

Interactions with Humans and Other Animals

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Interactions between fungi and animals are very different from those with plants, since both fungi and animals are heterotrophs. When you consider the broad diversity of Kingdom Fungi and their lifestyles, and the similarly broad diversity of the animal Kingdom, it comes as no surprise that interactions between the two are many and varied. Interactions can be direct or indirect, and can prove beneficial or detrimental to either or both of the interacting partners (Table 9.1). We begin by considering the interactions in which fungi use vertebrate tissues, concentrating on humans and invertebrates as a food source, causing detrimental effects to the animals. Fungi are themselves – as mycelium, fruit bodies and lichen thalli – fed on by animals. However, associations have also evolved between fungi and animals that, rather than having negative impacts, are based on mutual benefit. The benefits are commonly nutritional, but also often include other additional or even sole benefits, such as provision of a suitable environment, protection against antagonists, and carriage of fungal propagules.

FUNGI AND HUMANS: MEDICAL MYCOLOGY

A small number of fungi can directly affect vertebrates by colonising and growing on or within the skin, or inside the body. About 400 species of fungi are able to cause disease in humans. These diseases are called **mycoses**. A wider range of fungi cause humans problems by producing mycotoxins which, when ingested, give rise to **mycotoxicoses**. Also, some fungi are **allergens**. We deal first with allergens, then mycotoxicoses and finally mycoses.

Allergies

Fungal spores are extremely common in the air, with outdoor concentrations typically ranging between 200 and 10^6 spores m^{-3} (see also Chapter 3), the mean spore content outdoors being 100 to 1000 times greater than that of pollen. Outdoor spore concentrations

TABLE 9.1 Examples of the Wide Range of Interactions that have Evolved Between Fungi and Animals

Nature of symbiosis	Fungus	Animal	Effect on fungus	Effects on animal
Intracellular parasites	Microsporidia	Arthropods, fish and to a lesser extent other vertebrates	Nutritional	Death of cells and sometimes whole organisms
Pathogenic	Species of <i>Blastomyces</i> , <i>Histoplasma</i> , <i>Coccidioides</i> , <i>Paracoccidioides</i> (Ascomycota)	Humans ^a and other vertebrates	Nutritional	Death of cells, tissues and sometimes the whole animal
Pathogenic	<i>Pseudogymnoascus destructans</i> (Ascomycota)	Many bats, especially little brown myotis (<i>Myotis lucifugus</i>)	Nutritional	Epidermal tissues eroded and fat reserves lost, which are essential to hibernation, hence death
Pathogenic	<i>Batrachochytrium dendrobatidis</i> (Chytridiomycota)	Amphibians	Nutritional	Death; some species have become extinct
Pathogenic	Species of <i>Achlya</i> , <i>Saprolegnia</i> , <i>Pythium</i> (oomycetes)	Fish	Nutritional	A range of effects depending on pathogen: skin lesions, organ damage; blocked blood vessels and gills; mass mortality with some species
Pathogenic	<i>Entomophthora</i> (zygomycete) <i>Beauveria bassiana</i> , <i>Metarhizium anisopliae</i> (Ascomycota)	invertebrates	Nutritional	Death
Pathogenic	<i>Aspergillus</i> (Ascomycota)	Sea fan corals (<i>Gorgonia</i> spp.)	Nutritional	Depends on host immune status; lesions, galls and sometimes death
Biotrophic parasite and Mutualism	<i>Septobasidium</i> (Basidiomycota)	Scale insects	Nutritional and dispersal	Scales live within a mycelial mat on the surface of plants. Some scales are parasitized, others benefit from a buffered microclimatic environment and protection from predators
Predation	<i>Coprinus comatus</i> , <i>Hohenbuehelia</i> , <i>Hyphoderma</i> and <i>Pleurotus</i> mycelium	Nematoda and rotifers	Nutritional	Killing by toxins or trapping on adhesive on constricting ring structures; subsequent utilisation of whole body contents
Commensalism or mutualism: symbionts within invertebrate gut	<i>Asellariales</i> , <i>Harpellales</i> (Kickxellomycotina)	Freshwater, marine and terrestrial Crustacea, Insecta, Myriapoda	Obligate gut symbionts: nutritional/environmental	Slight loss of gut nutrients but possibly aid to digestion

TABLE 9.1 Examples of the Wide Range of Interactions that have Evolved Between Fungi and Animals—cont'd

Nature of symbiosis	Fungus	Animal	Effect on fungus	Effects on animal
Mutualism: symbionts within invertebrate gut	Yeasts in Saccharomycetes (Ascomycota) and Tremellales (Basidiomycota)	Coleoptera and Insecta	Nutritional and habitat	Nutrition: enzymes for digestion; provision of essential nutrients; detoxification of plant metabolites
Mutualism: ants and higher termites cultivate the fungus	<i>Attamyces</i> , <i>Leucoagaricus</i> and <i>Lepiota</i> spp. (<i>Basidiomycota</i>)	Attine ants Macrotermitinae	Provision of plant resources; maintenance of favourable abiotic and biotic environment; carriage of spores to new nests	Nutrition; ingested enzymes
Mutualism: females carry asexual spores to trees; larvae develop in colonised wood	<i>Amylostereum</i> spp. (<i>Basidiomycota</i>) <i>Ophiostoma</i> spp. (Ascomycota) <i>Ophiostoma</i> , <i>Ceratocystis</i> spp. (Ascomycota), <i>Entomocorticium</i> spp. (<i>Basidiomycota</i>)	Siricid woodwasps Ambrosia beetles Bark beetles	Carriage and inoculation into a suitable environment	Softening of wood; improved nutrition; ingested enzymes
Mutualism	Phallaceae (<i>Basidiomycota</i>)	Diptera	Spore dispersal	Nutrition: feeding on spore masses
Commensalism/ mutualism/ mycophagy: larvae burrow in wood colonised by the fungi	Wood-rotting species, e.g. <i>Laetiporus sulphureus</i> , <i>Trametes versicolor</i> and <i>Coniophora puteana</i> (<i>Basidiomycota</i>)	Death watch beetle <i>Xestobium rufovillosum</i>	May benefit from nutrient input in faeces; harm may accrue from comminution	Softening of wood; improved nutrition
Mycophagy within fruit bodies	Agarics and polypores (<i>Basidiomycota</i>)	Gamasid mites, Insecta and Coleoptera	Decreased reproductive output; spore destruction; spore dispersal	Nutrition and breeding ground
Mycophagy of mycelium	Mycelia of many soil fungi	Collembola, woodlice, nematodes, some millipedes	Morphological and enzyme production changes; increases and decreases in hyphal coverage and biomass; alteration of outcome of inter-specific mycelia interactions	Nutrition

^aSee Tables 9.4 and 9.5 for more examples of human pathogens.

vary with climate, especially temperature, moisture, and wind, and hence vary daily. Daily changes in fungal spore, as well as pollen, counts are monitored in many cities, which is useful for the huge population of asthmatics and people who suffer from allergic rhinitis (hay fever), because allergy symptoms tend to increase with spore concentrations (see below). In the United States, a simple scale for fungal spore concentrations has been developed by the National Allergy Bureau: below 6500 spores m⁻³ is categorised as low; 6500–12,999 spores m⁻³ qualifies as moderate; 13,000–49,999 spores m⁻³ is high, and greater than 50,000 spores m⁻³ is very high. The Spores of ascomycetes in the genera *Alternaria*, *Cladosporium*, and *Epicoccum*, and basidiomycetes in the genus *Ganoderma* are examples of common allergy-causing species.

Indoor air contains spores that have entered from outdoors as well as from those fungi growing indoors, but the concentration is usually half that of the outdoor environment. The indoor concentration depends on humidity, temperature, ventilation, the presence of decomposing material, carpets, pets, and plants. Unlike other allergic sources (e.g. pollen), fungal spores, and hyphal fragments are common in the air throughout the year, though there are seasonal peaks. In a study of badly infected buildings in Denmark, the most commonly occurring species were ascomycetes, especially in the genera *Penicillium* and *Aspergillus*, and to a lesser extent *Chaetomium*, *Cladosporium*, *Ulocladium*, and *Stachybotrys*. There has been a lot of concern, particularly in the United States, about the purported toxicity of certain fungi that grow in flooded homes. The spores of some of these indoor fungi, including a black-pigmented ascomycete, *Stachybotrys chartarum*, carry toxins that can cause a range of illnesses if they are absorbed in high concentrations. However, it is not clear how often people who inhale spores of this fungus in water-damaged buildings are exposed to levels of these mycotoxins that can cause illness. Nevertheless, the inhalation of large quantities of allergenic spores in these circumstances remains a serious public health concern. Fungal growth inside a building is indicated if the concentration of spores in indoor air exceeds the measurement for outdoor air on the same day, and/or if different fungi are identified in indoor and outdoor air.

The human body defends itself with its immune system that recognises and responds to different antigens, destroying, for example, potential pathogens. However, occasionally there is an overactive immune response, known as hypersensitivity, which causes more damage than the potential pathogen. There are different types of hypersensitivity (Table 9.2). In Type 1 (immediate hypersensitivity), for example, fungal antigens (proteins on the surface of fungi) are recognised by immunoglobulin E (IgE), and then termed allergens. Binding of the IgE with the allergen triggers allergic responses including asthma, eczema, hay fever, rhinitis (inflammation of nasal mucous membranes), and urticaria (nettle rash). Susceptible individuals can become sensitised by continual low-dose exposure to allergens. A wide range of fungi cause various allergic diseases (Table 9.2), with over 80 genera inducing Type 1 allergies in humans and over 20 genera producing allergic proteins. Allergies are a serious global health problem, with an estimated 10% of the human population showing allergic sensitivity to fungal spores, 300 million people suffering from asthma, and 250,000 deaths each year attributed to the illness. Fungal spores tend to be smaller than other allergen sources and can reach the alveoli. Unlike other allergen sources, some fungi may also colonise tissues (pp. 303–309).

TABLE 9.2 Types of Fungal Allergic Reactions Based on Information in Simon-Nobbe et al. (2008)

Type	Clinical manifestation	Allergic mechanism ^a	Examples of most prominent genera of inducers ^b
Allergic rhinitis	Nasal obstructions, pleuritis, rhinorrhea, and sneezing	Type 1 allergy	Ascomycota: <i>Alternaria</i> , <i>Aspergillus</i> , <i>Bipolaris</i> , <i>Cladosporium</i> , <i>Curvularia</i> and <i>Penicillium</i>
Asthma	In children: increased bronchial activity. In adults: severe asthma and even death	Type 1 allergy	Ascomycota: <i>Alternaria</i> , <i>Aspergillus</i> , <i>Cladosporium</i> , <i>Epicoccum</i> , <i>Helminthosporium</i> and <i>Penicillium</i>
Atopic dermatitis	Chronic skin inflammation	Type 1 allergy, associated with high levels of allergen-specific and total IgE	Ascomycete yeasts: <i>Malassezia furfur</i>
Allergic bronchopulmonary mycoses (ABPM)	Growth in bronchial lumen, which leads to persistent inflation. Bronchiectasis is induced in asthma sufferers	Type 1, 1II, 1IV	Ascomycota: <i>Aspergillus fumigatus</i> , <i>Candida albicans</i> , <i>Curvularia</i> , <i>Gotrichum</i> , and <i>Helminthosporium</i>
Allergic sinusitis	Multiple sinuses are affected; hyphae are detectable in mucus, but no tissue invasion	Type 1, 1II, 1IV, specific IgE and IgG antibodies and raised levels of total IgE	Ascomycota: <i>Alternaria</i> , <i>Aspergillus</i> , <i>Bipolaris</i> , and <i>Curvularia</i> .
Hypersensitivity pneumonitis (extrinsic allergic alveolitis)	Repeated allergen inhalation may lead to irreversible lung damage. Precipitating antibodies and antigen-induced lymphocyte stimulation occurs	Type 1II, 1IV	Ascomycota: <i>Aspergillus</i> and <i>Penicillium</i> Basidiomycota: <i>Lentinula edodes</i> , <i>Pleurotus ostreatus</i> , and <i>Serpula lacrymans</i>

^aThere are different types of allergic/hypersensitive reaction by humans. Type 1 is an immediate reaction causing an inflammatory response, as a result of immunoglobulin E (IgE) causing excessive activation of some white blood cells (mast cells and basophils). In Type 2, antibodies bind to antigens on the body's own cell surfaces. In Type 3 (immune complex) there is binding of an antibody (e.g. immunoglobulin G, IgG) to a soluble antigen. Type 4 is cell-mediated and does not involve antibodies.

^bThough Ascomycota and Basidiomycota are the most allergic fungi, other groups do have allergic members, for example, zygomycete genera, *Absidia*, *Mucor*, and *Rhizopus*.

Mycotoxicoses

Fungi produce an enormously wide range of metabolites, as described in detail in Chapter 5. Unsurprisingly, some of them are toxic. Not all toxic compounds produced by fungi are termed mycotoxins; those that are toxic mainly to bacteria are commonly called antibiotics, those toxic to plants are termed phytotoxins, and those that are found in mushroom fruit bodies are often referred to as mushroom toxins or poisons. The term mycotoxin is reserved for low molecular weight fungal secondary metabolites that are toxic to humans and other vertebrates in low concentrations. Not only is it challenging to define mycotoxins, it is also hard to classify them, and depends on the purpose of the classification. Clinicians tend

to categorise mycotoxins by effect (e.g. neurotoxins and immunotoxins) physicians by the illness they cause (e.g. St. Anthony's fire) organic chemists by chemical structure, biochemists by their biosynthetic origins, and mycologists by the fungi that produce them. Three to four hundred mycotoxins and mushroom toxins have now been identified, and they occur in families of chemically related metabolites. Only about 20, however, are the usual causes of vertebrate health problems (Table 9.3).

Humans and other animals are most commonly exposed to mycotoxins by unwittingly consuming them in contaminated food, and to mushroom toxins by mistakenly eating poisonous mushroom species. Food can be contaminated while growing, postharvest during storage, or indirectly via the food chain (e.g. in milk from cows that ate contaminated food). Mycotoxins contaminate up to 25% of the world's food supply. Exposure is most common in places where methods of food handling and storage are poor, malnutrition is a problem, and few regulations exist to protect populations from exposure. In many countries there is

TABLE 9.3 Some Mycotoxins and Mushroom Toxins

Toxin	Example producing species	Pathological effects
Mycotoxins		
Aflatoxins	<i>Aspergillus flavus</i>	Liver damage, liver cancer
Citrinin	<i>Penicillium citrinum</i>	Kidney damage
Gliotoxin	<i>Aspergillus fumigatus</i>	Immunosuppressant
Ochratoxins	<i>Aspergillus ochraceus</i>	Kidney damage
Patulin	<i>Penicillium expansum</i>	Kidney damage
Trichothecene: T-2	<i>Fusarium sporotrichioides</i>	Alimentary toxic aleukia
Trichothecene: Vomitoxin	<i>Fusarium graminearum</i>	Vomiting, anti-feedant
Zearalenone	<i>Fusarium graminearum</i>	Gynaecological disturbances
Fumonisins	<i>Fusarium moniliforme</i>	Oesophageal cancer
Ergot alkaloids	<i>Claviceps purpurea</i>	Vasoconstriction, gangrene, convulsions
Mushroom toxins		
Amanitin	<i>Amanita phalloides</i>	Liver damage
Phalloidin	<i>Amanita phalloides</i>	Liver damage
Muscarine	<i>Amanita muscaria</i>	Sweating, vomiting
Gyromitrin	<i>Gyromitra esculenta</i>	Liver and kidney damage
Orellanine	<i>Cortinarius speciosissimus</i>	Kidney damage
Coprine	<i>Coprinus atramentarius</i>	Alcohol poisoning
Psilocybin	<i>Psilocybe cubensis</i>	Psychotropic effects
Ibotenic acid	<i>Amanita muscaria</i>	Psychotropic effects

legislation governing permitted levels of mycotoxins in food, and food samples are tested to enforce these. In the UK, a maximum of $4\text{ }\mu\text{g kg}^{-1}$ of aflatoxin (produced by *Aspergillus* species) is permitted in human food and $50\text{ }\mu\text{g kg}^{-1}$ of patulin (produced by *Penicillium* species) in apple juice. Controlling mycotoxin production in food largely revolves around prevention by good agricultural practice and storage under conditions not conducive for fungal growth (e.g. low humidity for grain storage). In the future, plant breeding programmes and genetic engineering may produce crop plants with enhanced antifungal genes, and biocontrol strategies may be developed.

