

21st Century Guidebook to Fungi, Second Edition of the online version, by David Moore, Geoffrey D. Robson and Anthony P. J. Trinci

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Chapter 15: Fungi as symbionts and predators of animals

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Chapter 15: Fungi as symbionts and predators of animals

This Chapter deals with fungal co-operative ventures, including ant agriculture, termite gardeners, and agriculture in beetles. An important co-evolutionary story is that linking anaerobic fungi, the evolution of grasses and the rise of the ruminants. A fascinating story that links with human evolution since humans use cereal grasses as staple foods and selected their main food animals from among the ruminants. Finally, we look at the predatory nematode-trapping fungi.

Fungi have co-existed with animals and plants throughout the whole of the evolutionary time since these three groups of higher organisms originally separated from one another. Living together closely for this length of time has given rise to many co-operative ventures. We have already seen how many fungi have combined with plants as partners in mutually beneficial relationships such as mycorrhizas and lichens. In these symbiotic or mutualistic associations, the partners each gain something from the partnership so that the association is more successful than either organism alone. The organisms concerned (often two but sometimes more) live in such close proximity to each other that their cells may intermingle and may even contribute to the formation of joint tissues, as they do in the lichen thallus, which is one of the most ancient mutualistic associations of all and found in some of the most inhospitable environments.

Fungi have similarly close relationships with animals, which we will describe in this Chapter. There are several examples and the two about which we know most are:

- the **'fungus gardens'** created by leaf-cutter ants and termites (the most notable case being leaf cutter ants which cultivate and then graze upon hyphae, with neither fungus nor ant being able to live without one another), and
- the **chytrid** association with **ruminants**.

These two examples show the key features of symbiotic relationships, namely:

- they are mutually beneficial to the partners,
- the partners show behavioural and anatomical adaptations to enable the partnership, and

- the partners can be shown to have evolved in step (**‘coevolved’**) over a considerable length of time, even to the point that the fungi, and perhaps the animal, cannot survive independently of the partnership.

15.1 Fungal co-operative ventures

We have already discussed the circumstance in which fungi are used as **food for grazing animals**, from cattle weighing hundreds of kilograms to the microarthropods weighing hundredths of a gram (see [Section 11.1](#)). Lichens (*Cladonia* spp.) form an essential part of the winter diet of Caribou and Reindeer (*Rangifer tarandus*), and the reindeer, which are ruminants, have behavioural and anatomical adaptations enabling them to graze lichens under snow cover for their resident microbiota to digest the fragments (Kumpula, 2001; Turunen *et al.*, 2013). Fungus fruit bodies are eaten by many animals, but notably by mammals weighing less than three kilos. The dependence of animals on fungi as a food resource varies between species, but even mammals as large as deer and primates benefit by supplementing their diet with fungal fruit bodies (Hanson *et al.*, 2003; Trierveiler-Pereira *et al.*, 2016).

Many invertebrates use fungal mycelium as a major part of their nutrition, with approximately 80% of the tens of thousands of microarthropod species in forest soils being **fungivores** (or **mycophagous**) that depend on the fungal mycelium as their main or only food source. The many small animals involved have been mentioned in [Section 11.1](#) (see Fig. 11.11). At the microscopic level, some of the smallest grazers on fungi are the soil organisms discussed in [Section 11.2](#); collembola, mites, nematodes and larvae of mushroom flies. The integrated nature of mycelial networks ensures that in many fungi a morphological reaction occurs in response to grazing that serves to limit the damage caused. This implies that even at this level there is an evolutionary link between the grazing animal and the fungus. Indeed, collembola may even be perceived as mutualistic under some circumstances as they sometimes promote extra growth of the fungus as it overcompensates for grazing damage, producing a net increase in biomass, though this happens only when sufficient nutrients are available to the fungus (Bretherton *et al.*, 2006; Ngosong *et al.*, 2014; Pöldmaa *et al.*, 2016).

Another factor known to influence the presence of collembola in soil is the presence of **endophytic fungi** in the leaves of grass growing on the soil. Leaf litter infected with fungal endophytes appears to decompose faster than uninfected plant matter, possibly due to the presence of toxins changing the composition of soil communities to produce a higher proportion of detritivores (Lemons *et al.*, 2005). Endophytes deter larger animals, such as cattle, from grazing, thereby performing a **protective function for their host plants** (see [Chapter 13](#)) but in so doing they are also interacting with the animals by determining the palatability, digestibility or nutritional value of their food source.

