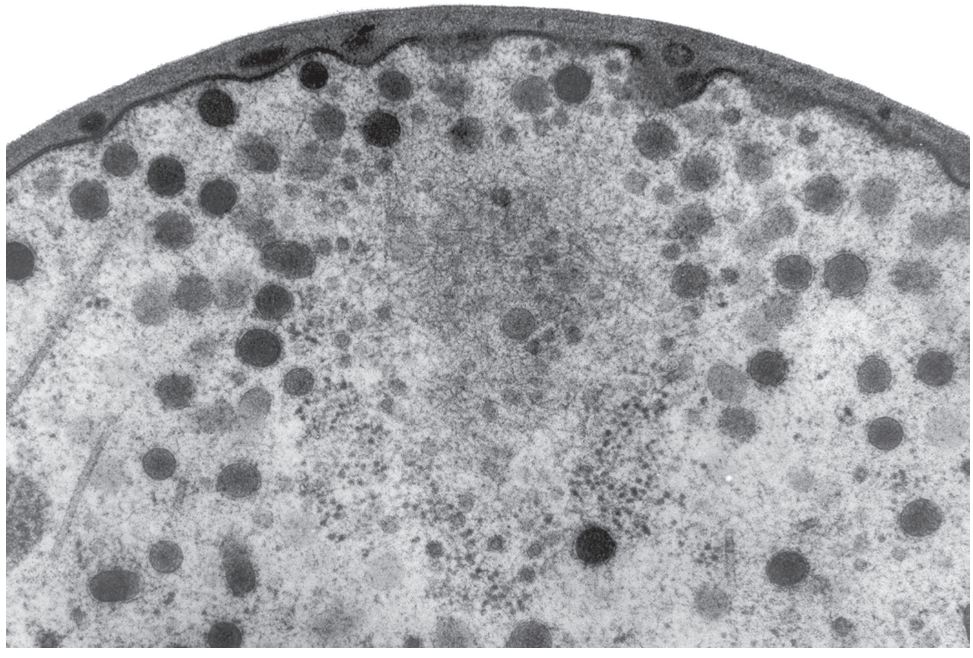
Mycologists are working to understand how all the cell wall ingredients are brought together at the hyphal tip, but there is a lot that remains to be learned. Hundreds of vesicles are delivered at the hyphal tip every second. These vesicles are produced behind the tip and are transported along the protein filaments of the cytoskeleton. The supply of vesicles must be coordinated with the demand for the new membrane and wall, with more vesicles arriving as the rate of extension increases, and fewer if the growth rate slows.

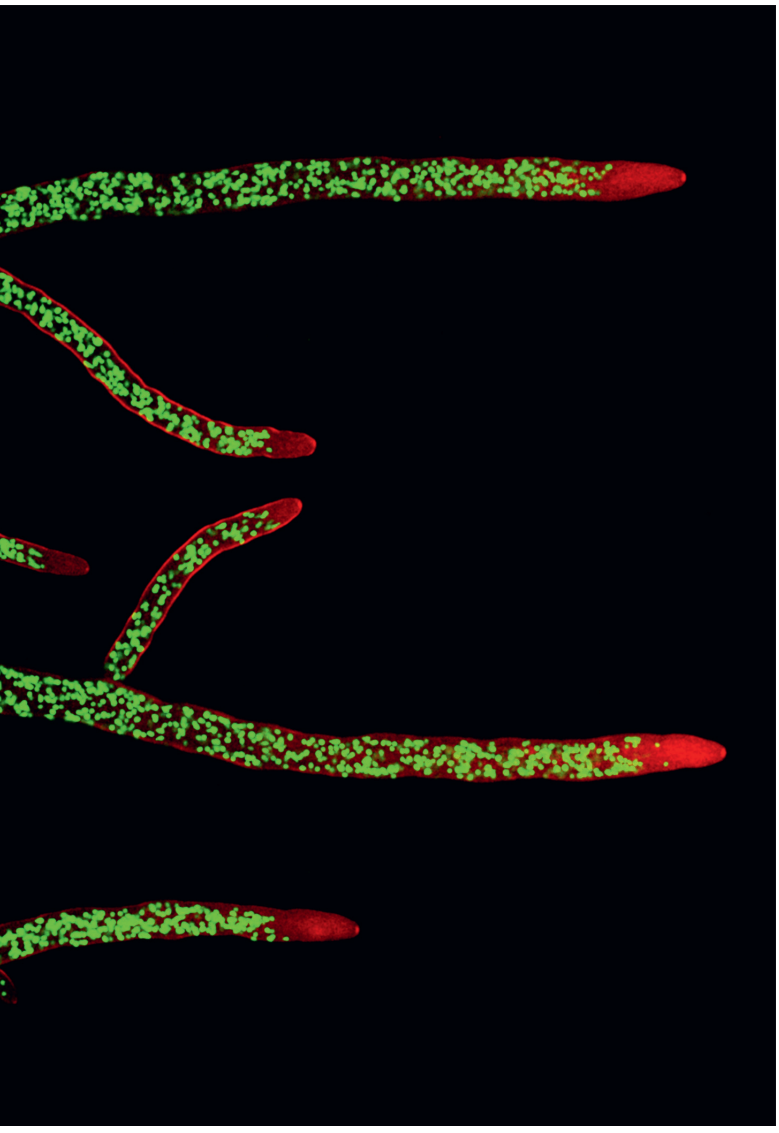
HYPHAL TIP GROWTH



← Hyphal tip, showing apical concentration of vesicles and the release of their contents (exocytosis).