The most notorious mycotoxins are the aflatoxins from *Aspergillus flavus* (hence 'A-flatoxin'), *Aspergillus parasiticus*, and *Aspergillus nomius*. These were first discovered in 1960 after the death, from liver disease, of over 100,000 turkeys in Norfolk, UK, followed by deaths of other farm animals. The cause turned out to be their food – ground peanut meal contaminated with *Aspergillus flavus*. There are four main aflatoxins B₁, B₂, G₁, and G₂, the first being the most important. They are not only acutely toxic but also cause chronic illness, being the most active natural carcinogenic substances known. Several *Aspergillus* species, notably *A. ochraceus*, also produce ochratoxin A (e.g. on cereals, especially barley, cocoa and coffee beans). Kidney damage is the main problem, but ochratoxin A is also a liver toxin, an immune suppressant and a carcinogen. Patulin is produced by a range of *Aspergillus* and *Penicillium* species, but the main problem is from *Penicillium expansum* causing soft rot of apples and other fruit, and is often found in unfermented apple juice. The trichothecenes are a family of over 60 sesquiterpenes, produced by a range of genera including *Fusarium*, *Phomopsis*, *Stachybotrys*, and *Trichoderma*. Trichothecene T-2, which has been the most studied, is produced by *Fusarium sporotrichoides* and *Fusarium poae*, growing on millet left in the fields under snow. It caused a terrible epidemic of alimentary toxic aleukia (involving degeneration of bone marrow, haemorrhaging, necrosis of the alimentary tract and blood abnormalities) in the former Soviet Union, in the 1940s. The zearalenone family of mycotoxins, produced by *Fusarium* species, particularly *Fusarium graminearum* and *Fusarium culmorum*, mimic oestrogen, and in some formulations can be called drugs. The ergot alkaloids, with lysergic acid as a structure common to all, are a toxic cocktail produced in the sclerotia of *Claviceps* (Ascomycota) species – pathogens of various grasses. Human ergotism (St. Antony's fire) caused by eating bread made with flour from infected cereals, especially rye, was common in the Middle Ages, with major epidemics in Russia as late as 1927 and the last reported outbreak in France in 1951. The disease takes two forms – convulsive ergotism affects the central nervous system, while the second form results in gangrene in the extremities. It is still an important animal disease.

There are relatively few toxic mushroom fruit bodies (Table 9.3). The most toxic – *Amanita phalloides* (the death cap) – produces two closely related families of bicyclic peptide toxins, the most abundant of which are α -amanitin and phalloidin. They have completely different actions: α -amanitin specifically inhibits RNA polymerase II, preventing mRNA synthesis, while phalloidin irreversibly binds to filamentous actin, disrupting cell structure. Both toxins cause liver damage, and eventually death. A single fruit body contains only a few thousandths of a gram, but this is sufficient to kill an adult human. This potency is being harnessed, and has been successful in arresting pancreatic cancer in mice, by linking the α -amanitin toxin to an antibody that attaches to cell surface EpCAM protein found on cancer cells. Other toxic examples include *Amanita muscaria* (fly agaric) and species of *Clitocybe* and *Inocybe*, which produce muscarine, an

acetylcholine analogue which binds to nerve synapses causing continuous stimulation. The fly agaric also produces the amino acid ibotenic acid, whose decarboxylated derivative muscimol causes hallucinations and dizziness. *Tricholoma equestre* and *Russula subnigricans* cause destruction of muscle tissue, coma and heart failure, the toxin from the latter being cycloprop-2-ene carboxylic acid. *Gyromitra esculenta* (false morel) is also fatal when uncooked, as a result of the toxin gyromitrin, which gives rise to toxic hydrazines. Some species of *Cortinarius* produce orellanine, which causes kidney damage. *Psilocybe* species produce psilocybin, which is hallucinogenic for several hours (p. 162), and some people also experience nausea and panic attacks.

Mycoses

Fungal diseases can be categorised according to increasing severity as superficial, subcutaneous, or systemic. The latter can be further divided depending on whether the causative organism is a true pathogen able to invade tissues of an otherwise healthy host, or whether it is an opportunist able to invade tissues of a debilitated or immunocompromised host (Table 9.4). There are around half a dozen extremely unpleasant fungal diseases, including aspergillosis, candidiasis, histoplasmosis, coccidiomycosis, and blastomycosis. Ironically, with advances in some medical treatments there has been a rise in life-threatening fungal diseases, especially in the areas of transplant and chemotherapy, where the immune system is suppressed.

There are three main attributes that fungi need to cause disease in humans: (1) the ability to grow well at 37°C; (2) the ability to utilise many different carbon and nitrogen sources, and to scavenge limiting elements (e.g. iron); and (3) the ability to recognise and adapt to the conditions within the human host, which are very different from those outside. After first briefly considering superficial and cutaneous infections, we will describe some of the main fungi that are able to invade living humans (i.e. those that exhibit these three main attributes). There will also be other fungi that share these attributes and could grow in the expanding population of immunocompromised patients, some of which are already starting to emerge as pathogens (e.g. filamentous species of *Acremonium*, *Alternaria*, *Bipolaris*, *Fusarium*, *Penicillium marneffei* and *Pseudoallesheria*, *Scedosporium prolificans*, and the yeast-like *Candida krusei*, *Rhodotorula rubrum*, and *Trichosporon* species).

Superficial Infections

There are fungi on the skin all of the time, most of which are commensal, causing no harm, though others are capable of causing disease. Many of the commensals are yeast forms, while the pathogens can be in yeast or mycelial form. Species in the genus *Malassezia* live permanently on the skin, at population densities varying between individual people and between sites, often with less than 4 cm^{-2} on hands and feet, and up to 10^4 cm^{-2} on chest and backs, with maximum densities occurring between late teens and early middle age. Most have an absolute growth requirement for lipids and, hence, are prevalent in areas rich in sebaceous glands (e.g. chest, back, face, and scalp). *Malassezia furfur* is the cause of dandruff. *Malassezia* species are also able to cause skin complaints, including pityriasis versicolor and seborrhoeic dermatitis. Pityriasis versicolor is scaly pigmented lesions, typically on the upper trunk, containing both yeast and mycelial forms, and often the most common fungal infection in hot climates, as warmth and humidity favour development. Seborrhoeic dermatitis is also seen as scaly lesions in around 3% of the immune competent population but about 80% of HIV-positive patients.

TABLE 9.4 Categories of Fungal Infections of Humans Based on Severity of Effects

Infection/mycosis	Definition and general description	Examples of disease	Causal fungus	Phylum
Superficial	Superficial infection of skin or hair shaft; no invasion of living tissue	Seborrhoeic dermatitis, dandruff, folliculitis pityriasis	<i>Malassezia furfur</i> (lipophilic yeast)	Basidiomycota
Cutaneous	Superficial infections of the hair, skin, or nails. No living tissue is invaded, but a variety of allergic or inflammatory response occurs in the host due to the fungus and its metabolic products	Candidiasis of skin, mucous membranes and nails/Thrush Tinea/ringworm	<i>Candida albicans</i> <i>Epidermophyton, Microsporum,</i> <i>Trichophyton</i>	Ascomycota Ascomycota
Subcutaneous	Chronic, localised infections of skin and subcutaneous tissue following accidental implantation of the fungus, mostly saprotrophs from soil or plant material	Chromoblastomycosis Entomophthoromycosis Mycotic mycetoma Sporotrichosis	<i>Phialophora, Cladosporium</i> <i>Basidiobolus ranarum</i> <i>Exophiala</i> and others <i>Sporothrix schenckii</i>	Ascomycota zygomycete Ascomycota Ascomycota
Systemic – Dimorphic/True pathogen	Able to invade and develop in tissues of an otherwise healthy host with no recognisable predisposing factor (i.e. can overcome the physiological and cellular defences of the human host). Primary site of infection is usually pulmonary. The morphology outside of the host differs from that inside the host	Blastomycosis Coccidioidomycosis Histoplasmosis Paracoccidioidomycosis	<i>Blastomyces dermatitidis</i> <i>Coccidioides immitis</i> <i>Histoplasma capsulatum</i> <i>Paracoccidioides brasiliensis</i>	Ascomycota Ascomycota Ascomycota Ascomycota
Systemic – Opportunistic	Infections occur almost exclusively in immunocompromised patients, (e.g. AIDS, advanced cancer, post-organ-transplant, following steroid/antibiotic/ chemo-therapy). Incidence is rising	Aspergillosis Candidiasis (candidosis) Cryptococcosis Hyalohyphomycosis Phaeohyphomycosis Pneumocytosis Penicilliosis Zygomycosis	<i>Aspergillus fumigatus</i> <i>Candida albicans</i> <i>Cryptococcus neoformans</i> Non-pigmented, conidial fungi (e.g. <i>Fusarium</i> spp.) darkly pigmented conidial fungi (e.g. <i>Cladosporium, Curvularia</i>) <i>Pneumocystis jirovecii</i> <i>Penicillium marneffei</i> <i>Rhizopus, Mucor, Absidia</i>	Ascomycota Ascomycota Basidiomycota Ascomycota Ascomycota Ascomycota

Fungal pathogens have emerged independently in different phyla. They have also emerged independently many times within phyla, for example in Ascomycota ranging amongst diverse species such as *Pneumocystis jirovecii* (archiascomycete), *Candida* spp. (hemiascomycetes), and *Aspergillus* spp. (euascomycetes). They emerged in three different ways: (1) from commensals to pathogens (e.g. *Candida* spp. on skin and mucosal surfaces) which can be transmitted to other humans; (2) fungi that are not part of the normal human mycobiota, but can be transmitted from person to person (e.g. lung-colonising *Pneumocystis* spp.) transmitted as aerosols and inhalation, and *Malassezia* spp. and dermatophytes; and (3) opportunistic fungi with no human to human transmission, infection being from the natural environment, though how they can be so well adapted to a human host, yet lacking direct transmission is unclear.

Cutaneous Infections

About 20 species in three genera – *Epidermophyton*, *Microsporum*, *Trichophyton* – all with the ability to utilise keratin, grow in the non-living tissues of hair, nails, and skin, in the region above the layers where keratin is deposited. They cause a complex of diseases known clinically as tinea (ringworm) in humans and other vertebrates, and are spread in a keratin-tissue fragment containing viable fungus. Some are **anthropophilic**, largely growing on humans but occasionally other animals (e.g. *Trichophyton rubrum* and *Trichophyton tonsurans*). Others are **zoophilic**, primarily found on other mammals, but can be transmitted to humans via direct contact (e.g. *Microsporum canis*, in cats and dogs). A third group are **geophilic**, decomposing keratin rich tissues in soil, but can also form infections in humans (e.g. *Microsporum gypseum*). The lesions caused by ringworm vary considerably in appearance, but often there is inflammation, swelling, and vesicles. Spreading ring-like lesions, from which the disease gets its name, are found on face, scalp, limbs, and body (Figure 9.1a). On the scalp, skin becomes scaly and hair is lost. When nails are infected they become discoloured, raised, thickened, and crumbly. In temperate regions, 75% of all tinea diseases are foot ringworm (athlete's foot). In the UK, 10–15% of the population have foot ringworm and 5% nail diseases. It is more prevalent in men than women, is higher in sufferers of diabetes and the immunosuppressed, and increases with age to about 25% in the elderly.

There are around 200 *Candida* species ubiquitous in the natural environment, commonly associated with plants and animals, but only a dozen or so associated with human disease, the most common of which are *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*. They are carried innocuously by many people on the skin, in the mouth, vagina and gastro-intestinal tract. *Candida albicans* and other *Candida* species can cause cutaneous infections at many sites on the body, especially those that are moist, such as folds of flesh and armpits (Figure 9.1b). Infection of the mouth and vagina is commonly called thrush, because of the white yeast plaques that it forms on the surface of mucous membranes. Oral infections are most common in babies and the elderly; about 75% of women will have a vaginal infection at some time. Mostly, *Candida* is in balance with other skin microbes, but the balance can be shifted by antibiotic therapy and immune suppression, occurring in almost all AIDS sufferers. These cutaneous infections are relatively simple to treat. However, *Candida* can cause a different and serious disease if the cells enter and spread within the body (see Systemic opportunistic infections, below); such diseases are not a consequence of cutaneous infection.

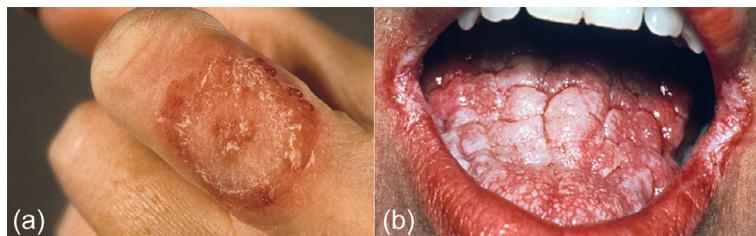


FIGURE 9.1 Human pathogens: cutaneous infections. (a) Ringworm is caused by *Tinea* species infecting the skin, seen here on a finger. (b) Candidiasis of the tongue and mouth corners of an immune deficient human adult.
Source: (a) © Roy Watling; (b) courtesy of www.doctorfungus.org © 2007

Subcutaneous Infections

Subcutaneous infections mainly occur in the tropics and subtropics, as a result of a saprotrophic fungus being implanted via a wound, and the majority of infections occur in people who walk barefoot, farmers, gardeners, florists, and miners. There have also been increases in recent history as a result of blast injuries from war-settings introducing soils, and fungal infection, into human tissue. Chromoblastomycosis, mycetoma, and sporotrichosis are the most common diseases, and are caused by ascomycetes, but some Entomophthoromycotina (entomopathogenic fungi; pp. 314–320) (e.g. *Basidiobolus ranarum* and *Conidiobolus coronatus*) also form subcutaneous infections (Table 9.5). An outbreak of 3000 cases of sporotrichosis occurred in a South African gold mine in the 1940s, contracted from infected pit props.

Systemic-Dimorphic/True Pathogens

Systemic mycoses are usually acquired by inhalation, starting in the lungs and subsequently affecting the whole body. The four main examples are blastomycosis, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis. The fungi involved are all dimorphic, switching between yeast and mycelial phases. In each case the yeast is the pathogenic state and the filamentous form is saprotrophic. The dimorphism is regulated by temperature, with mycelial growth in nature at 25–30 °C, and yeast growth in tissue or enriched media at 37 °C (body temperature). Some of these diseases are confined to certain geographical regions.

Blastomycosis, caused by *Blastomyces dermatitidis*, is found in the United States in the Mississippi and Ohio River valleys, and in Canada in states that border the St. Lawrence Seaway and the Great Lakes (Figure 9.2). Outbreaks of the disease are associated with activities around water courses, since the fungus grows in moist soil with rotting plant material. It is, however, difficult to isolate, hence its ecology in nature is unclear. Even in areas where it is prevalent, only one or two people in 100,000 get the disease. Infection occurs when the spores become airborne, entering the lungs and multiplying there, causing an acute disease resembling pneumonia, chronic tuberculosis, or lung cancer, and can result in acute respiratory distress. The fungus can also spread to other organs via blood and lymph. The disease can be fatal, particularly in patients with compromised immune systems. **Histoplasmosis** occurs in the same region (Figure 9.2), and is caused by *Histoplasma capsulatum* var. *capsulatum*, which grows well in nitrogen-rich wild bird, chicken, and bat guano. The disease is similar to blastomycosis, and again in most cases no obvious symptoms are produced. In Central and West Africa, the disease takes a different form, causing deep mycoses in the skin and bones. It is caused by a different variety of the fungus – *Histoplasma capsulatum* var. *duboisii* – likely a different species.