Most of the above discussion concentrates on animals using fungi as a nutritional resource, but that last paragraph raises ‘host protection’ as another function for associations between microbes and animals which has been described as **defensive symbiosis**. All organisms are threatened by aggressive encounters with predators, pathogens and parasites, but it is becoming increasingly evident that microbial symbionts can make important contributions to the arsenal of chemicals the host can use to defend themselves.

Many of these defensive compounds are produced by the host animals themselves, but they do take advantage of the immense chemical potential of microbial secondary metabolism and these **microbial defensive symbioses are widespread**. Unfortunately, most are bacterial symbionts and defensive symbiotic partnerships between animals and fungi are rarely studied. Yet they are a

promising research target as fungi have a vast biosynthetic potential and are already a rich source of antibiotics (Flórez *et al.*, 2015).

Another promising research topic that has emerged recently concerns the ***fungal associates of sessile marine animals***; corals, sponges and tunicates. Metagenomic and transcriptomic analyses have revealed residential as well as transient and variable fungal communities. The ecological function of fungi in association with sessile marine animals is complex and there is evidence for the full range of detrimental and beneficial interactions between fungi and their marine hosts. Based on evidence from terrestrial ecosystems a rich variety of relationships is likely to be found in sessile organisms of the marine environment. The evaluation of marine animal-fungal symbioses under changing environmental conditions may prove to be critical for predicting marine ecosystem responses to global pollution and climatic change (Yarden, 2014); [Chapter 16](#) will describe a case where the relationship has tipped over into an emerging infectious disease.

Our next topic in this Chapter, **fungus farming** (or **gardening**) by animals, is a classic mutualism, but this time with the fungus encouraging grazing rather than attempting to deter the fungivore.

15.2 Ant agriculture

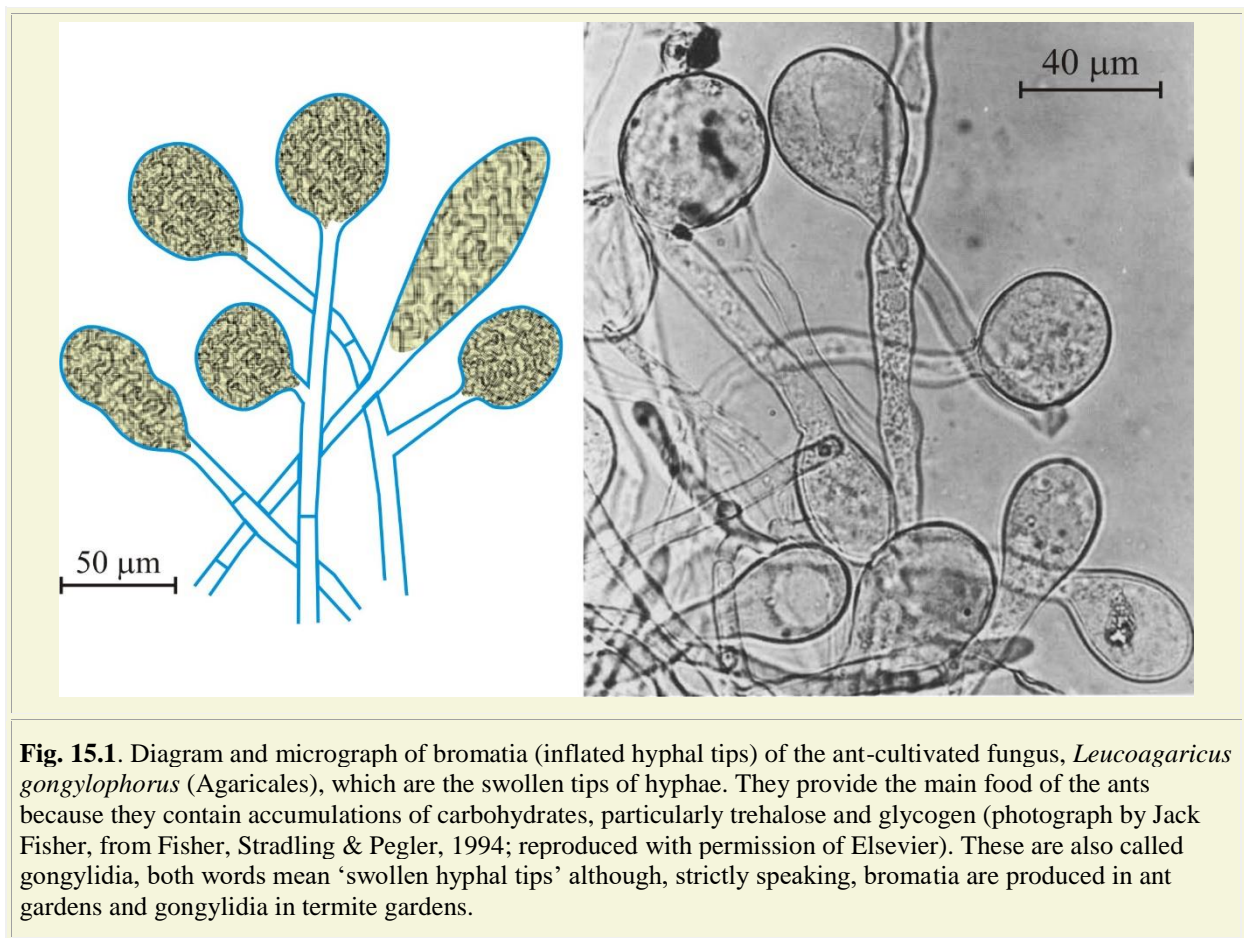
Attine ants are a group of more than 200 fungus-growing ant species living in the Neotropics (Central and South America) that have a rather unusual ability to cultivate fungi. Most use leaf-litter debris for fungal cultivation, but the leaf-cutter ants (*Atta* spp. and *Acromyrmex* spp.) cut and collect fresh leaves to grow two genera of fungi, *Leucocoprinus* and *Leucoagaricus* in the family Agaricaceae (Basidiomycota: Agaricales) (Mueller & Rabeling, 2008). This ant-fungus mutualism shows how successful, and how complicated, a relationship like this can be (Mueller *et al.*, 2001; Sánchez-Peña, 2005).