↓ Transmission electron micrograph of hyphal tip of *Botrytis cinerea* (Ascomycota), with central Spitzenkörper and vesicles moving toward and fusing with the plasma membrane.



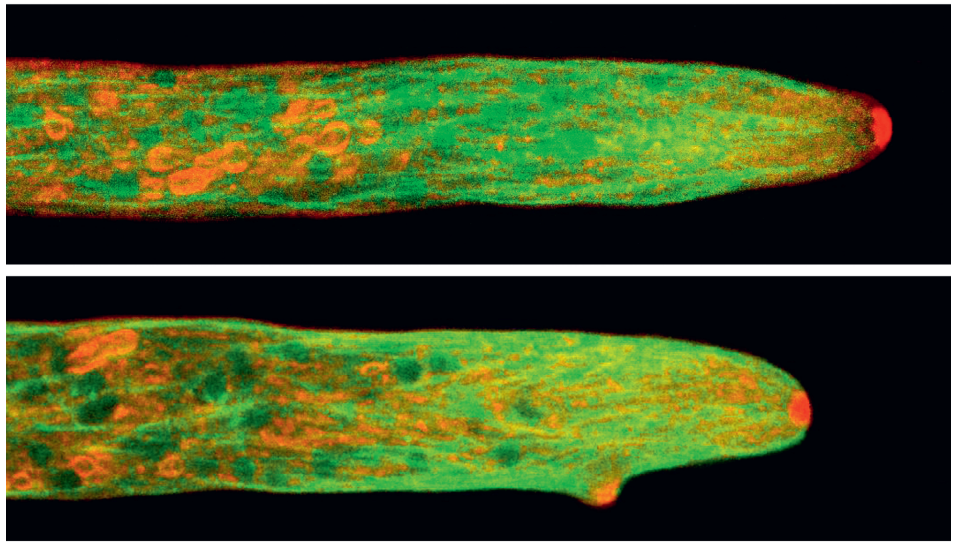


← Hyphae stained with fluorescent dyes to show nuclei in green and vesicles in red.

The Spitzenkörper

A collection of vesicles at the hyphal tip called the Spitzenkörper (which is German for apical body) plays a critical role in the supply of vesicles. This is visible as a dark spot using a light microscope. Some studies suggest that this works as a holding pen for vesicles arriving at the tip before they merge with the cell membrane. The position of the Spitzenkörper is associated with changes with the direction of the extending hyphal tip and new accessory Spitzenkörpers develop behind the growing tip at the sites of branch emergence. The positioning of new branches is controlled so that they do not develop too close to the tip of the primary hypha, but the precise location of each branch seems to be more flexible. Detailed observations on growing hyphae suggest that the Spitzenkörper works as a guide that controls the speed, shape, and direction of hyphal growth, as well as the development of branches, which raises the question: What controls the behavior of the Spitzenkörper?

→ Stained hyphal tips highlight vesicles associated with the Spitzenkörper at the tips of the main hyphae, and with an emerging branch in B. The cytoskeleton is stained in green.