Coccidioidomycosis, commonly known as ‘valley fever’, is a disease found in southwest United States, Central America, northern South America and Argentina (Figure 9.2). The ascomycetes responsible – *Coccidioides immitis* and *Coccidioides posadasii* – thrive in dry, salty soils typical of desert areas (though it is not a problem in the deserts of Africa or Asia). *Coccidioides immitis* is endemic to the southern deserts and central valley of California, and probably Baja California, while *Coccidioides posadasii* is endemic to southern Arizona, New Mexico, northern Mexico, western Texas, and some parts of South America. In the soil, these fungi are associated with heteromyid (a family of rodents) burrows, and grow as septate mycelium that produces arthroconidia (p. 67), which can be inhaled by humans when the spores rise in dust storms. In the lungs, the arthroconidia enlarge to form large multinucleate spherules (80 µm), which form many small (2–5 µm) uninucleate endospores, which can spread the infection

TABLE 9.5 Subcutaneous Fungal Infections

Disease	Example causative species	Type of fungus	Natural habitat	Disease symptoms	Prognosis and therapy
Chromo-blastomycosis	<i>Cladophialophora carrionii</i> , <i>Fonsecaea compacta</i> , <i>F. pedrosoi</i> , <i>Phialophora verrucosa</i>	Filamentous Ascomycota	Soil and woody plant matter	Localised crusted, verrucoid, ulcerated lesions form. Satellite lesions can spread through lymph. Sometimes there is dissemination to the brain	Infections are usually localised. Early stages are treated by topical anti-fungals or surgical removal. Advanced infections may require long systemic treatments with itraconazole or terbinafine
Entomophthoromycosis	<i>Basidiobolus ranarum</i> , <i>Conidiobolus coronatus</i>	Zygomycete (Entomophthorales)	Soil and plant litter	<i>B. ranarum</i> causes gradually enlarging granulomas in arms and trunk; <i>C. coronatus</i> typically colonises nose tissues	<i>B. ranarum</i> has been treated with amphotericin B, potassium iodide, and itraconazole; surgery is often necessary
Mycetoma	<i>Acremonium falciforme</i> , <i>A. redifei</i> , <i>Aspergillus nidulans</i> , <i>Exophiala jeanselmei</i> , <i>leptosphaeria senegalensis</i> , <i>Madurella mycetomatis</i> , <i>M. grisea</i>	Filamentous Ascomycota	Ubiquitous, mainly soil	Localised infections of cutaneous and subcutaneous tissues. Lesions are locally invasive tumour-like abscesses. Lesions rupture resulting in ulcers, swelling, and distortion of the infected part of the body	Mycetomas are resistant to chemotherapy, often leaving surgery as the only option
Sporotrichosis	<i>Sporothrix schenckii</i>	Ascomycota, dimorphic, filamentous and conidia-forming at 25 °C or less, switching to cigar-shaped yeast cells at 37 °C	Globally in soil	Usually localised lesions of cutaneous and subcutaneous tissues, typically following lymphatic pathways. Sometimes sporotrichosis can cause osteoarthritis, pulmonary infections and meningitis. Internal dissemination is by yeast cells. Peru has highest incidence of infections	Prolonged (3–6 months for local lesions; at least 12 months for osteoarticular disease) treatment is needed, but usually responds. Local hypothermia, and oral azoles and is effective

Information from a variety of sources, including <http://www.doctorfungus.org/> (accessed 30 Nov 2011).

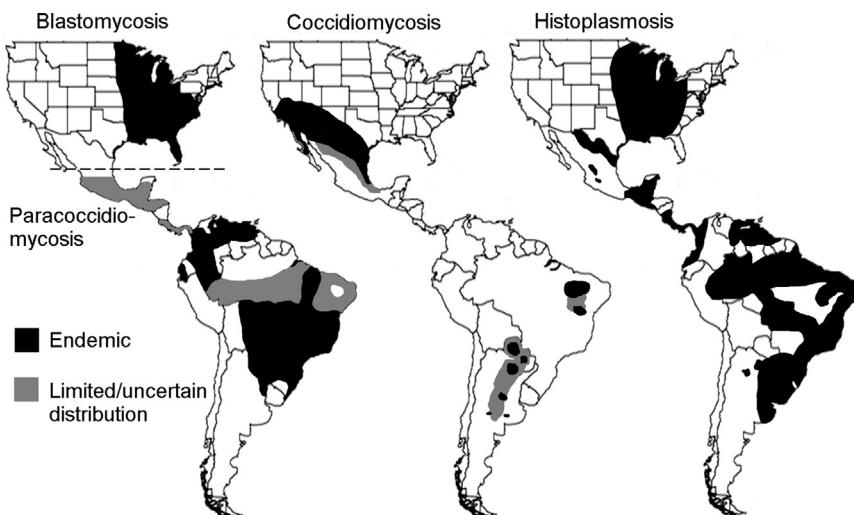


FIGURE 9.2 Approximate distribution of some systemic dimorphic pathogens endemic in North and South America. Source: Information from Hector and Laniado-Laborin (2005) and Colombo et al. (2011).

in the surrounding tissues. Millions of people have been infected, but about 60% of infections are asymptomatic; 30% experience a range of symptoms, including coughs, for several months but are ultimately self-limiting; less than 10% require medical intervention. In severe cases, the fungus can spread to adrenal glands, bones, central nervous system, joints, lymph nodes and skin, and the disease can be fatal. There has been a steady increase in reported cases from about 1500 in 1998 to over 20,000 in 2013.

Paracoccidiomycosis – caused by *Paracoccidioides brasiliensis* – is largely confined to South and Central America (Figure 9.2). Infection occurs by inhalation of spores, though their origin is uncertain, as the fungus has rarely been isolated from soil. There is a rare acute form that affects juveniles of both sexes, which sometimes leads to death from fungal growth in the liver, spleen or bone marrow. Skin testing shows that a high proportion of the population of both sexes has come into contact with the fungus by the age of 20 years. However, most cases of pulmonary infections in adults are males (often >50:1 males:females) between 30 and 50 years old. This remarkable difference in the frequency of infections between males and females results from inhibition of the dimorphic switch from mycelium/conidium to yeast by physiological concentrations of the female sex hormone oestradiol; growth of yeast cells when formed is unaffected. The cytosol of yeast and hyphae contains a high-affinity binding protein with similar properties to mammalian receptor proteins responding to steroid hormones, and so there may be a signal transduction pathway operating in the fungus similar to those of mammals. Also, there are major differences in yeast and mycelial cell walls: in hyphae, β -(1-3)-glucan is the major component, whereas it is α -(1-3)-glucan in yeast. The latter may play a role in evasion of host defences.

Systemic-Opportunistic Infections

Since the 1980s mortality from fungal diseases has been increasing, largely reflecting the increase in immunocompromised patients, but also due to increased travel to tropical regions

and an increased clinical awareness and improved diagnosis. The most common systemic opportunist fungal pathogens in immunocompromised patients are species of *Candida* and saprotrophic (i.e. act as saprotrophs in the natural environment outside of human bodies) *Aspergillus* and zygomycete species. Also, though relatively infrequent in western countries, the basidiomycete yeast *Cryptococcus neoformans* causes very high (up to 100%) mortality in AIDS sufferers in regions where HAART (highly active antiretroviral therapy) is not available (i.e. around 400,000 per annum worldwide). Over 40 million people in the world suffer from HIV/AIDS, but in developed countries AIDS has become a chronic illness rather than rapidly terminal, thanks to the introduction of HAART. Nonetheless, globally around 3 million people die from it each year. *Pneumocystis jirovecii* (= *carinii*) and *Penicillium marneffei* have emerged from obscurity to being major pathogens of AIDS patients. All of these fungi can be thought of as opportunists, growing inside humans if they happen to find themselves there, but are usually found living in the natural environment.

Aspergillosis covers a broad spectrum of diseases caused by species of *Aspergillus*. Of over 200 *Aspergillus* species, less than 20 cause disease in humans, other mammals and birds, the most common being *Aspergillus fumigatus*, and to a lesser extent *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus terreus*. These are all saprotrophs, which are extremely common in nature. They produce large quantities of small (2–4 µm), airborne conidiospores, to which we are all exposed, yet mostly do not succumb to disease. Until the mid-twentieth century, disease was usually associated with occupations in which people were exposed to abnormally high spore loads, such as agricultural workers frequently handling hay and agricultural produce stored in confined spaces, giving rise to disease commonly called farmer's lung. Reduction of spore load, due to changes in harvesting methods and improved storage protocols, reduced the incidence of the disease. It is, however, now a major concern because of its lethal invasion of immunocompromised patients, and is one of the most frequently acquired nosocomial (i.e. hospital-acquired) infections following immunosuppressive therapy, with around 700,000 deaths world-wide each year caused by *Aspergillus* infections.

There are three main types of disease caused by *Aspergillus* species: allergic bronchopulmonary aspergillosis, pulmonary aspergilloma, and invasive aspergillosis. Allergic bronchopulmonary aspergillosis is a hypersensitive reaction to spore and hyphal surface antigens, leading to asthmatic reactions, and is particularly common in asthma and cystic fibrosis patients. With pulmonary aspergilloma, *Aspergillus* forms balls of hyphae plus host cells, tissue debris, and other substances in cavities within the lungs. In this form of disease, the fungus does not usually invade surrounding lung tissue; in about 10% of cases it resolves itself without treatment, and only rarely does it enlarge. Invasive aspergillosis starts from the primary focus of infection, which is usually the lower respiratory tract, resulting from inhaled spores, though less commonly invasion can be via the sinuses or skin through catheter insertion sites etc. From the primary focus, *Aspergillus* invades blood vessels and is transported to other organs, particularly the brain. Unlike several other human pathogenic fungi, *Aspergillus* species do not have a yeast-mycelial dimorphism, but grow strictly in filamentous form. Though *Aspergillus fumigatus* is the major cause of invasive aspergillosis, cases resulting from *Aspergillus terreus* are increasing, and *Aspergillus lentulus* is emerging; *Aspergillus terreus* and *Aspergillus lentulus* both have low susceptibility to currently available antifungal drugs.

Candidiasis. While *Candida albicans* and a few other species of *Candida* usually cause, at worst, cutaneous infections (see above), sometimes they penetrate through the skin or

mucosal surfaces, eventually reaching the bloodstream. From there they can be disseminated to organs, including the brain, liver, and kidneys, eventually leading to death. How the yeast cells enter the bloodstream is not entirely clear, but routes include damage to the intestinal tract by chemotherapy and surgery, and direct entry via catheters and intravenous lines. The immune system can eradicate low numbers of yeast, though not if the immune system is compromised. It is the fourth most common nosocomially acquired bloodstream infection. It is difficult to attribute mortality rates to *Candida* infection, because almost all sufferers of invasive candidiasis have an underlying illness, but it probably ranges between 15% and 50%, much higher than other systemic infections (e.g. MRSA, methicillin-resistant *Staphylococcus aureus*). The annual financial cost in the United States alone in 2002 was estimated at US \$1.7 billion. *Candida albicans* is the most common cause of candidiasis, followed by *Candida glabrata* and *Candida parapsilosis*. *Candida glabrata* rapidly develops resistance to antifungal drugs, which may select for this species. *Candida parapsilosis* commonly grows from biofilm on plastic surfaces, and probably enters via catheters and intravenous lines. In Asia, *Candida tropicalis* is a particularly common cause of candidiasis.

Several traits of *Candida albicans* are putative virulence factors. (1) The ability to adhere to host tissue, so as not to be dislodged by the bloodstream or host secretions (e.g. sweat and saliva). *Candida albicans* produces **adhesins** that bind to a range of host proteins, including fibronectin, and carbohydrate moieties of membrane glycoproteins and glycolipids. Als (agglutin-like sequence) proteins and Hwp (hyphal wall proteins) are also adhesins. (2) *Candida albicans* produces a wide range of extracellular enzymes that break down host proteins, including antibodies, lipases, and phospholipases. (3) The ability to switch between phenotypes is significant not only in *Candida* spp. but also in other invasive pathogenic fungi. Though it is frequently referred to as the yeast/mycelial dimorphism, *Candida albicans* is polymorphic also growing as pseudohyphae (Figure 9.1). Yeast forms are typical outside the body. Both yeast and hyphae are found in tissues, and the different forms may be important at different stages or in different types of infections; some mutants unable to switch forms have a reduced ability to cause disease. The switch from yeast to filamentous morphology is critical for invading host tissue. It can be induced *in vitro* with blood serum and nutrients, but the very complex interacting signal networks, found by genetic analysis, indicate that morphogenesis in *Candida* can respond to many different chemical and physical cues (p. xxx). This reflects a mode of development highly adapted to the multiple and changeable niches presented by host tissues.

***Cryptococcus* infection** in humans is acquired by inhalation of spores or yeast cells from the natural environment, especially soil containing pigeon guano (*Cryptococcus neoformans* var. *neoformans* and *Cryptococcus neoformans* var. *grubii*), and where eucalyptus trees and decaying wood are present (*Cryptococcus neoformans* var. *grubii* and *Cryptococcus gattii*). *Cryptococcus neoformans* var. *neoformans* and *Cryptococcus neoformans* var. *grubii* have a worldwide distribution in the natural environment, and cause the vast majority of cryptococcal infections in humans with underlying immunosuppression. On the other hand, *Cryptococcus gattii* causes the majority of infections reported in immunocompetent hosts. *Cryptococcus* species are not obligate human pathogens, and also occur in other organisms, including domesticated and wild animals, insects, and amoebae, but there is no evidence of direct transmission between animals and humans, nor between humans. *Cryptococcus* is the most important life-threatening fungal infection of AIDS patients. It can colonise the host respiratory tract without causing disease,

and can be cleared or enter a latent phase, which may subsequently be reactivated and disseminated in the blood to cause systemic infection – cryptococcosis. It most commonly infects the brain and central nervous system, causing meningoencephalitis, but can cause localised infection in any organ. *Cryptococcus neoformans* is usually isolated, from both patients and the environment, as budding yeast. In its filamentous form it can undergo monokaryotic fruiting or mating, the latter involving fusion of two haploid cells with different mating type alleles, a and α (Chapter 4). Over 98% of both clinical and environmental isolates are the α -mating type, which is more virulent than the a-mating type. *Cryptococcus neoformans* can also form thick-walled chlamydospores (p. 67.) that could act as long-term survival structures in the natural environment. The production of polyphenoloxidase, which converts phenolic substrates into melanin, is a virulence factor. Melanin and the thick, acidic, mucopolysaccharide capsule of the yeast inhibit phagocytosis.

Penicillium marneffei is endemic to Southeast Asia and has emerged as a significant mycosis in humans since the 1980s with the rise of AIDS. In the north of Thailand, about 25% of AIDS patients are infected with it. It causes systemic infections resulting in considerable mortality. Bamboo rats (*Rhyzomys*) are a possible source of infection, as surveys have shown a high prevalence in these animals. However, the fungus is present, and may grow, in soil in the endemic region, and soil is currently assumed to be its main environmental reservoir. Of over 200 species in the genus *Penicillium*, *Penicillium marneffei* is the only species that is highly pathogenic, and the only species that has a temperature-dependent dimorphism, growing intracellularly as a fission yeast.

Pneumocytosis caused by *Pneumocystis jirovecii*, is one of the major opportunistic pathogens of immunocompromised patients. Though previously mistaken as a protozoan, *Pneumocystis jirovecii* is an ascomycete (Taphrinomycotina), but a rather unusual one. It has cholesterol, not ergosterol, in its cell membranes, which negates the use of amphotericin B and azole antifungal drugs. Its yeast-like cells cluster together in host tissue; its vegetative form is probably haploid, replicating asexually. Following fusion, a diploid zygote is formed, and meiosis occurs in a cell termed the precyst (terminology from the time when it was thought to be a protozoan), giving rise to the early cyst. Eight spores develop within the cyst, which should be called an ascus, and the spores ascospores. The mature ascus ruptures, and the ascospores are released to germinate into feeding forms. This lifecycle information comes from growth in animals. It is currently not possible to grow it in artificial culture, so little is known of its ecology and physiology, nor what infective agent is released into the environment. Though its DNA has been detected in air and water samples, it is probably not viable and close host proximity (such as vertical transmission between mother and child) is likely necessary for transmission to occur. Strains from humans, rodents, rabbits and other animals are genetically distinct, with high host specificity, as shown by cross-infection experiments. It appears to be passed from human to human by breathing in spores, which germinate and invade the alveoli, resulting in extensive damage to the alveolar epithelium. It only causes problems in premature babies, malnourished infants, and immunocompromised patients, where it causes pneumonia and, in a few cases (<3%), lesions in the lymph nodes, liver, spleen or bone marrow.