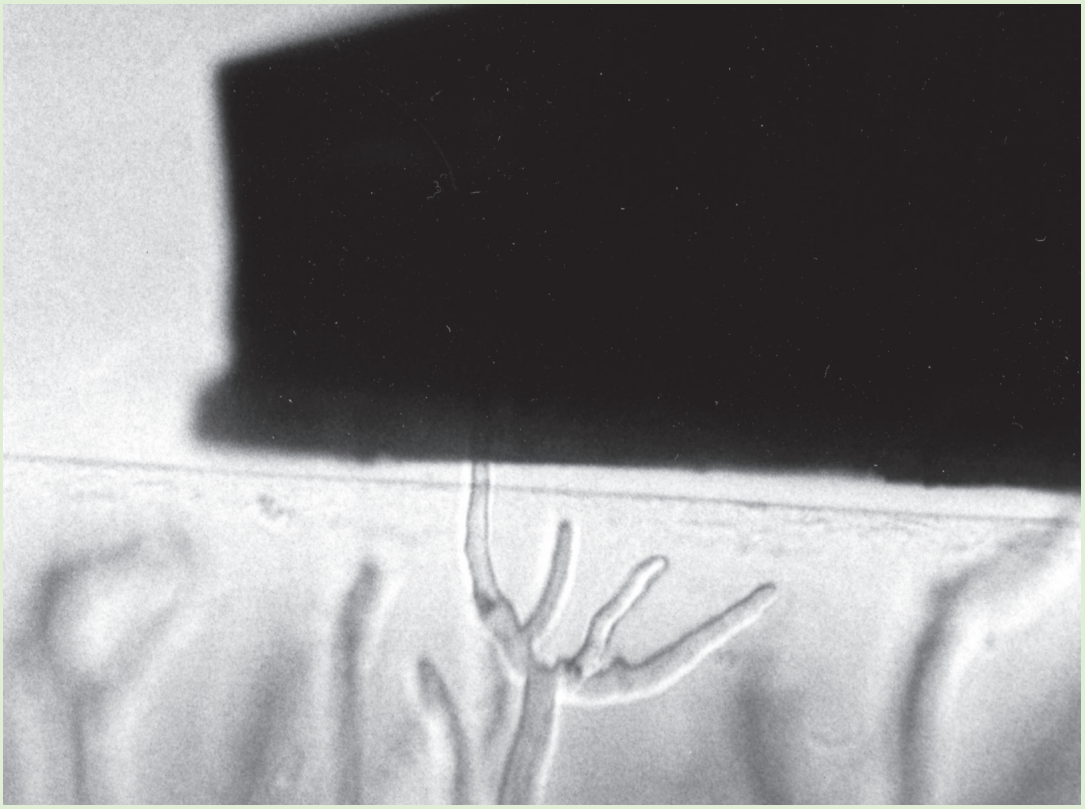


A

B

Research methods for hyphal growth

Many different approaches are used in research on hyphal growth. The behavior of living hyphae is studied with light microscopes and different components inside these cells can be stained with fluorescent dyes to see how they move and interact with one another. This is called live cell imaging, and the detailed internal structure of hyphae is examined at higher magnifications with electron microscopes. Biochemical analysis of the composition of the cell walls of hyphae coupled with genetic manipulation provides information on the way that hyphae operate. Physiological techniques allow mycologists to measure the pressure inside hyphae, the force exerted by single hyphae, and many other physical characteristics. A clearer picture of how hyphae work has come from bringing together the findings from these experimental methods, but many of the mechanisms that govern hyphal tip growth remain an enigma.

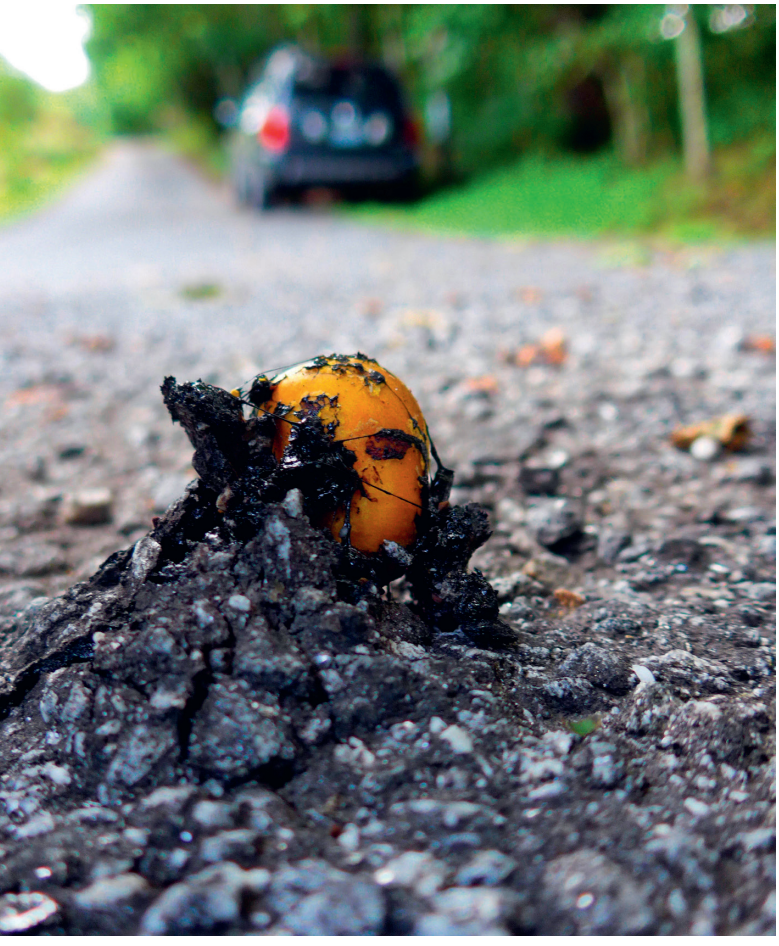


↑ A single hyphal tip emerging from agar pushing against the black beam of a highly sensitive strain gauge to measure the force that the fungus exerts.

Cytoplasmic flow in hyphae

The cytoplasm inside hyphae is a watery fluid containing dissolved molecules and ions, and suspended organelles including mitochondria, and nuclei. The movement of this fluid carries the organelles along the length of the hyphae toward the extending tips.

We can think of the mycelium as a complicated network of pipes through which the fungus controls the flow of its cytoplasm. As the cytoplasm flows in one direction through the hyphae toward the growing tips, smaller organelles and vesicles travel in both directions along the filaments of the cytoskeleton. Proteins that act as motors power these bidirectional movements. Experiments demonstrate that the mass flow of cytoplasm is driven by a gradient of hydrostatic pressure within the growing colonies.



← Mushroom pushing its way through a road surface using the force from its pressurized hyphae.

Hyphae under pressure

The fluid cytoplasm of the hypha is pressurized by osmosis, which pushes the cell membrane against the inner surface of the cell wall. Osmosis happens in response to the difference in the concentration of salts and sugars between the cytoplasm inside the hypha and the surrounding fluid. For example, a hypha growing in soil contains more of these dissolved solutes than the water percolating between the soil particles. This difference in chemical concentrations causes a net flow of water into the hypha, which becomes pressurized to a few atmospheres of hydrostatic pressure. Hyphal pressure can rise to twenty to eighty times the pressure in our arteries: 4 to 8 bars in a hypha, versus 0.1 to 0.2 bars in our bloodstream. This pressure is evident when a hypha is damaged on a microscope slide and cytoplasm bursts through the cell wall.

The pressure in hyphae (and other cells that develop substantial pressure) is called turgor. Bacteria and plants are examples of other organisms with walled cells that develop turgor. Amoebas and other single-celled protists that do not have cell walls use contractile vacuoles to expel water that diffuses into the cytoplasm by osmosis.

Hyphal penetration

The smooth cylindrical form of hyphae with domed tips is shaped by the interaction between the pressurized cytoplasm and the mechanical resistance of the cell wall. We know this because a hypha that is punctured and loses its pressure collapses and its cell wall becomes

The role of enzymes

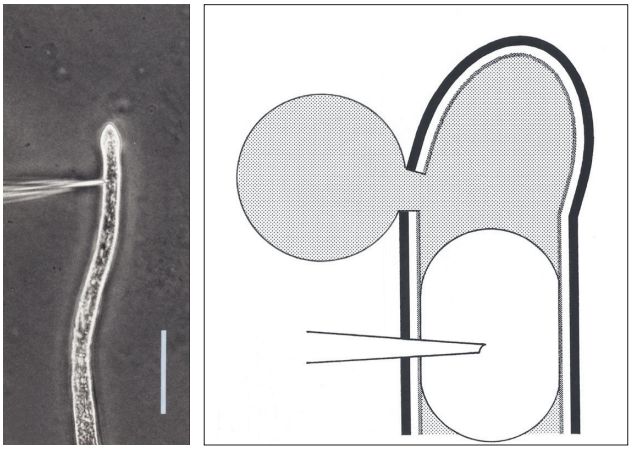
Invasive growth also relies on the release of enzymes from the tips of hyphae that perform the dual role of digesting their food and reducing resistance to penetration. Enzymes released from hyphae into woody tissues, for example, break down some of the tough materials as they release sugars and other small molecules that the fungus absorbs as its food (see Chapter 5, page 154). As this happens, the wood is weakened, which allows the hypha to push through the remaining obstacles using its pressure. The same thing happens when hyphae invade animal tissues, although the enzymes are different. In this case, the fungus breaks down proteins and fats as it colonizes the skin and other tissues. Some fungi feed on the bodies of dead animals, while pathogenic species infect living animals (see Chapter 8, page 256).

↑ Mycelium of a wood-decay mushroom extending underneath the bark of a fallen tree.

wrinkled. It follows that the healthy hypha relies on its pressure to maintain shape, and this pressure is essential for extending the cell wall at the growing tip. Turgor is one of many characteristics of hyphae that must be in place for growth to occur.

The filamentous shape of the hypha and the pressurization of its cytoplasm likely evolved to allow these cells to invade solid sources of food, including the tissues of plants and animals. Some of the pressure within a hypha is exerted on its surroundings as it grows and relaxes the cell wall at its tip. This process of wall loosening allows the hypha to adjust the force that it uses to overcome the physical resistance of its food or simply to push through obstacles in its path. This invasive growth mechanism is a critical feature of fungal behavior and contrasts with the way that yeasts grow on surfaces and in fluids by producing buds.

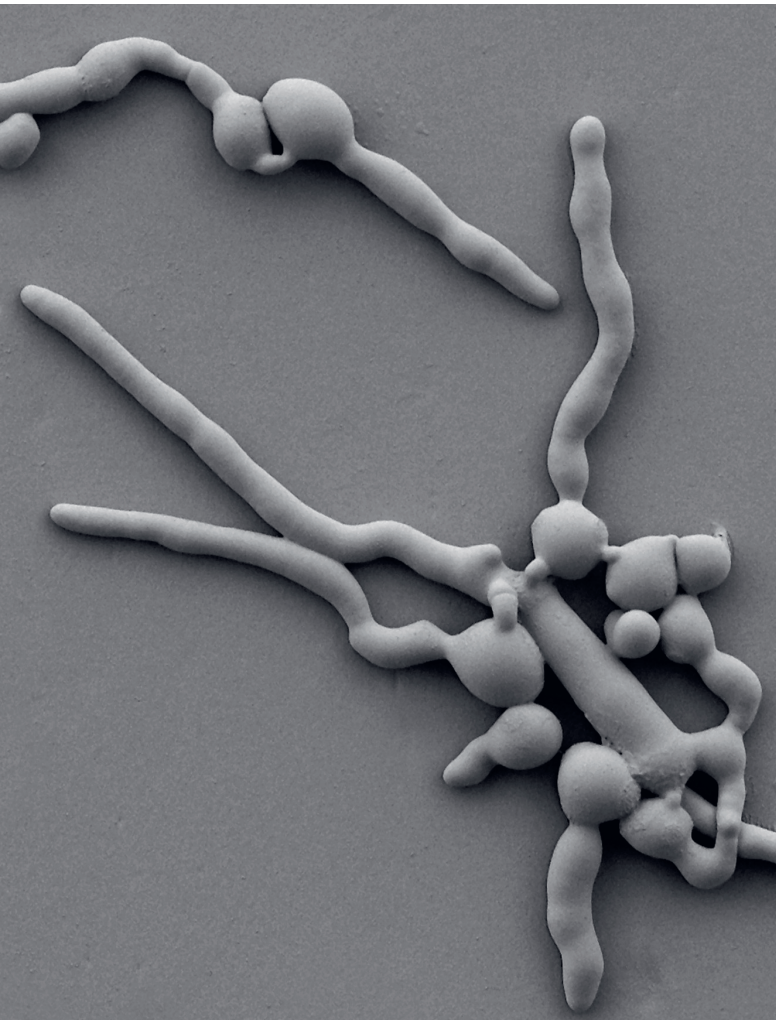
↓ Measurement of hyphal turgor pressure with a pressure probe (A). The pressure probe measures cell wall strength by injecting silicone oil into the hypha until the wall bursts (B).



A B

The mycelium

When fungal spores germinate, one or more slender germ tubes or germ hyphae extend from the spore. Each hypha grows away from the spore before it sprouts its first branch. More branches emerge as growth continues and branches form new branches to produce an expanding mycelium of hyphae.



↑ Early stage in mycelium development in the ascomycete fungus *Neurospora crassa*, showing hyphae emerging from spores.

Mycelia that form on hard surfaces like wet rocks grow in two dimensions; mycelia that develop in soil or penetrate plant tissues grow in three dimensions. Mycelia range in size from microscopic colonies on plant leaves to huge mycelia that occupy hundreds of hectares of forest soil.