Zygomycosis, though hitherto rare, zygomycoses are increasing, especially in immunocompromised patients, and are often lethal due to resistance to many common antifungal drugs. With *Mucor circinelloides*, sporangiospore size dimorphism is linked to virulence.

The larger sporangiospores are virulent, germinating inside the host (demonstrated in wax moth (*Achroia*)) and lysing macrophages, whereas the smaller spores are not.

Dimorphisms are common in human pathogenic fungi. *Cryptococcus neoformans* infections occur in the yeast mode, the hyphal mode being present in nature and the phase when sexual reproduction takes place. *Cryptococcus neoformans* forms giant cells (up to 50 µm) in the lungs of infected hosts, formation being enhanced in the presence of opposite mating types, as a result of signalling by a mating pheromone, somewhat analogous to quorum sensing. Cell signalling circuits may govern both virulence and mating. With *Candida albicans*, the yeast phase is critical for spread within the host, whereas the hyphal form allows the fungus to escape from macrophages following phagocytosis, and also to form biofilms on catheters, etc. As mentioned earlier, *Candida* spp. exist as yeasts, pseudohyphae and filamentous forms (Figure 5.15). Switching between morphological forms is obviously also crucial in the pathogens categorised as systemic/dimorphic true pathogens (e.g. *Paracoccidioides brasiliensis*). On the other hand, other pathogens (e.g. *Aspergillus fumigatus*) are strictly filamentous. Clearly, there is no simple rule that one morphological form is pathogenic and others are not.

Antifungal Agents for Treatment of Mycoses

The main treatment of fungal infection is chemotherapy, with drugs whose main actions are (1) inhibition of plasma membrane synthesis or disruption of plasma membrane integrity; (2) disruption or inhibition of cell wall biosynthesis; and (3) inhibition of metabolism and disruption of mitosis (Table 9.6). These antifungal agents also differ in structure, solubility, spectrum of activity, extent of fungistatic/fungicidal activity, and ability to induce resistance. Superficial and cutaneous fungal diseases of skin usually respond well to topical antifungal creams, including various azoles, terbinafine, and amorolfine. Fluconazole or Amphotericin are taken internally in severe *Candida* and other systemic fungal infections.

PATHOGENS OF OTHER VERTEBRATES

Though much of our attention inevitably falls on humans, all other mammals have fungal pathogens, some closely allied to those affecting humans. Just one rather different example is given below – the emerging bat white nose syndrome. Birds too are subject to fungal diseases, again often similar to those affecting mammals (e.g. aspergillosis and candidiasis are the most common). Reptiles also have fungal diseases, the integumentary system being most affected, with lesions often containing soil saprotrophs; systemic mycoses are rare. Neither birds nor reptiles are considered further here. With regard to amphibians, the devastating emerging chytridiomycosis disease overshadows all others. Fish, too, are affected by fungal diseases, especially caused by members of the fungus-like oomycetes. Both chytridiomycosis of amphibians and some oomycete diseases are described below.

Bat White-Nose Syndrome

An emerging fungal disease of bats – White-nose syndrome – is having devastating effects on bat populations. The recently (2006) discovered psychrophilic *Pseudogymnoascus destructans* (Ascomycota: Helotiales; previously known as *Geomyces destructans*) is causing

TABLE 9.6 Commonly Used Antifungal Drugs, Their Mode of Action, and Susceptible Fungi

Type of drug	Mechanism of action	Susceptible fungi
Drugs active against plasma membrane, synthesis or integrity		
Allylamines (naftifine and terbinafine) and thiocarbamates (tolnaftate)	Inhibits activity and formation of lanosterol	<i>Asperillus, Acremonium, Arthrographis, Fusarium, Penicillium, Trichoderma</i>
Azoles (imidazoles and triazoles) and echinocandins (cifofungin)	Inhibits ergosterol synthesis	<i>Aspergillus, Candida, Cryptococcus</i>
Folimycin (concanamycin A)	Inhibits V-type proton-ATPase	Various fungal species
Hydroxypyridones	Inhibition of ATP-synthesis and uptake of essential components	Various fungal species
Octenidine and pirtenidine	Inhibition of ergosterol biosynthesis	<i>C. albicans, Saccharomyces cerevisiae</i>
Polyenes (Amb and nystatin)	Auto oxidation of ergosterol; formation of free radicals which damage the plasma membrane	Species of <i>Aspergillus, Candida, Coccidioides, Cryptococcus, Histoplasma, Saccharomyces</i>
Sphingofungin	Interrupts sphingolipid synthesis	Various fungal species
Active against cell-wall components		
Aureobasidin-nikkomycin polyoxins	Inhibition of chitin synthesis and assembly	<i>Candida</i> and <i>Cryptococcus</i> spp.
Benanomycin A-pradimicin A	Membrane disruption causing leakage of intracellular potassium; calcium-dependent complexing with saccharides of mannoprotein	<i>Aspergillus</i> spp., <i>Candida</i> spp., and <i>Cryptococcus neoformans</i>
Echinocandins (caspofungin, micafungin, anidulafungin)	Inhibition of cell-wall glucan synthesis	<i>Candida</i> spp.
Active against cellular anabolism		
5-Fluorocytosine	Inhibition of pyrimidine metabolism	Species of <i>Asperillus, Candida</i> , and <i>Cryptococcus</i>
Sordarin (sordaricin methyl ester)	Disrupts placement of tRNA from A site to P site; disrupts movement of ribosomes along the mRNA thread	<i>C. albicans</i> and <i>S. cerevisiae</i>

From Abu-Elteen and Hamad (2011).

massive mortality in hibernating bats in eastern North America. Many bat species are affected, but the little brown myotis (*Myotis lucifugus*), which was formerly among the most common bat species, is now facing extirpation in that region. *Pseudogymnoascus destructans* hyphae replace hair follicles and associated sweat and sebaceous glands; the very obvious white mycelial growth on nose, ears and wing membranes is reflected in the name of the disease. The fungus erodes the epidermal tissues, and infected bats have no fat

reserves, which are crucial for surviving hibernation. *Pseudogymnoascus destructans* is psychrophilic, growing optimally at 5–10 °C, with little growth above 15 °C; the temperatures in infected bat hibernacula range between 2 and 14 °C all year round, allowing continual fungal growth.

Chytridiomycosis of Amphibians

Perhaps the most devastating of all vertebrate pathogens is the chytrid *Batrachochytrium dendrobatidis* (shortened to *Bd*), which first came to notice in 1987 when the golden frog (*Atelopus zeteki*) was extirpated in Costa Rica. It causes a disease of amphibians that has resulted in serious declines of over 200 species, and the extinction of at least three species, the Panamanian golden frog, the Australian gastric brooding frogs (*Rheobatrachus* sp.), the sharp-snouted day frog (*Taudactylus acutirostris*), and probably many more. The disease has spread worldwide, but Southeast Asia has a much lower incidence, and the island of Madagascar, the most amphibian-rich place on the planet, appears currently to be free from the disease (Figure 9.3a). It spreads rapidly, as seen following extirpation of amphibians in Costa Rica in 1987, moving through Central America, and reaching Panama by 2008 (Figure 9.3b). This movement is too rapid to be spread by frogs and toads themselves, so this must be by another agent, perhaps on the feet of birds. Global spread is due to the human trade in amphibians, but the origin of this emerging disease is still uncertain. Patterns of genetic diversity of *Bd* isolates from infected populations indicate that it is a recently emerged lineage (*BdGPL*) that evolved in the twentieth century, as the product of recombination in a single mating between closely related strains; isolates from worldwide locations are extremely closely related, and the two other strains that have been discovered (*BdCAPE* and *BdCH*) are much less virulent.

The motile flagellate zoospores of *Bd* seek the amphibians and penetrate the skin, where they form sporangia (Figure 9.3c). Symptoms of colonisation are not usually obvious to the naked eye, though sometimes there are skin lesions. There is epidermal hyperplasia (abnormal increase in the number of normal cells) and hyperkeratosis (thickening of the skin), and possibly increased skin shedding. The mechanism by which *Bd* causes death is related to disruption of electrolyte transport across the epidermis, that is critical in maintaining homeostasis. Not all species succumb to the disease and some species and populations in the wild have survived initial declines. This variation is likely to be due to differences in virulence between *Bd* strains, environmental conditions, host behavioural characteristics and immune responses. Rate of production and load of zoospores is probably an important determinant of the rate of disease development and mortality, as mortality occurs above a threshold of infections. Possible treatments, on a small scale, include application of antifungal drugs (e.g. itraconazole) to all of the tadpoles in infected populations, and application of 'probiotic' bacteria that produce antifungal compounds that kill *Bd* on amphibians' skin.

Recently, a new species of chytrid that is pathogenic to amphibians, *Batrachochytrium salamandrivorans*, was discovered causing dieoff in Dutch and Belgium fire salamanders. This chytrid is suspected to have been introduced to Europe from another region of the world, and suggests that there is probably a large number of species of these chytrids waiting to be discovered, as well as to emerge as pathogens.

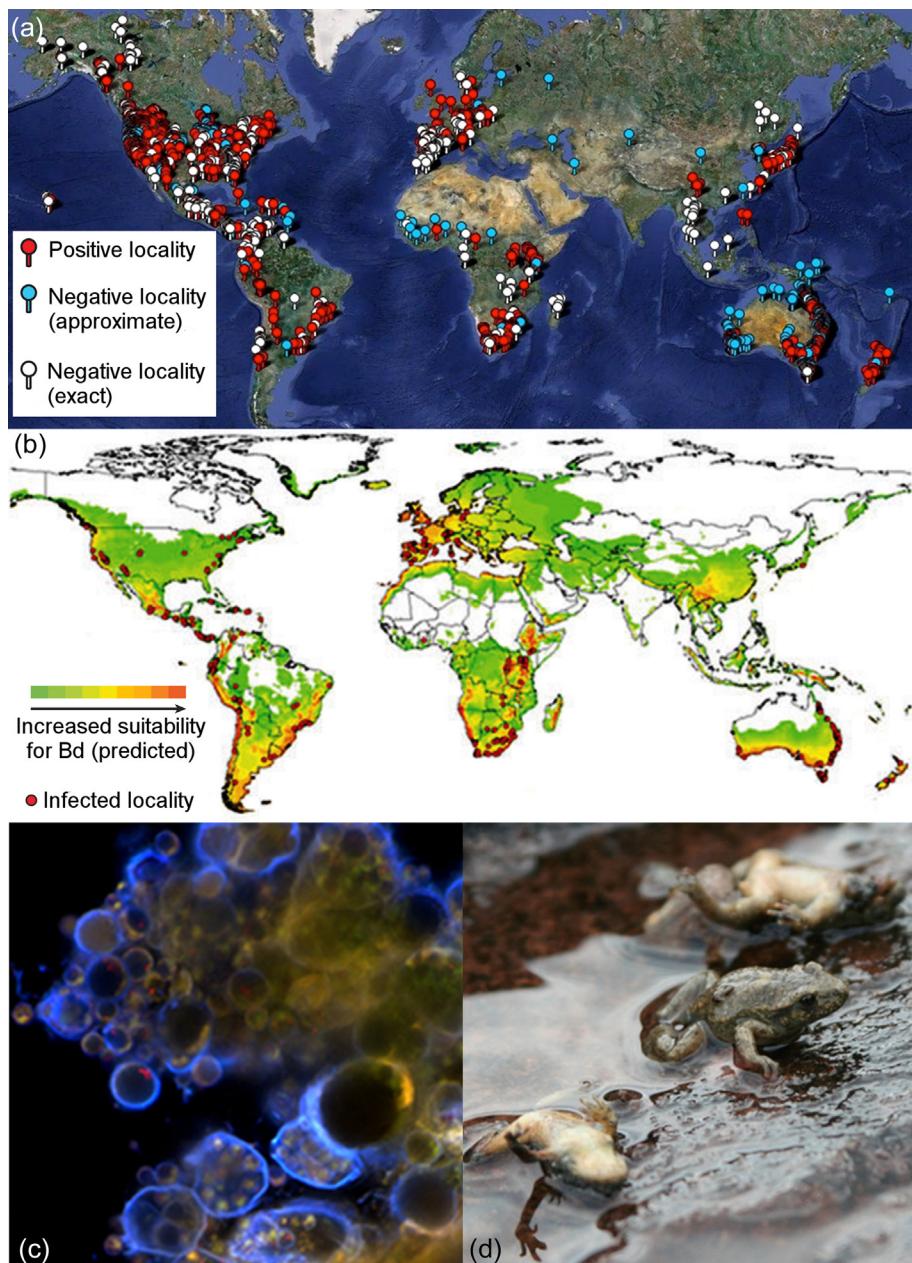


FIGURE 9.3 (a) Chytridiomycosis of amphibians caused by *Batrachochytrium dendrobatidis* (*Bd*) is now widespread across much of the globe, though Southeast Asia is still largely free of the disease, as seen in this screen shot from the Global Mapping Project (<http://bd-maps.net>). (b) The disease is predicted to spread even more widely by the Climate Envelope Model developed by D. Rödder, J. Kielgast, J.B. Schmidlein, et al. (unpublished data). (c) Laser-scanning confocal micrograph of *Bd* in culture. Blue stained structures are metabolically active sporangia. (d) Pyrenean Midwife toads *Alytes obstetricians* suffering from lethal chytridiomycosis. Source: Panel (b) from Fisher et al. (2009), (c) from Fisher et al. (2009), (d) © Mat Fisher.

Pathogens of Fish

Oomycetes (Kingdom Stramenopila) are perhaps the most widespread ‘fungal’ disease of fish, especially species of *Achlya* and *Saprolegnia* (Saprolegniales), but also Saprolegniales species in the genera *Aphanomyces*, *Calyptotrichia*, *Dictyuchus*, *Leptolegnia*, *Pythiopsis* and *Thraustotheca*, *Pythium* (Peronosporales), and *Leptomitus* (Leptomitales). Saprolegniasis is a disease of the epidermis of fish (Figure 9.4). It typically starts on the fins or head and often spreads over the entire body, being visible as white or grey mycelial patches. Spores commonly enter the fish body via damaged gills. In Salmonids, saprolegniasis is associated with stress. *Saprolegnia* species can also infect fish eggs, swimming from dead to live eggs via positive chemotaxis. Ulcerative mycosis and epizootic ulcerative syndrome, which can cause mass mortality, are attributed to *Aphanomyces invadans*. Distinct skin lesions, which appear as red-spots, black marks, or red-centred, white-rimmed deep ulcers, contain hyphae that can sometimes penetrate deeply into the fish beyond the muscles, damaging the brain, vertebrae, and other organs. Branchiomycosis is a widespread disease, especially in warmer climes, and can cause major problems in carp farms. The disease obstructs the blood vessels in the gills, and appears initially as flecks on the gills. The gills later become grey-white, and can even drop off exposing underlying cartilage. *Branchiomyces sanguinis* is associated with carp (*Cyprinus*), tench (*Tinca*), and sticklebacks (*Gasterosteidae*), and *Branchiomyces demigrans* is common on pike (*Esox*) and tench.

Ichthyophonus is one of the most well-known diseases of fish, especially marine, killing over 80 species. Unlike most fish pathogens that are facultative, the causal agents *Ichthyophonus hoferi* and *Ichthyophonus gasterophilum* are obligate pathogens. Their phylogenetic position is not, however, clear. Fish are usually infected via the digestive tract. Fins disintegrate and can fall off; organs including liver, kidneys and spleen can be colonised, causing swelling, distension of the body and accumulation of exudates; eyes bulge and erode when colonised.