The largest mycelia are produced by species of *Armillaria*, which attack trees and shrubs by growing from plant to plant in the soil. One celebrated mycelium of the honey mushroom *Armillaria ostoyae* studied in Oregon is spread over an area of 2,400 acres (900 hectares or 9 square kilometers; about the same size as Logan International Airport, in Boston, Massachusetts). It is thought to be 8,000 years old.

Hyphal networks

Although the tips of individual hyphae within the same mycelium extend as separate filamentous cells, the formation of branches creates a system of interconnected tubes. As the largest hyphae grow outward, they become separated like spokes on a wheel. Crosswise connections between the spokes are formed by the fusion of branches, which leads to the development of an integrated web. We have seen how pressure gradients cause the flow of the cytoplasm toward the growing tips of hyphae and molecular motors power the movement of vesicles. The operation of these mechanisms over the larger distances within a mycelium allows the fungus to transport materials from one part of the mycelium to another. This allows one cluster of hyphae that are absorbing plenty of food to supply other cells that have exhausted the nutrients in their vicinity.

Nutrient flow in mycelia

The transfer of nutrients across mycelia has been studied by tracking the distribution of radioactive tracers in fungi grown in trays of sand. Nutrients take minutes or hours to diffuse along hyphae but are also carried more swiftly in the flow of cytoplasm toward the tips. Analysis of the movement of the radioactive tracers shows that nutrient flow through the hyphae and cords (see page 65) of some fungi increases and decreases with a pulse rate lasting many hours. Water is also transported through tubular vacuoles.

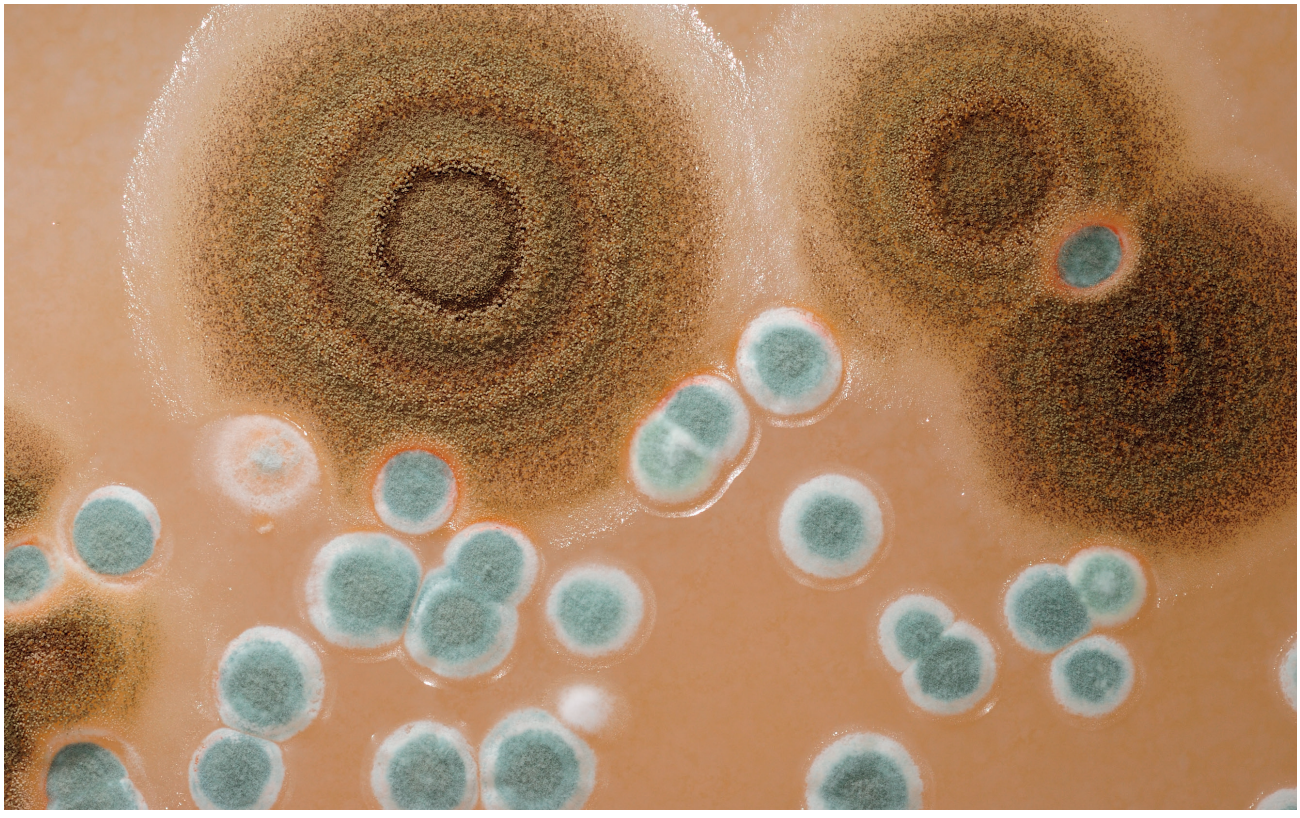
Separate mycelia of some species of wood-decay fungi fuse when they make contact and water and nutrients can be transferred between these colonies. Cooperation of this kind makes sense because the blended mycelium can be regarded as a single individual—something similar to the body of a multicellular animal. The situation is more complicated when nutrients are transferred between the mycelia of mycorrhizal fungi and the root systems of plants. The sharing of resources in these mutualistic relationships is a subject of active interest among experts on mycorrhizas (see Chapter 6, page 187, for a critical discussion of the "Wood-Wide Web").

↑ Logan International Airport in Boston, Massachusetts, USA, is about the same size (2,400 acres/900 hectares) as the largest discovered clones of *Armillaria ostoyae*.

↓ Fruiting bodies of *Armillaria* sp. are connected by an extensive network of hyphae and rhizomorphs.

Hyphal sensitivity and cellular consciousness

Hyphae detect microscopic ridges on leaf surfaces, steer their tips around obstacles, and shrink themselves to grow through constrictions. These expressions of the sensitivity of the hypha are concentrated in its tip where the disturbance of proteins in the cell membrane triggers signaling within the cytoplasm that results in changes in growth patterns.



Contact between mycelia of the same species and subsequent fusion of hyphae is another example of hyphal sensitivity involving the recognition of genetic compatibility and a slew of developmental modifications. The wounding responses described earlier in the chapter (see page 55) offer a third illustration of fungal sensitivity. Part of the reaction to a wound is an immediate automatic response to the loss of cytoplasm, which is like the clotting reaction produced by preformed proteins in our bloodstream. But these reactions in the fungus are followed by a series of more complicated growth processes in which branches may emerge on either side of a damaged hyphal compartment to restore growth. These and many other features of fungal behavior are expressions of cellular irritability that fall along a spectrum of complexity from bacterial behavior to nerve impulses in animal brains.

Do fungi possess intelligence?

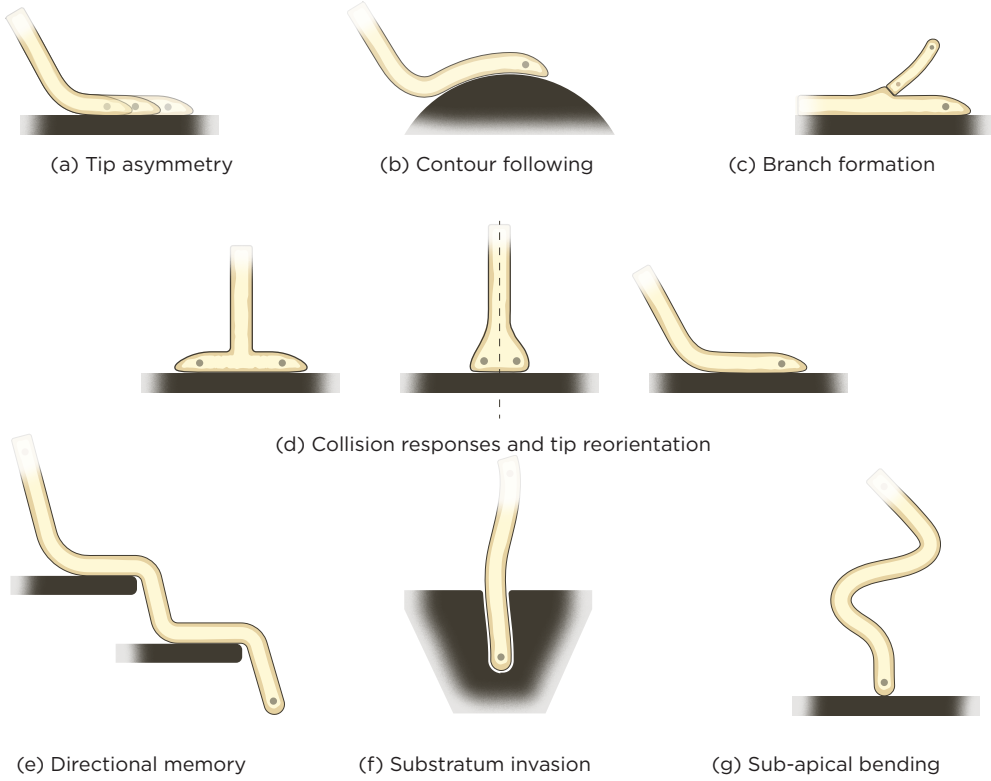
Recent experiments on hyphal and mycelial behavior have encouraged the idea that fungi possess a simple form of intelligence or consciousness. It seems logical to

reject this notion at first because we are so accustomed to the human version of consciousness. Ethological research has extended theories of consciousness to other brained animals, and there are striking examples of complex behavioral responses among animals without brains when they search for food, evade noxious chemicals and predators, and react to physical injury. Expanding this inquiry to single-celled organisms including amoebas and to fungi, we find that there is no clear definition of consciousness that allows us to separate conscious from unconscious organisms. All organisms are responsive to their environment and all of the underlying mechanisms have a cellular nature.

This inclusive view of consciousness is encouraged by evidence for fungal learning and memory among wood-decay fungi. Mycelia of these fungi that are successful in finding and decomposing small blocks of wood grow in the same direction when they are placed in fresh trays containing soil. They appear to remember the position of wood blocks in the earlier experimental test, which suggests that mycelia use a simple form of spatial navigation and memory. The fungus is not thinking in the sense that a brained animal thinks, but some of the underlying cellular processes are the same.

Billions of hyphae can grow in a cubic meter of the richest grassland soil or in compost used to cultivate mushrooms. These numbers are comparable to the density of neurons in the human brain, but this does not mean that fungi are capable of processing information like an animal nervous system. Nervous systems amplify their processing power by forming synaptic connections that allow each nerve cell to interact with thousands of neighbors. Cross connections between hyphae certainly boost the connectivity between cells in a mycelium, but the potential for communication is limited to the slow passage of chemical signals. Nevertheless, fungal ethology or behavioral ecology is an exciting area of research.

HYPHAL RESPONSES TO CONTACT WITH SURFACES



↖ Fungal colonies can detect and respond to other individuals of their own or other species.