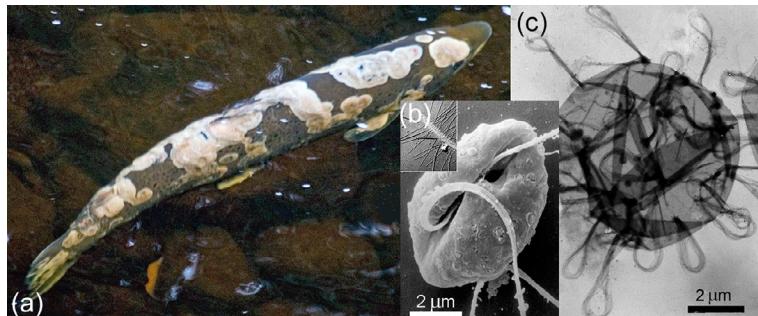


FIGURE 9.4 Saprolegniosis – oomycete (Kingdom Stramenopila) pathogens of fish and their eggs. These fungus-like organisms cause serious losses to fish both in commercial hatcheries and fish farms and can threaten wild stocks of salmonids when they return to their spawning grounds. (a) Mature brown trout (*Salmo trutta*) showing characteristic white lesions of *Saprolegnia parasitica*. (b) Secondary zoospore of *Saprolegnia parasitica*, with ventral groove from which flagella emerge. The hairs which decorate the anterior flagellum are shown in this electron micrograph inset. (c) A secondary cyst case of *Saprolegnia parasitica*, showing bundles of hooped spines that characterise isolates of the fish pathogenic species. Source: © Gordon Beakes.

KILLERS OF INVERTEBRATES

There are probably fungal pathogens of all invertebrate species, but those of insects have been most studied. We will first describe these and then mention the intriguing nematode-killing fungi, the recently emerging aspergillosis of coral, and crayfish plague.

Entomopathogens

There are estimated to be in excess of 1000 species of fungi parasitic on insects, in about 90 genera. Insect pathogenesis as a way for fungi to obtain nutrition has arisen *de novo* in all of the major fungal groups (Table 9.7). There are only a few Chytridiomycota that are parasitic on soil- or aerial-inhabiting invertebrates, partly because they are largely dependent on free water for dispersal. The zygomycete order Entomophthorales, and the ascomycete Hypocreales comprise a vast number of entomopathogens, but there are relatively few basidiomycete entomopathogens. The most studied fungal entomopathogens are *Beauveria bassiana* and *Metarhizium anisopliae* (Hypocreales).

Fungal entomopathogens are dispersed as spores, which must land on the cuticle of an insect host and remain there until they can germinate. Spores are usually adhesive, for instance *Entomophthora* spp. have mucilaginous coats, *Verticillium* spp. have slime drops, the zoospores of *Coelomomyces psorophora* produce host specific secretions that attach them to susceptible mosquitoes, and conidia of *Metarhizium anisopliae* produce an adhesion-like protein, MAD1. Spores of most fungal entomopathogens require nutrients to be available on the surface of the cuticle before they germinate, and the fungus' lipolytic activity helps. Different spore types (e.g. aerial conidia, submerged conidia, blastospores) have different adhesion properties and different cell wall surface carbohydrates, which affect pathogenesis as a result of differences in insect immune system recognition.

Germination is influenced by temperature, humidity, UV light, nutritional, and chemical environment. Fungal pathogens also have to be able to tolerate the toxic compounds present in the cuticles of some insects (e.g. caprylic or capric acids in Japanese silkworm, *Bombyx mori* and the rice stem borer, *Chilo suppressalis*). Following germination, hyphae must then enter the host. Some invade immediately upon germination, while others grow extensively over the host's surface first (e.g. *Metarhizium anisopliae* on wireworms, *Hyalius pales*). Extensive surface growth is correlated with hard host cuticle, and may allow the pathogen to detect thinner cuticle areas and build up inoculum potential. As with some plant pathogens, some fungal entomopathogens produce an appressorium, at the tip of the germ tube, prior to penetration (Figure 9.5). The site of penetration is variable and includes the arthrodial membranes between joints and between segments, direct penetration through cuticle, via the more vulnerable ventral surface, sense organs, and spiracles. Only a few others invade via the gut (e.g. *Smittium morbosum* in mosquito larvae), as digestive enzymes make this an inhospitable environment. Cuticle thickness is a major determinant of where entry occurs; *Entomophthora* spp. penetrate any part of the thin cuticle of flies, mosquitoes and aphids, but enter larger insects with thicker cuticle via arthrodial membranes. Penetration is brought about by a dual mechanical and enzymatic process.

When the fungus has succeeded in entering the insect, mycelial growth may be localised around the point of entry in the epidermis, but ultimately the body is usually extensively

TABLE 9.7 Fungal Pathogens of Insects

Kingdom/Phylum/Subphylum	Examples of pathogenic fungal species	Host
Fungus-like oomycetes	<i>Lagenidium giganteum</i> ^a	Mosquito larvae
	<i>Aphanomyces labis</i>	Mosquito larvae
	<i>Pythium flevoense</i>	Mosquito larvae
Chytridiomycota	<i>Myiophagus</i> spp.	Scale insects
Blastocladiomycota	<i>Catenaria</i> spp.	Small flies (but mostly nematodes)
	<i>Coelomycidium</i> spp.	Scale insects, beetle larvae, dipteran pupae
	<i>Coelomycetes</i> spp.	Obligate pathogens requiring two aquatic hosts, mosquito larvae and crustaceans (e.g. copepods bugs) at different life cycle stages
Zygomycetes: Mucoromycotina	<i>Sporodiniella umbellata</i>	Pathogens of weak insects
Zygomycetes: DKH clade	<i>Smittium morbosum</i> Some <i>Harpellales</i>	Mosquito larvae Adult black flies (Simuliidae)
Zoopagomycotina	<i>Zoopagaceae</i>	Trap hosts with adhesives and produce a restricted haustorium
	<i>Cochlonemataceae</i>	Rotifers, amoebae, rhizopods
Entomophthoromycotina	<i>Basidiobolus</i> and <i>Conidiobolus</i> spp.	Range of insects (and vertebrates)
	<i>Entomophthora, Pandora, Zoophthora</i> spp.	Range of insects
Ascomycota: Hypocreales	<i>Beauveria</i> spp. ^a	Broad host range
	<i>Metarrhizium</i> spp. ^a	Broad host range
	<i>Hypocrella</i> spp.	Broad host range
	<i>Cordyceps</i>	Broad host range
	<i>Ophiocordyceps</i>	Broad host range
	<i>Laboulbeniales</i>	Obligate haustorial ectoparasites of insects and a few other arthropods
	<i>Lecanicillium</i> ^a	
Basidiomycota (only Septobasidiales)	<i>Auriculoscypha, Ordonia, Septobasidium, Uredinella</i> spp.	Scale insects

^aRegistered biocontrol agent.

Information extracted largely from Vega et al. (2012).

colonised in the **exploitation phase** (Figure 9.5a). Most fungal entomopathogens produce dispersal structures, such as blastospores or hyphal fragments, which circulate in the haemolymph before forming mycelium. Successful entry into the host does not ensure colonisation since insects have immune systems that can respond to invasion by eliminating or confining the pathogen. Exploitation is usually **necrotrophic**, with host death resulting from

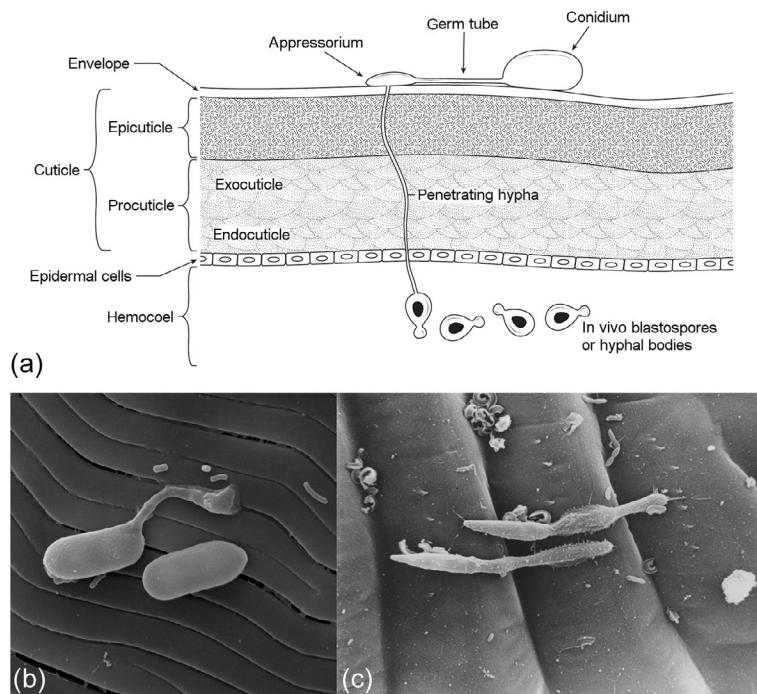


FIGURE 9.5 (a) Fungal pathogen infection of insects. A spore lands on the insect cuticle, adheres and then germinates, forming a germ tube and an appressorium. From the appressorium, a penetrating hypha enters. An appressorium forms, and from its under surface a fine hypha penetrates through the insect cuticle. Hyphae sometimes branch within the procuticle. The hypha breaches the epidermis and reaches the haemocoel, where it produces blastospores, which spread in the haemolymph before forming mycelium. (b) Two conidia of *Aschersonia* on the cuticle of whitefly. The upper conidium has germinated and produced an appressorium. (c) Germinated conidia of *Metarhizium anisopliae* on a tick. Source: (a) From Vega et al. (2012), (b, c) © Tariq Butt.

fungal toxins (e.g. destruxins), toxic proteases, or chronic disruption of host physiology following extensive mycelial development. Zygomycetes tend to colonise as mycelia whereas ascomycetes form budding, blastospores that colonise the haemocoel (body cavity), and then other tissues by mycelial spread. Some pathogens only colonise certain tissues, for example, *Entomophthora erupta* is confined to the abdomen of the green apple bug (*Lygus communis*), where it utilises the internal contents and posterior leg and wing muscles. Because the muscles of the pre- and mesothorax remain uncolonised, the insect is still able to move around even with ballistospores discharging from the ruptured abdomen, reminiscent of the sci-fi movie, *Alien*.

The parasitic relationship is sometimes **hemibiotrophic**, being initially biotrophic before becoming necrotrophic, or completely **biotrophic**. *Septobasidium* spp. (Basidiomycota) are epiphytic on tree bark, forming colonies with a tough outer layer of interwoven hyphae. Within the lower layers of mycelium armoured scale insects (Diaspididae) form a labyrinth

of tunnels and chambers, in which they live and move about. A proportion of the scale insects are infected with the fungus; hyphae form coiled structures, analogous to haustoria, within the haemocoel of the host. Hyphae emanate from the natural orifices of the host, interconnecting with the external mycelia network, but this does not appear to interfere with the mobility of the insect. The lifespan of the scale insects is often lengthened, the insects remaining in a juvenile condition, resembling the effects of biotrophic parasites on host plants (p. 262). Nutritional advantages to the fungus are obvious, but since scale insects are provided with an environment buffered from external climate and protected from predators, and only some are colonised, the relationship could be considered mutualistic to the population.

Most fungi that are biotrophic parasites of insects and other arthropods have a small body size. They are commonly restricted to the host surface, with haustoria penetrating the host cuticle. Many have lost structures usually found in related taxa, even certain lifecycle states (e.g. sexual or occasionally asexual states). The Laboulbeniomycetes (Ascomycota) is the only group of insect ectoparasites that has diverged into many clades (over 2000 species, with 80% parasitizing beetles (Coleoptera)), appearing to be evolutionarily very successful. Unlike most fungi, Laboulbeniomycetes have a thallus with determinate growth ([Figure 9.6](#)). Some are dioecious; in some species the male comprising as few as three cells though the thalli of others can have several thousand cells. When a potential host makes contact with a mature thallus, the fungus releases sticky spores that attach to the host. The thallus that develops is attached to the host by an enlarged basal cell (foot cell), and nutrients are abstracted from the host through a peg-like or root-like haustorium below the surface of the cuticle. Spores are produced by the thallus and spread to other parts of the host's body, as well as to new hosts. There is usually no obvious major damage to the host. Like most obligate fungal biotrophic parasites of plants and animals, the Laboulbeniomycetes are largely highly host specific, with some even being specific to a particular sex of host, and others specific to certain parts of the body. For example, *Stigmatomyces baeri* usually develops on the upper side of the female fly host, but on the ventral surface of the male host.

The final stages of the life cycle of fungal entomopathogens are **exit** and **survival**, enabling the fungus to spread to a new host food source ([Figure 9.7](#)). Prior to death, the behaviour of parasitized insects is often altered by the fungus in a way that benefits the fungus, which is sometimes referred to as a zombie fungus. With fungi that cause 'summit disease', the infected insects (e.g. grasshoppers) congregate at the top of plants during late afternoon ([Figure 9.7e and h](#)), because of interference with the nervous system and demands for oxygen which is depleted as spiracles are blocked by hyphae. Fungal entomopathogens of above-ground insects commonly anchor the host in an exposed aerial position, using hyphae which grow into the plant. Sap-feeding insects are further anchored by their stylets, and many insects clasp plant tissues and each other when rigour mortis sets in ([Figure 9.7](#)). On the other hand, the zombie fungus *Ophiocordyceps unilateralis* causes the infected canopy nesting ants to move to the forest floor, where humidity and temperature are conducive for fungal growth; the ants affix themselves with their mandibles to the undersides of leaves. With some entomopathogens, volatile attractants lure potential hosts to the vicinity of the fungus; healthy green apple bugs (*Lygus communis*) are attracted to, and insert their stylets into, the spore masses of *Entomophthora erupta* on the dorsal surface of infected insects.

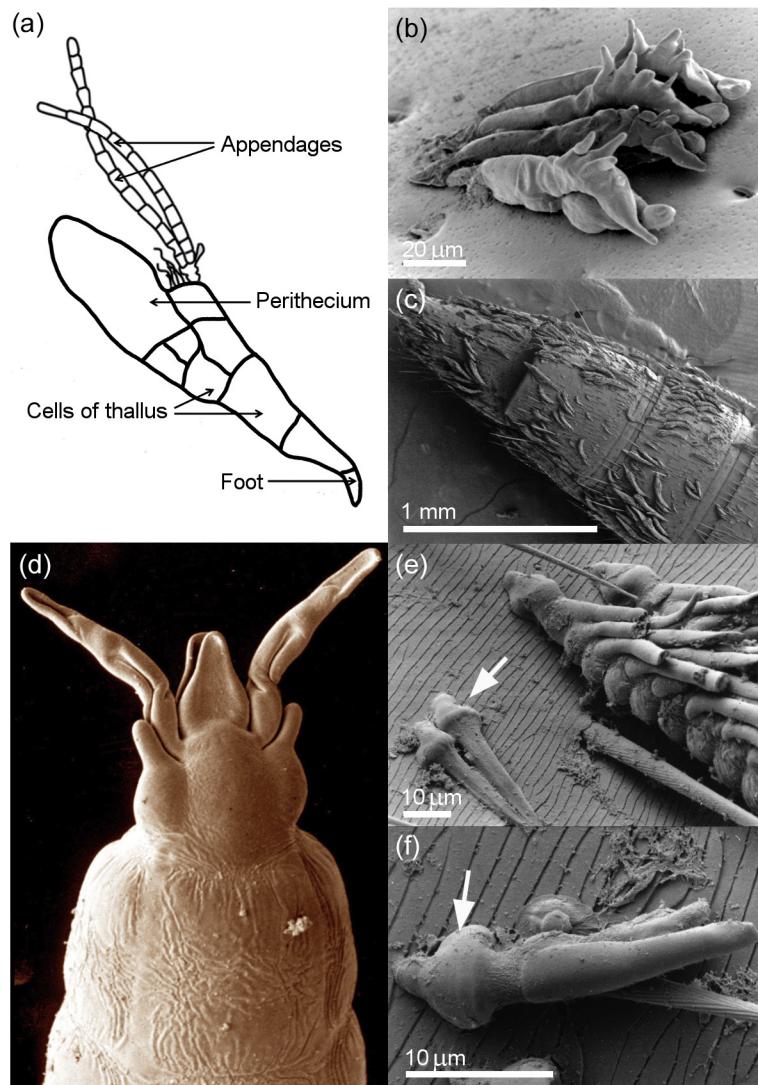


FIGURE 9.6 Laboulbeniales are biotrophic parasites on invertebrate hosts. Their simple, determinate thalli are very different from other fungi. Each ascospore has a foot cell – a swollen structure at the base – that attaches the spore to the insect cuticle. The foot cell acts like an appressorium and a fine penetration peg arises from it. The penetration peg grows in a very limited way into the insect cuticle – but presumably sufficiently to enable the fungus to tap into nutrients from the host (a) Diagram showing main features. Scanning electron micrographs of: (b) young thalli of *Hesperomyces* sp. on a two spot ladybird (*Adalia bipunctata*); (c) many thalli of *Rhacomyces philonthinus* on the cuticle of a rove beetle (*Philonthus* sp.); (d) the apex of a *Hesperomyces* sp.; (e) *Rhacomyces philonthinus* ascospores (arrowed) and thalli; (f) *Rhacomyces philonthinus* ascospores (arrowed). The horn-like appendages are elaborate in some species, and are thought to play a role in ascospore discharge. Source: All images © Alex Weir and Gordon Beakes.