→ Hyphal responses to growth on different surfaces.

Hyphal strands, cords, and rhizomorphs

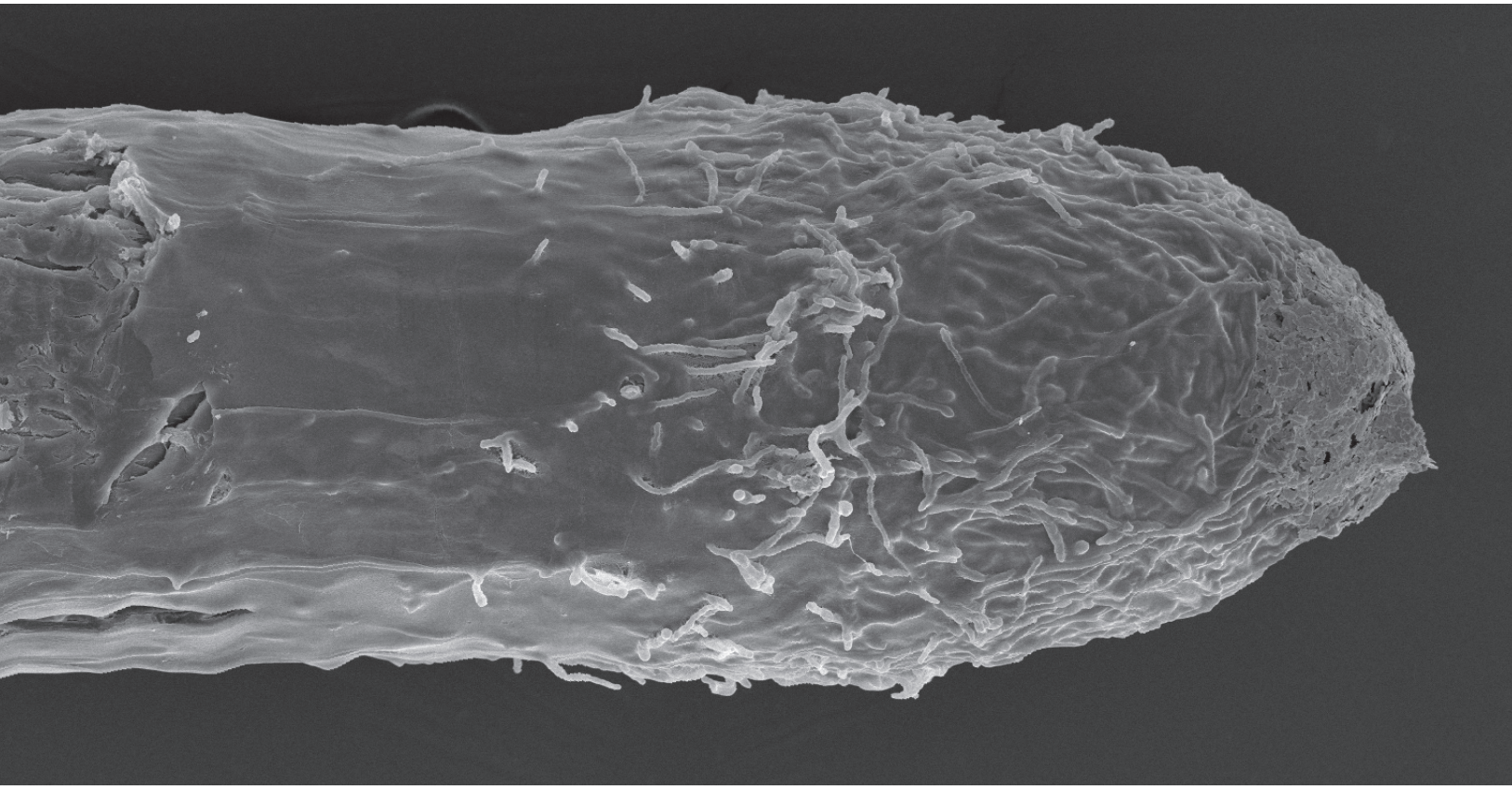
Some fungi produce multicellular assemblies of hyphae called mycelial strands, cords, and rhizomorphs. There are some variations in the definitions of these structures, but strands are the simplest structures, cords are larger, and rhizomorphs are thick, root-like organs.

← Characteristic black "shoestring" rhizomorphs of *Armillaria* growing under tree bark.

The term organ is applied to rhizomorphs because they are composed of hundreds or thousands of cells that grow in a highly coordinated fashion. Mushrooms are another example of fungal organs (see Chapter 4, page 104).

Strands and cords

The simplest strands are formed by bundles of a few hyphae that grow in parallel and are woven together with thin branches or tendril hyphae. According to some definitions, strands are transformed into cords when more hyphae become bundled together and develop cores of empty vessel hyphae. Cords are composed of hundreds of hyphae and reach a maximum diameter of a few millimeters. They are often found behind the tips of multiple hyphae that are spread out into the shape of a fan. Masses of white cords thread themselves through rotting wood or underneath wet leaf litter. Hyphae growing inside cords are likely to gain some protection from dehydration and the transfer of water through



↑ Rhizomorph tip of *Armillaria gallica*.

these active interior cells supports the growing tips as the older parts of the mycelium begin to dry out. In this way, the fungus can use cords as bridges over drier materials and reach wetter locations where they can resume the active decomposition of plant debris.

Rhizomorphs

Rhizomorphs have a more complicated structure than cords and are produced by a limited selection of wood-decay fungi and phytopathogenic species that cause plant diseases. The largest rhizomorphs can reach diameters of ⅜in (1 cm) and bear a superficial resemblance to plant roots. Rhizomorphs have rounded tips and are coated with mucilage, which probably helps to keep the hyphae hydrated and acts as a lubricant as they push through compacted soil. Hyphae organized as a layer beneath the rhizomorph surface operate as the active force-generating cells that allow the rhizomorph to push forward. This tip growth process distinguishes rhizomorphs from cords. The force exerted by the rhizomorph is derived from turgor pressure through the concerted loosening of the cell walls of multiple hyphae that run all the way into the tip. Hyphae on the outside of the rhizomorph are pulled along as these active cells elongate and larger inactive hyphae deeper within the rhizomorph surround a central gas-filled core.

Armillaria species that produce giant mycelia are examples of fungi that form rhizomorphs. These spread as branching networks of blackened "shoestrings" underneath tree bark. In the laboratory, rhizomorphs of *Armillaria gallica*, the "honey mushroom," were found to grow in length almost three times faster than individual hyphae (3.5 mm day^{-1} vs. 1.5 mm day^{-1}), which may explain how this "humongous fungus" can form giant clones covering hundreds of hectares of forest (see page 63).

Reproductive structures

The most diverse and the most complex fungal forms are all associated with reproduction (which is the subject of the next chapter). Diversity and complexity are manifested in both multicellular fruiting bodies and the individual cells that produce and release spores.

Multicellular fruiting bodies are formed by hyphae. Fruiting bodies are produced mainly by members of the Dikarya (see Chapter 1, page 40). They include the flask-shaped perithecia and cup-shaped apothecia of ascomycetes and the umbrella-shaped mushrooms of basidiomycetes. In each case, mycelial hyphae are redirected from their role in feeding to the formation of sex organs that function in the production and release of spores. Energy that has been stored from the feeding phase is committed to this reproductive process with the transfer of cytoplasm from the radiating filaments of the mycelium to the converging hyphae of the fruiting body.

← Delicate fruit bodies of the inkcap mushroom *Coprinopsis lagopus*.

Mushroom development begins with the knotting of a small group of hyphae into a little pellet or fruiting body initial. This grows as cytoplasm is transferred from the supporting mycelium and the cells in the initial grow into a primordium that will develop into a mature fruiting body. In some species, such as the model mushroom *Coprinopsis cinerea*, a miniature cap and stipe are differentiated from the surrounding tissue early in development and then rapidly expand as their hyphae swell with water. Other mushrooms do not form such "embryonic" structures and grow by the gradual aggregation of many apically growing (rather than inflating) hyphae. The diversity and evolution of fungal fruiting bodies is discussed in Chapter 4 (see page 112).

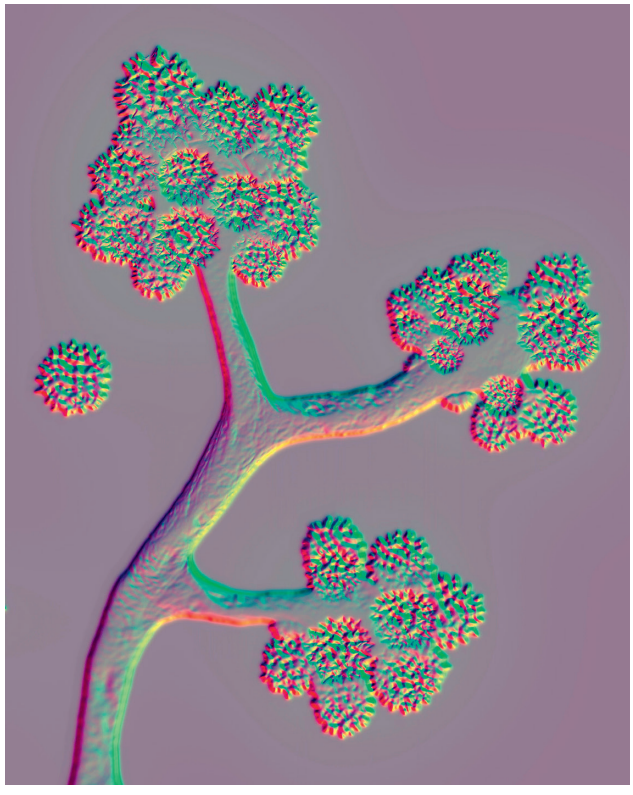
The mycelium is usually out of sight, hidden within the substrate that it is exploring and digesting. We can detect the presence of a mycelium by the presence of fruiting bodies. A circular arrangement of fruiting bodies suggests the extent of an individual mycelium and is called a fairy ring.

Spore-producing cells

Hyphae become differentiated into the asci and basidia that produce spores inside or on the surface of the fruiting bodies of ascomycete and basidiomycete fungi. The biomechanics of spore discharge has shaped the evolution of fruiting bodies and is discussed further in Chapter 4 (see page 106). Hyphae of ascomycete and

basidiomycete fungi also produce asexual spores or conidia when they grow as separated stalks or conidiophores as well as more complex multicellular asexual fruiting bodies called conidiomata. Species of Mucoromycota produce different structures, with asexual spores cleaved from the cytoplasm of sporangia at the tips of sporangiophores and sexual spores maturing within zygosporangia.

Fruiting bodies and spore-producing cells exhibit tremendous diversity at both macro- and microscopic scales, but they all result from developmental modifications among tip-growing hyphae that look much the same. Hyphae are the stem cells of the fungi.



↑ Circular "fairy ring" of mushrooms growing in the homogeneous environment of a lawn.

→ Sporangiophore of *Cunninghamella* (Mucoromycota) tipped with spiny sporangioles that contain multiple spores.

(continued...)

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