FIGURE 9.7 Pathogens of invertebrates have different ways in which they survive and spread in time and space. (a) *Metarhizium anisopliae*, the green muscardine fungus, is a common insect pathogen, and has been used extremely effectively as a bioinsecticide. Here the white mycelium is seen emerging from the body sutures of a chrysomelid beetle, with subsequent formation of green conidia. (b) Fruit bodies of *Ophiocordyceps amazonica* emerging from a tropical forest grasshopper that it has killed. (c) *Beauveria bassiana*, a bioinsecticide, has mummified this caterpillar. (d) The asexual stages of several different *Hypocrella* spp. emerging from whitefly (Aleyrodidae hemiptera) colonies that they have killed, on bamboo leaves. (e) Spores of an *Erynia* sp. bursting through the abdominal sutures of an onion fly (*Delia*), which has climbed to an elevated position on vegetation – classic symptoms of a ‘summit’ disease. (f) Not only do fungi kill insects but also arachnids. Here a *Cordyceps* sp. is emerging from a trapdoor spider. (g) *Lecanicillium lecanii* causing mass mortality of coffee green scale pest (*Coccus viridis*), another fungus effectively used as biopesticide. (h) *Ophiocordyceps nymphaoides* emerging from the neck region of a ground dwelling ponerine ant that has climbed a tropical forest shrub and died clinging to it. Source: All images © Harry Evans.

Pathogen dispersal is usually via spores, often with different spore types fulfilling different roles such as spread within the insect population during the active season, and survival from one season to the next. Pathogens in the Entomophthorales have a range of spore types; ballistospores are shot off, by sudden pressure release, but if they fail to hit a suitable target, sticky secondary spores emerge from the ballistospores. Other spore types are also produced for survival, and in some for dispersal in water. Spore release is often timed to coincide with times when potential hosts are abundant. Species of *Erynia*, for example, that parasitize the biting blackflies (simuliids) of water courses, release ballistospores from corpses anchored to rock surfaces above water at precise times in the late afternoon, coinciding with arrival of healthy flies. Some Ascomycota (e.g. *Hypocrella* spp.) also produce different spore types, with ascospores being shot off and behaving similarly to ballistospores of the Entomophthorales. If these ascospores fail to land on a suitable host, they produce sticky spores on needle-like projections. Resting/survival spores are often produced by pathogens within the host, and released when the cadaver disintegrates. Others (e.g. *Cordyceps* and *Hirsutella* spp.) form pseudosclerotia (p. 61). The type of infection propagule can affect insect mortality. For example, mortality of tobacco budworm (*Heliothis virescens*) following infection by *Beauveria bassiana* is greater and earlier when infected by blastospores than by other conidia.

The success of entomopathogenic fungi in nature has led to the development of their use as biopesticides (p. 423). Understanding their biology and ecology is important to the successful application and formulation of such products. It would be wrong to assume that all fungal entomopathogens are obligate, and additional roles have recently been discovered for some. Several, including *Beauveria* species, could be endophytes (pp. 234–239). Others, including *Beauveria bassiana*, are antagonistic to plant pathogens, and *Lecanicillium* species are parasitic on fungal pathogens of plants. Species of *Beauveria*, *Isaria*, and *Metarrhizium* are common members of the soil mycobiota and may grow in the rhizosphere on exuded carbon sources.

Nematophagous and Rotifer-Trapping Fungi

There are over 300 species of fungi from Ascomycota, Basidiomycota, Chytridiomycota, Mucoromycotina and also fungus-like oomycetes that obtain their nutrition by predation or parasitism of nematode adults, instars, eggs or cysts (Table 9.8). These fungi have attracted considerable attention for over 100 years, perhaps, in part, because of the fascinating and dramatic mechanisms which some of them adopt for capturing their prey, but also because of their potential as biocontrol agents of plant-parasitic nematodes. They are found worldwide from the tropics to the Arctic and Antarctica, as saprotrophs in soil, mosses, dung, and decomposing wood and leaf litter. There are at least three different categories of **predatory fungi**: (1) those which form trapping structures; (2) endoparasites which infect nematodes as spores whose saprotrophic phase is predominantly within the nematode body; and (3) parasites of cyst nematodes that almost exclusively infect the females, eggs or larvae (Table 9.8).

Those in the first category form extensive mycelia that produce a variety of trapping structures depending on species, including adhesive pegs, knobs, rings, and three-dimensional networks of loops, and non-adhesive constricting rings, which attract and capture nematodes (Figure 9.8) and rotifers. The constricting ring traps are particularly spectacular; nematodes are captured when their bodies pass through the three-celled noose. When triggered by the touch of a nematode's body within the noose, rapid flow of water into the cells causes them to expand instantaneously, securing the nematode. Nematode-trappers are found in diverse

TABLE 9.8 Examples of Nematophagous and Rotifer-Trapping Fungi and Fungus-Like Organisms, and the Ways in which they Obtain their Prey

Predacious mechanism	Fungal species	Fungal phylum	Ecological characteristics
Spontaneously produced adhesive knobs, branches, and non-constricting rings	<i>Dactylaria candida</i> , <i>Dactylaria gracilis</i> , <i>Monacrosporium cionopagum</i>	Ascomycota	Slow growing soil saprotrophs; great predacious ability
Adhesive networks inducible by the presence of nematodes	<i>Arthrobotrys conoides</i> , <i>Arthrobotrys oligospora</i>	Ascomycota	Fast growing soil saprotrophs; weak predacious ability
Constricting rings	<i>Arthrobotrys dactyloides</i>	Ascomycota	Soil saprotroph
Adhesive projections	<i>Hyphoderma</i> spp. <i>Pleurotus</i> spp. <i>Hohenbuehelia</i> spp.	Basidiomycota	Adhesive projections – stephanocysts – on basal hyphae of fruit bodies Hour glass-like projections, with a viscous adhesive and immobilising toxin Similar structure to <i>Pleurotus</i> All are wood decay fungi
Endoparasites; conidia are either ingested or adhere to cuticle	<i>Meristacrum</i> spp. <i>Drechmeria coniospora</i> <i>Harposporium</i> spp. <i>Meria coniospora</i> <i>Catenaria anguillulae</i>	Zygomycete Ascomycota Ascomycota Ascomycota Chytridiomycota	Adhesive conidia on conidiophores that protrude from the nematode Adheres to mouthparts Hook onto mouthparts. Lodge in oesophagus Endoparasites are mostly obligate Zoospores colonise at natural openings
Endoparasites of cyst nematodes	<i>Dactylella oviparasitica</i> <i>Nematophthora gynophila</i>	Ascomycota Oomycete	Egg parasite On females and cysts of <i>Heterodera</i> spp.
Endoparasites; infective gun cells	<i>Haptoglossa</i> spp.	Oomycete	Obligate parasites of bacterivorous nematodes and rotifers
Toxin production	<i>Arthrobotrys</i> spp. <i>Pleurotus ostreatus</i>	Ascomycota Basidiomycota	Many species that produce trapping structures also produce toxins
Rotifer predators	<i>Cephaliphora navicularis</i> <i>Sommerstorffia spinosa</i>	Ascomycota oomycete	Adhesive knobs, often attached to mouthparts of bdelloid rotifers Adhesive pegs catch hard-bodied rotifers

fungal groups (Table 9.8), but the majority belong to a monophyletic group within the family Orbiliaceae (Ascomycota). In the Orbiliaceae, the trapping mechanisms have evolved along two main lineages, one which produces adhesive trapping structures and the other constricting rings. Some fungi produce traps spontaneously, whereas others require environmental triggers. In some (e.g. *Arthrobotrys oligospora*) traps tend to form when nitrogen is limiting.

There is a sophisticated chemical 'dialogue' between the fungal predator and its nematode prey. The presence of nematodes stimulates trap formation, the triggers being small peptides, with a high proportion of non-polar and aromatic residues. On the other hand, the mycelium of many, perhaps all, species often produces chemical attractants to nematodes, and trapping organs can provide further attractants. The initial event in capture by fungi is mediated by lectins on the trap and carbohydrates on the surface of the nematodes. Different fungal species

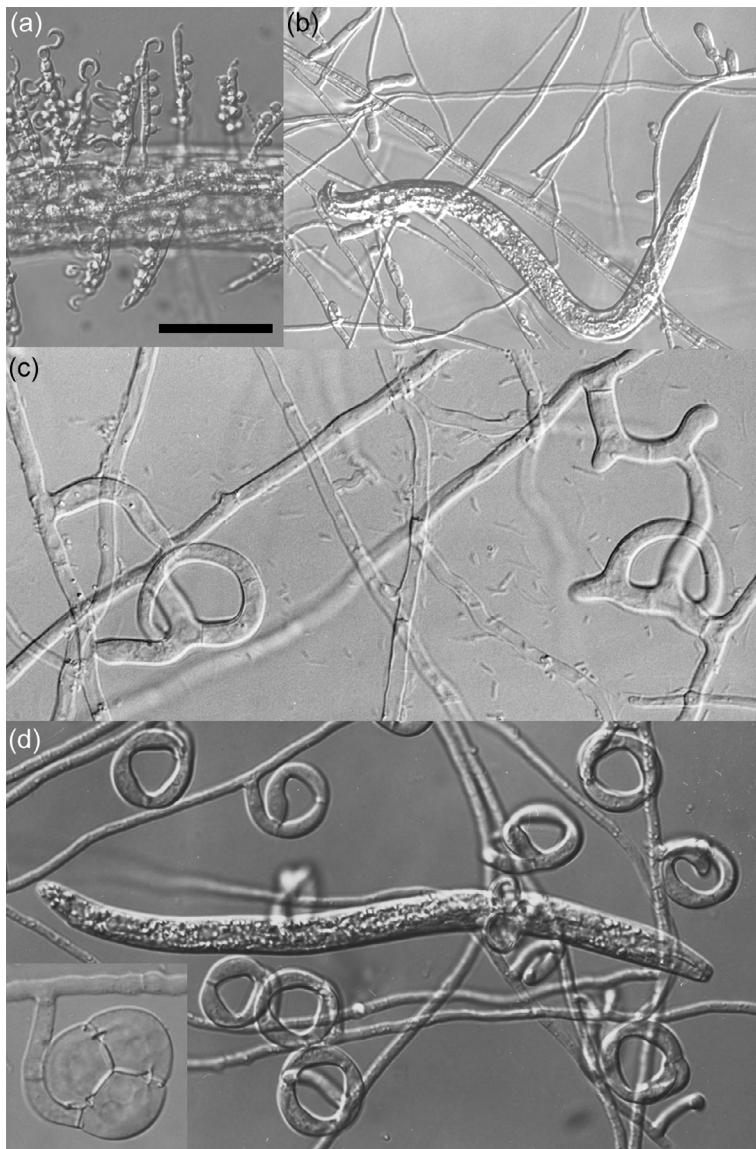


FIGURE 9.8 Nematode trapping fungi. A range of fungi are able to capture and utilise the contents of nematodes, using a range of different mechanisms. Some employ (a) conidia that lodge in the mouth or gut of the nematodes, germinate, grow, and eventually emerge producing more conidia, such as the hook-shaped conidia of *Harposporium anguillulae*. Others form adhesive structures, such as (b) small projections on hyphae of *Monacrosporium cionopagum* or (c) adhesive reticulate networks of *Monacrosporium* sp. (d) The constricting ring traps, such as those of *Drechmeria coniospora*, are a more complex mechanism. They comprise three celled loops, the cells of which rapidly expand when triggered (d and inset – *Monacrosporium doedycoides*) by the passage of a nematode. In all cases, when the nematode has been captured, hyphae penetrate the cuticle and digest the body content of the nematode. Scale bar = 50 µm (a); 100 µm (b, d); 20 µm (c). Source: © John Webster.

have different carbohydrate-binding proteins with different specificities for carbohydrates – 2-deoxyglucose in the case of *Dactylaria candida* and N-acetylglucosamine for *Arthrobotrys oligospora*. After contact with the nematodes, the prey is immobilised within a few hours by production of a nematotoxin (e.g. serine protease PII by *Arthrobotrys oligospora*). The wood decay basidiomycete *Pleurotus ostreatus* produces hour-glass shaped projections on its hyphae that exude an adhesive substance, and a toxin – ostreatin (2-decenedioic acid) – that immobilises prey within a minute. Other wood decay fungi can grow into nematodes through body orifices (e.g. *Amylostereum* species grow into the vulva of *Deladenus*, Figure 9.9a).

When the nematodes are attached to the fungal surface, a penetration peg then pierces the cuticle using mechanical pressure and hydrolytic enzymes. Inside the nematode's body, the penetration tube swells to form an infection bulb from which hyphae develop and spread, producing extracellular enzymes that rapidly digest the nematode. They spread by producing non-infective conidia.

In contrast to trap formers, most **endoparasites** form limited mycelium outside of their hosts. Rather, they produce infective zoospores or conidia that are either sticky and adhere to the host's cuticle or mouthparts, or are non-adhesive and lodge in the oesophagus when ingested. The spores germinate on or in the host nematode or rotifer, mycelium permeates and digests the body, and infective conidia are produced on hyphae that emerge (Figure 9.8a). The infective zoospores of the Chytridiomycota and fungus-like oomycetes form thalli within the host; zoospores are produced within these thalli and are subsequently released to the outside. Perhaps the most remarkable example of a parasitic infection structure is the 'gun cell' of *Haptoglossa* spp. (oomycete) (Figure 9.10).

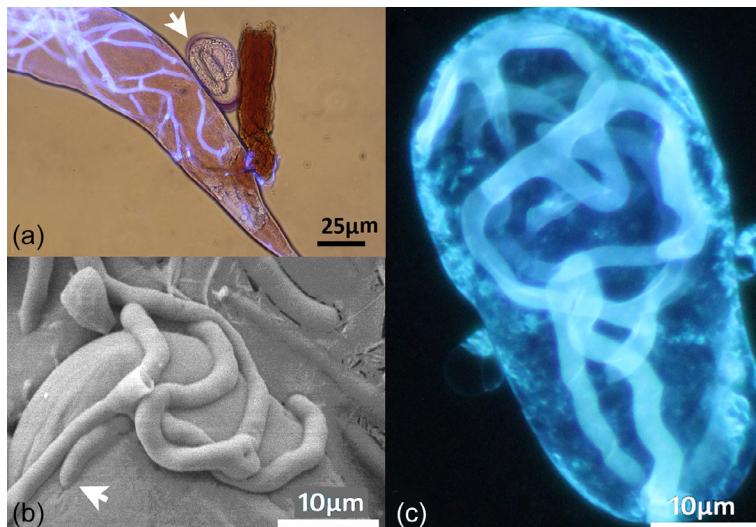


FIGURE 9.9 (a) Hyphae (fluorescence stained) of the wood decay fungus *Amylostereum areolatum* within the body of a nematode *Deladenus siricidicola*, having entered through the vulva. Note the healthy, unparasitised egg (arrowed). (b, c) Parasitism of *Deladenus siricidicola* eggs by *Amylostereum areolatum*. (b) Cryogenic scanning electron micrograph of *Amylostereum areolatum* hyphae growing over the egg surface. The point of penetration is arrowed. (c) Fluorescence stained hyphae within a parasitized egg. Source: From Morris, E.E., Hajek, A.E., 2014. Eat or be eaten: fungus and nematode switch off as predator and prey. *Fungal Ecol.* 11, 114–121.

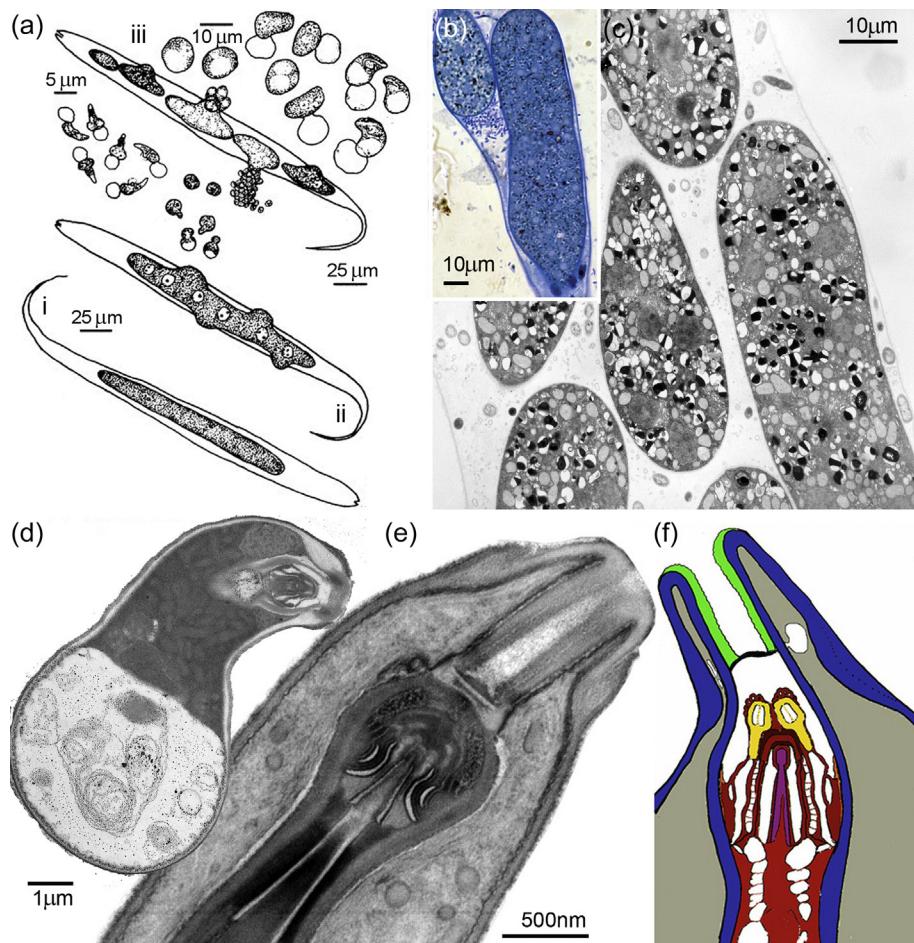


FIGURE 9.10 Fungus-like oomycete gun-cell-forming parasites of nematodes. Fungus-like species in the oomycete genus *Haptoglossa* produce extremely complex infection structures. (a) Life-cycle of the parasite; light (b) and electron (c) micrographs of young thalli showing typical densely packed cytoplasm; Gun cell of (d) an unnamed species and (e) *Haptoglossa erumpens*; (f) Diagrammatic summary of gun cell needle chamber apparatus in *Haptoglossa dickii*. Once infection has occurred a sausage-shape thallus develops within the body of the nematode (a(i), b, c), sporangia start to form (a(ii)) and large and small spores emerge from mature sporangia (a(iii)). The two spore types give rise to the two different infection gun cells. The spores germinate to form an infection cell (d, e, f), which matures to form an intracellular needle-shaped ‘missile’, within a looped, invaginated, infection tube, with a large vacuole at the base (d). The inverted tube containing the needle apparatus has associated restraining apparatus (f). This tube everts within a fraction of a second, penetrating the host’s cuticle, and injecting the parasite’s cytoplasm into the nematode. Source: (a) From: Glockling, S.L., Beakes G.W., 2000. Video microscopy of spore development in *Haptoglossa heteromorpha*, a new species from cow dung. *Mycologia* 92, 747–753; (b, c) © Gordon Beakes; (d, f) from Beakes, G.W., Sekimoto, S., 2009. *The evolutionary phylogeny of oomycetes – insights gained from studies of holocarpic parasites of algae and invertebrates*. In: Lamour, K., Kamoun, S. (Eds.), *Oomycete Genetics and Genomics: Diversity, Interactions and Research Tools*. John Wiley & Sons, Hoboken, pp. 1–24; (e) from Beakes, G.W., Glockling, S.L., Sekimoto, S., 2012. *The evolutionary phylogeny of the oomycete fungi*. *Protoplasma* 249, 3–19.

Parasites of nematode eggs and cysts are taxonomically different to those that parasitize adults, but are usually soil saprotrophs found in plant roots, though some wood decay fungi can also parasitise nematode eggs (Figure 9.9). Specific morphological features are not apparent on the mycelium, but appressoria-like swellings are sometimes seen on hyphae. Zoospores have a role in others, and these are attracted to their hosts.

Aspergillosis of Coral

Marine invertebrates also suffer from fungal disease. In the Caribbean and Florida Keys, there is an ongoing epizootic among the sea fan corals (*Gorgonia* species), which was first reported in 1995, though an epidemic in the 1980s may have been the same disease. Lesions, galls, and a purpling of the coral tissue occur, that can lead to death. Over 50% of sea fan tissue has been lost due to mortality arising from infection with *Aspergillus sydowii*, a soil saprotroph, though the marine strain is different. In inoculation experiments, *A. sydowii* from terrestrial sources was not pathogenic to Gorgonian coral. Infection induces a generalised defence response in the host, including production of antifungal compounds in the vicinity of infection. Like opportunistic pathogens of humans (see above), pathogenicity depends on host immune status. Elevated temperature has been hypothesised to drive outbreaks of the disease; production of antifungal compounds is much greater at elevated temperatures, but *A. sydowii* grows optimally at 30 °C. However, it appears that prevalence of the disease has declined steadily since the 2000s.

FUNGI AS FOOD AND HABITAT FOR ANIMALS

Obtaining carbon and energy from photosynthetic organisms often necessitates the breakdown of complex molecules (e.g. cellulose), plant defence compounds (e.g. polyphenols) and lignin, yet few organisms aside from basidiomycete fungi possess the necessary biochemical machinery to breakdown the latter. Further, the nutritional value of plants is often relatively low. The carbon:nutrient ratio of different species of plants and different plant parts vary considerably, but carbon:nitrogen (C:N) and carbon:phosphorus (C:P) ratios of leaves are usually in the range 25:1 to 100:1 and 450:1 to 1850:1, respectively, and for wood range from 350:1 to 500:1 and 1250:1 to >3500:1. Thus, a lot of resources must be consumed to obtain small amounts of nitrogen and phosphorus, which are essential building blocks for making enzymes, proteins, DNA, etc. When fungi decompose plant tissues the nutrient content increases, as the carbon is lost during respiration as CO₂. Also, mycelium itself has seven times lower C:N and C:P ratios (35:1 and 505:1) than undecayed wood. It, therefore, comes as no surprise that many organisms feed directly on fungi, mycophagy being most prevalent amongst members of the phylum Arthropoda, although there are also many examples within Mollusca, Enchytraeidae, Annelida, Collembola, and Nematoda. Many invertebrates also consume fungi indirectly when they eat decaying plant material. The benefits of feeding on fungal-decayed rather than undecayed plant material are seen with the death watch beetle, *Xestobium rufovillosum* (Anobiidae), which typically attacks wood colonised by certain basidiomycetes (e.g. *Laetiporus sulphureus*, *Trametes versicolor*, and *Coniophora puteana*). The length of time it takes for the beetle to complete its lifecycle is related to the state of decay of the wood: in undecayed

wood the larvae develop very slowly or not at all, but in decayed wood the life cycle is completed within 10–17 months. This is not only because nutrients are more concentrated in the decayed wood but also because softer decayed wood is easier to consume. Many mutualistic associations have evolved between invertebrates and fungi, based on fungal improvement of nutrition (e.g. ants, higher termites, ambrosia beetles, and wood wasps, pp. 330–334).

Mycelium

Not all mycelia are equally palatable to invertebrates, as some contain toxic chemicals or produce toxic volatiles. When mycelium is eaten, its morphology, foraging patterns, physiology, and biochemistry often change dramatically, though effects differ depending on fungal species, resource status, grazing intensity (density), and invertebrate species (Figure 9.11), probably reflecting differences in patterns of grazing. Some invertebrates preferentially feed on individual hyphal tips at the colony margins, some on swathes of hyphal tips, and others in patches within the colony. Nematodes feed, not by severing hyphae like most, but by inserting stylets and ‘sucking out’ contents. Changes take place in the immediate vicinity of grazing and also several cm from the site of grazing. Counterintuitively, grazing does not always decrease mycelial growth; very low density grazing sometimes stimulates hyphal coverage, and when heavy grazing ceases there can be catch-up or even over-compensatory growth.

Mycelium is also eaten by man, as part of fermented foods (e.g. tempeh, pp. 410–411). Meat substitutes are also made from fungal mycelium – *Fusarium venenatum* is cultured on an industrial scale, formed into the texture of meat and marketed as Quorn® (p. 416).

Fruit Bodies

Some macroscopic fruit bodies are perennial (e.g. polypore brackets) while others are ephemeral lasting for only a few days (e.g. Agaricales). Both provide food sources and breeding grounds for a diversity of invertebrates, including nematodes, enchytraeids, mites, Collembola (springtails), Coleoptera (beetles), and Diptera (flies). Insects that feed on agarics are mostly polyphagous (i.e. a wide variety of species are eaten) because the fruit bodies are unpredictable and ephemeral resources. On the other hand, half of the insects feeding in polypore brackets are monophagous, which probably results from evolution of physiology to cope with the specific chemical defences, and mouth parts to cope with the physical structure of the host bracket. Some *Drosophila* species are resistant to the toxin amanitin, found in some poisonous fleshy fruit bodies (e.g. the death cap *Amanita phalloides*) and are able to feed on it, whereas other *Drosophila* are not. While some invertebrates use living fruit bodies, others use those that are decaying, with different invertebrate species found at different stages of decay. The invertebrates that are polyphagous on brackets tend to colonise them after they have been decaying for some time, by which time the defence chemicals have presumably decreased. Early colonisers, that tend to be monophagous, have the advantage of less competition and a more nutritious environment, but they have a narrower choice of fungi. Many fruit bodies produce a bouquet of volatile organic compounds (VOCs), the composition of which can change during ageing, which are attractive to the flies and beetles that feed and breed in the fruit bodies, and are at least partly responsible for the partitioning of resource use between species.

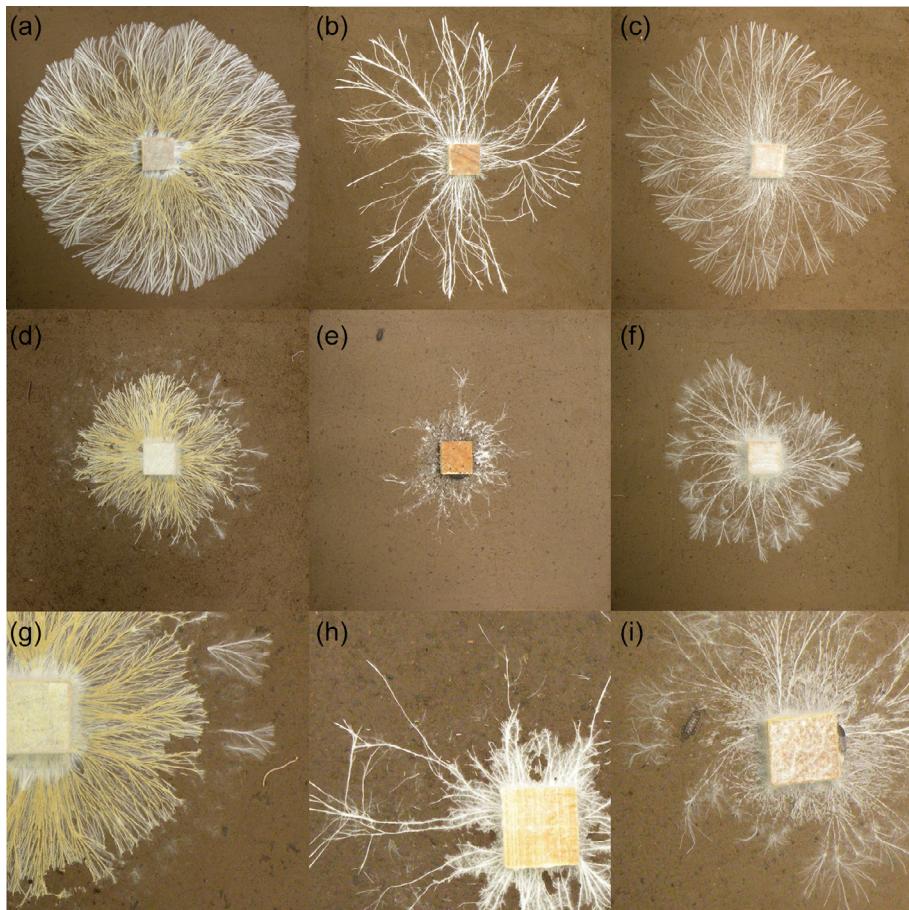


FIGURE 9.11 Many invertebrates graze on mycelium because of its high nutritional quality. Grazing can have dramatic effects on the fungus, but this depends on the extent of grazing, the invertebrate and fungus species, and the size of the mycelium. Digital images showing un-grazed mycelia of (a) *Hypholoma fasciculare*, (b) *Resinicium bicolor* and (c) *Phanerochaete velutina*, and the respective effects of 10 days of grazing by (d) 5 millipedes *Blaniulus guttulatus*, (e) 5 woodlice *Oniscus asellus* and (f) 60 collembola *Folsomia candida* on 24 × 24 cm trays of woodland soil. Invertebrates vary in their styles of grazing; (g) millipedes (Myriapoda), seen here on *Hypholoma fasciculare*, graze in an arc rather like a windshield wiper; (h) collembola, seen here on *Resinicium bicolor*, graze on finer cords and hyphae; and (i) woodlice (Isopoda), seen here on *Phanerochaete velutina*, often graze in straight lines, like a lawn-mower.

Source: All images © Tom Crowther.

Invertebrate feeding on fruit bodies can decrease reproductive fitness of fungi by decreasing the area of functional hymenium, causing altered morphology due to gall formation, and by damaging/destroying spores during passage through the gut. Fungi have evolved a variety of defence mechanisms, including chemicals, as already mentioned, and stephanocysts that kill nematodes, Collembola and other small invertebrates. In contrast, some fungi are dependent on invertebrates for spore dispersal; for example, species of stinkhorn (Phallales) smell of rotting flesh, which attracts Diptera to feed on the sticky head of spores, dispersing

them elsewhere on their legs and bodies (p. 92). Some species can only germinate following passage through the gut of an appropriate invertebrate (e.g. spores of a *Ganoderma* spp. must pass through a fly larva gut).

Fruit bodies also form an important part of the diet of some vertebrates. Some are opportunistic mycophagists, others (e.g. squirrels and several marsupials) concentrate on fruit bodies when they are available, and a few are obligately mycophagous (e.g. the marsupial rat kangaroos – *Potorous longipes* and *Potorous gilbertii*). Some marsupials and rodents are the main dispersers of spores of hypogeous mycorrhizal fungi – truffles. Like other animals, humans also eat fungi because they are nutritious – high in protein and low in fat – and some are tasty or of medicinal value (pp. 403–404). *Agaricus bisporus* is cultivated in 70 countries worldwide, accounting for 38% of mushroom sales. It is the fungus traditionally cultivated in Europe and North America but others, favoured more in Asian cuisines, are gaining in popularity, including the wood-rotting oyster fungi (*Pleurotus* spp., 25% of world sales with China leading in cultivation and consumption), the paddy straw mushroom (*Volvariella volvacea*, 16% of world sales), and shiitake (*Lentinula edodes*, 10% of world sales).

Lichen Thalli

Lichens are grazed by many invertebrates, including gastropods, and are often inhabited by invertebrates. Lichens produce carbon-based secondary compounds (CBSCs) that defend against lichenivory. Gastropods are selective in the regions of thalli upon which they graze, avoiding parts high in CBSCs, such as the sorelia of *Lobaria scrobiculata*, and preferring parts low in CBSCs, such as the cephalodia of *Nephroma arcticum* and the cortex and photobiont layers of lichens in general.

MUTUALISTIC ASSOCIATIONS BETWEEN FUNGI AND ANIMALS

Many invertebrates and ruminants have taken feeding on fungi one step further and over millennia, have coevolved with specific fungi forming innumerable liaisons in which both partners benefit (mutualism). High quality food is made available to the animals, and sometimes they also acquire fungal enzymes (e.g. cellulases) that remain active in the gut. Occasionally animals also benefit from an improved environment. Benefit to the fungus usually accrues from animals either bringing organic resources to the fungus or taking the fungus to suitable organic resources – often in specialised organs, and in some cases includes the animal creating a suitable microclimatic environment and even reducing competitors. Some of the most studied and intriguing associations are described below. Symbioses based largely on nutrition of both partners include fungi in the guts of invertebrates and vertebrates, and the farming of fungi by leaf-cutting ants and higher termites. Symbioses based on vectoring fungi to appropriate habitats and nutrition of the vector include those with ambrosia beetles and siricid woodwasps. Other symbioses are based on fungal nutrition and provision of suitable environment for the invertebrate, including the relationship between *Septobasidium* and scale insects (p. 318), and the jet black ant (*Lasius fuliginosus*) – aphid – fungus association (p. 332).

Fungi as Gut Symbionts

Some fungi live only in the guts of invertebrates and vertebrates. The *Asellariales* and *Harpellales* (in Subphylum **Kickxellomycotina**; formerly the Trichomycetes, though this grouping was phylogenetically heterogeneous, also containing protozoa) are obligate symbionts in the guts of freshwater, marine and terrestrial crustaceans, insects and millipedes, distributed worldwide. They have a small determinate body, which attaches to the gut lining by means of a holdfast. The vast majority do not penetrate the gut peritrophic membrane, though there are exceptions, e.g. *Stachylina minuta*, but it does not reach the cuticle. They appear to feed by absorption of nutrients as gut fluids flow over them, and may aid in food digestion, so the majority are commensal (cause no harm) or mutualistic. However, a few gut-inhabiting fungi are parasites/pathogens; some produce cysts in the ovaries of adult black flies, others cause sterilisation of the host, and *Smittium morbosum* kills mosquito larvae by preventing them from moulting. The invertebrate gut is a harsh and transient environment, because of digestive enzymes, the lining is shed at ecdysis (moult), and older regions of the peritrophic membrane break up and are expelled with faeces. To cope with this problem, Kickxellomycotina grow rapidly between moults and membrane breakdowns, converting almost all thallus material to spore production. Spores expelled at ecdysis are likely to be reingested when the host eats its shed skin. Kickxellomycotina (pp. 32–33) growing in aquatic hosts often have adaptations, such as long appendages on spores, that keep them in the vicinity of hosts.

Over 650 species of yeasts, mostly (>75%) Saccharomycetes (Ascomycota) and a few Tremellales (Basidiomycota), have only been cultured from the guts of beetles. As more and more insects are examined, more yeast species new to science are being discovered. The location of yeasts in invertebrate guts varies between species, some being found in the crop at the anterior end, others in the midgut and others in the hindgut. In anobiid beetles the fore part of the midgut comprises a group of blind sacs like a bunch of grapes – **mycetomes** – lined with large cells that contain many yeast cells. The yeasts provide enzymes for digestion, detoxify toxic plant metabolites, and provide essential nutrients, as many of them are able to synthesise a wide variety of B-complex vitamins. The symbioses can be intra- or extracellular, and yeasts are not only found in the gut, but sometimes in the fat body, eggs, haemolymph, and blood.

The symbiosis seems to be more important to the insect than to the yeasts; the yeasts obviously have a nutrient rich environment, but a major benefit may be as a means of dispersal to different habitats. In *Drosophila*, no more than three yeast species are usually isolated from individual flies, and this varies seasonally, apparently reflecting what species are present on feeding substrata. In rice planthoppers, yeasts are found in the fat body and are vertically transmitted by movement of the yeast to the primary oocyte, where eggs become infected. In some beetles, vertical transmission occurs when the female smears yeast cells onto the egg shells, which are then consumed when the larvae hatch.

The rumen and the rest of the digestive tract of herbivorous vertebrates provide a rich supply of food to microbes, though conditions are unfavourable to most fungi. In the rumen, temperatures are 39–40.5 °C, raised above mammal body temperature by fermentation; conditions are largely anaerobic (headspace atmosphere of around 65% CO₂, 30% CH₄, 4% N₂, H₂, 0.6% O₂), with any O₂ rapidly used by facultative anaerobes; the pH is continually

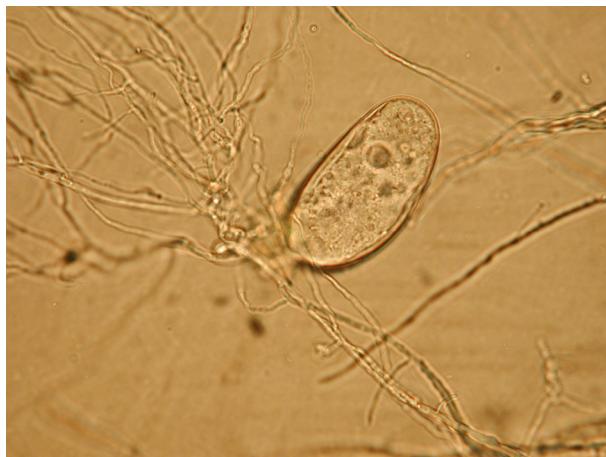


FIGURE 9.12 Neocallimastigomycota inhabit the rumen. The rhizoids grow into and breakdown plant fragments. Source: © Gareth W. Griffith.

being modified by host food and microbial metabolism but is buffered around pH 6.4–6.8 by bicarbonate rich saliva; and redox potential is extremely low. In the rumen fluid, anaerobic bacteria (10^4 – 10^5 ml $^{-1}$) and ciliate protozoa (10^{10} – 10^{11} ml $^{-1}$) predominate, but in the entire rumen content Neocallimastigomycota (p. 30) can form up to 20% of the microbial biomass. Anaerobic fungi in six genera – *Anaeromyces*, *Caecomyces*, *Cyathomyces*, *Neocallimastix*, *Promyces*, and *Orpinomyces* – are invaders of newly ingested plant material. Zoospores are attracted to plant material. They encyst and form a thallus with rhizoids that penetrate into the plant material (Figure 9.12). The rhizoids secrete a range of enzymes that break down cellulose, xyloans, starch, and other polymers. The nucleus from the zoospore is retained in the cyst and then in the sporangium, which is separated from the anucleate rhizoids by a septum. Zoospores are subsequently released from the sporangium, to infect more plant material. Newborn ruminants do not have the complex rumen microbial community found in mature animals, but colonisation occurs rapidly before the rumen becomes functional, probably from resistant stages of Neocallimastigomycota in faeces and possibly from saliva during licking by the mother. Also, several species are found as spores in air samples.

Ants and Higher Termites

Obligate mutualisms between fungi and social insects, in which the former are housed in the nest of the latter, have evolved independently twice; once in attine ants on the American continent, 50 million years ago (mya), and once in higher termites (Macrotermitinae) of central African tropical rainforest, 24–34 mya, later spreading to savanna and to Asia. The leaf-cutting genera of the attine ants are associated with a diversity of basidiomycete lineages, including *Attamyces*, *Leucoagaricus* spp., and *Lepiota* spp., whereas the termites are associated with a single genus *Termitomyces*. No reversions to the ancestral ant and termite life style are known, indicating the huge benefit of these mutualisms to the animals. There have, however,

been fungal reversals from the ant/fungus mutualism, though not for termite fungi. The basis of the mutualisms is that the worker ants and termites provide the fungus in the nest with organic matter (Figure 9.13). In the case of the ants, this is portions of leaves, and with the termites it is comminuted (i.e. broken into fine fragments), dead grass and wood. The insects do not have the enzymes necessary to digest lignocellulose, but the fungi do. Fungal decomposition of the organic matter results in nitrogen- and phosphorus-rich fungal biomass that provides the insects with most of their food. For example, the N concentration in the nests of *Macrotermes bellicosus* with *Termitomyces* was 2 times greater in the fungus comb (the main part of the fungus garden constructed from primary faeces that is subsequently completely consumed) and 20 times greater in the mycotêtes (fungal nodules; asexual fruit bodies – coremia) than in the original food. The fungus-growing termites eat the mycotêtes, which mix with consumed organic matter in the gut and are then deposited in the faeces, on the



FIGURE 9.13 Mutualism between fungi and ants and termites. (a) Fungus garden from the nest of *Macrotermes bellicosus*. (b) A close up showing the mycotêtes (white spheres of fungal material) that are consumed by the termites. While in some species sexual fruit bodies are only produced when the termites abandon the nests, in others they are regularly produced, as in (c) *Termitomyces reticulatus*, seen here growing straight from the underground fungus gardens of *Odontotermes badius*. Note the pseudorhiza connecting the fruit body with the fungus garden. Source: (a) © Karen Machielsen; (b) Photo taken by Prof. Renoux © Duur Aanen; (c) from Aanen and de Beer (2007).

top of the fungus comb. In contrast, with the fungus-growing ants, all fruiting is suppressed and the fungus spreads vegetatively from the older, bottom to the newer, upper regions of the fungus garden; small colonised fragments are added, together with faecal droplets. These droplets stimulate growth of the fungus and contain incompatibility compounds that are antagonistic towards genetically different fungal symbionts from other ant colonies brought in by foraging workers, maintaining a monoculture. Both ants and termites acquire cellulases and hemicellulases when they consume the fungus. These acquired enzymes survive gut passage and are concentrated in the faecal droplet that is deposited on fresh plant material, preparing it for fungal colonisation and increasing the initial mycelial growth. Contaminant fungi are kept out by: physical removal of spores by licking; chemical secretions from the ants; and antibiotics secreted by actinomycetes (*Streptomyces*) growing on the ants' bodies, targeted at a virulent Ascomycota mycoparasite (*Escovopsis*) that specialises in attacking fungus gardens. Similar grooming and antibiotic secretions maintain a monoculture in the nest of termites. However, *Leucoagaricus* spp., *Lepiota* spp., and other Basidiomycota associated mutualistically with the ants are more competitive than in the termite-fungus mutualism, being able to survive for approaching 12 days following abandonment of nests, as opposed to almost immediate replacement in termite nests. The fungal symbionts also benefit from a fairly constant microclimatic environment. In African savanna, above-ground temperatures can vary by 35 °C between winter night and summer daytime, but in the nests they remain in the narrow range of 29–31 °C, maintained by the ventilation system within the mounds.

When new colonies are founded it is essential that the fungal symbiont establishes within the new nest. Young ant queens take asexual propagules, in an infra-buccal pocket, from the natal nest on their mating flights, and use this to start the garden in the new colony (vertical transmission). Some termites also transmit the fungus to the new colony via a single parent in a similar way. *Termitomyces* spp. are carried to new colonies by queens in the genus *Microtermes* and by kings of *Macrotermes bellicosus*, but other Macrotermitinae acquire their symbionts while foraging: sexual spores are consumed and survive passage through the gut, and are then deposited in a faecal pellet on the new fungus comb. With such horizontal transmission, it might be expected that it would be easy to exchange fungal symbionts between termite lineages but this rarely happens between genera. Basidiomycota symbionts of ants fruit sexually only rarely. *Termitomyces* spp., however, produce large basidiocarps on the soil surface. These connect, via pseudorhiza, with the nest sometimes 2 m below ground (p. 64, **Figure 9.13**). *Termitomyces titanicus* produces the largest known edible fruit bodies, weighing up to 2.5 kg with caps greater than 60 cm diameter.

The highly successful mutualistic relationships between fungi and leaf-cutting ants and termites are based on invertebrate nutritional requirements and both provision of food and habitat for the fungus. An intriguing mutualistic relationship between the ascomycete *Cladosporium myrmecophilum* and the jet black ant appears to be based on improvement of the ant's environment rather than on nutrition. This common European ant, which lives in hollow tree trunks or beneath tree stumps, makes its nest from a cardboard-like material called carton. Carton comprises small wood and soil particles, honeydew (secreted by aphids that are farmed by the ant) and mycelium of the fungus. The fungus is inoculated into new walls by workers adding fragments from old nest walls. The ants do not appear to eat the fungus, though mycelium only emerges from nest walls in the absence of ants, implying that the ants prevent mycelial emergence in some way.

Ambrosia Beetles, Bark Beetles, and Wood Wasps

Bark and wood are difficult to break down and are relatively poor in nutrients, thus again it comes as no surprise that many invertebrates that feed on these plant tissues associate with fungi. In doing so, they obtain nutritional benefits of concentrated nitrogen sources and essential nutrients, including vitamins and sterols. The extent of dependence on fungi ranges from opportunistic to facultative to obligate. In bark beetles (that feed on fungi) and ambrosia beetles (that feed in subcortical tissues), mycophagy has evolved many times. In many species, when the adults lay their eggs in wood, fungi are also deposited. These fungi have been transported in specialised structures on the body of the beetle, termed **mycangia**. The most developed mycangia are invaginations of the beetle integument that are lined with secretory cells or glands. Less developed structures include shallow pits, deeper pits, and tubes not associated with glands, and setae. The glandular secretions contain amino acids, fatty acids, phospholipids, and sterols that support growth of fungal propagules, protect them from desiccation and may act against fungi not symbiotic with the beetle. Most mycelial fungal symbionts are *Ophiostoma* species (Ascomycota), with some *Ceratocystis* species (Ascomycota) and a few *Entomocorticium* species (Basidiomycota). These ascomycetes are well-adapted to arthropod dispersal, extruding ascospores from long-necked perithecia at a height where they are likely to contact the invertebrate's body, and having sticky spores shaped to allow multiple contact points. Asexual conidia are also vectored by the beetles, and in those beetles that produce sac mycangia, it is only these spores that are carried; asexual spores are found only in the galleries housing pupae, though ascomata form in old, disused galleries. Chlamydospores and yeasts of the basidiomycetes are found in mycangia, but not conidia or basidiospores. The mycelial fungi often have species-specific associations with beetles. Ascomycete yeasts are also symbionts of bark beetles, though adult beetles often carry several species; *Pichia capsulata* and *Pichia pini* are prevalent yeast associates with most bark beetle species.

The larvae of ambrosia beetles (of which there are around 3400 species) develop in woody xylem. The fungal symbionts penetrate the wood beneath the beetle tunnels, and line the walls with a thin mycelial cover or separate mycelial cushions, in both cases comprising short erect hyphae with swollen tips or chains of cells. Both larvae and adults feed only on fungi. Unlike ambrosia beetles, bark beetles lay their eggs in the inner bark of trees and feed on the phloem, which is rich in nutrients. Nonetheless many bark beetles, especially species of *Dendroctonus* and *Ips*, are associated intimately with fungi, as both larvae and adults feed on mycelium, yeasts, and conidia. Young adults of *Ips avulsus* and *Ips calligraphus* also seek out and ingest entire perithecia of *Ophiostoma ips*. The species of mycangial fungus carried by a bark beetle can have differential effects on the beetle. For example, many more progeny are produced by *Dendroctonus ponderosae*, and emerge sooner, when *Ophiostoma clavigerum*, rather than *Ophiostoma montium*, is the food source.

Woodwasps (Siricidae), like the wood-boring beetles, carry basidiomycete fungal symbionts (*Amylostereum* species) in a pair of pouches – mycangia, at the base of the ovipositor, and inoculate the fungus together with eggs into wood. Three species of *Amylostereum* – *Amylostereum areolatum*, *Amylostereum chailletii* and *Amylostereum laevigatum* – are involved, and the relationship is obligatory and species-specific. These fungi cause white rot, softening the wood and improving the relative nutrient content, so that wood wasp larvae can feed by burrowing through decomposing wood. The fungus benefits by being carried to and

inoculated directly into a suitable resource. Asexual oidia are carried by the woodwasps, but the fungi can also spread via basidiospores. In the northern hemisphere clones are common amongst isolates of *Amylostereum areolatum*, but less common in *Amylostereum chailletii*, indicating that woodwasps are more important in spreading the former than the latter.

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