Fungus cultivation evolved apparently only once in the attines, about 45-65 million years ago. The ants actively inoculate their nest with the fungus and then cultivate it by providing it with pieces of leaves, pruning the hyphae and removing intruder fungi. As a reward, the fungus provides bundles of specialised hyphae that the ants use as a food source; these are the **gardening ants**. The ants are engaged in an agricultural activity; they collect fresh leaf biomass to convert it to compost in order to cultivate a particular fungus that then provides the main food source for the nest (Vega & Blackwell, 2005). Schultz & Brady (2008) point out that **agriculture is a specialised symbiosis** that is known to have evolved in only four animal groups: ants, termites, bark beetles, and humans.

Gardening ants, which collect and compost plant material, occur in Central and South American tropical rain forests where they are the **dominant herbivore** (only humans destroy more trees). They destroy the forests, of course, but also damage crops, devastating tracks up to 30 cm wide. An ant colony may contain several million ants, cover 8 m² and may be 1 m deep. Leaf cuttings (several mm square) are pulped by ants and the chewed-up plant material + saliva + faeces are turned into compost for a fungal garden which is spawned with mycelium carried from parts of an established garden. The garden is a monoculture of a member of the order Agaricales which does not normally produce fruit-bodies. Under the care of the ants, the mycelium produces hyphae with swollen tips rich in lipids and carbohydrates (bromatia; these are also called gongylidia, both words mean 'swollen hyphal tips' although, strictly speaking, **bromatia** are produced in **ant gardens** and **gongylidia** in **termite gardens**) (Fig. 15.1).

These swollen hyphal tips are cropped by ants and are the main food for the larvae; the larvae depend entirely on the fungus to digest the leaves (Bass & Cherrett, 1996). The first genomic studies of the attine leaf-cutting ant genera *Atta* and *Acromyrmex* demonstrated losses of two

genes in the arginine synthesis pathway (argininosuccinate synthase and argininosuccinate lyase) although all other (non-leaf cutting) ants, including species in the same subfamily (Myrmicinae) as the attine ants, have functional copies of those genes.

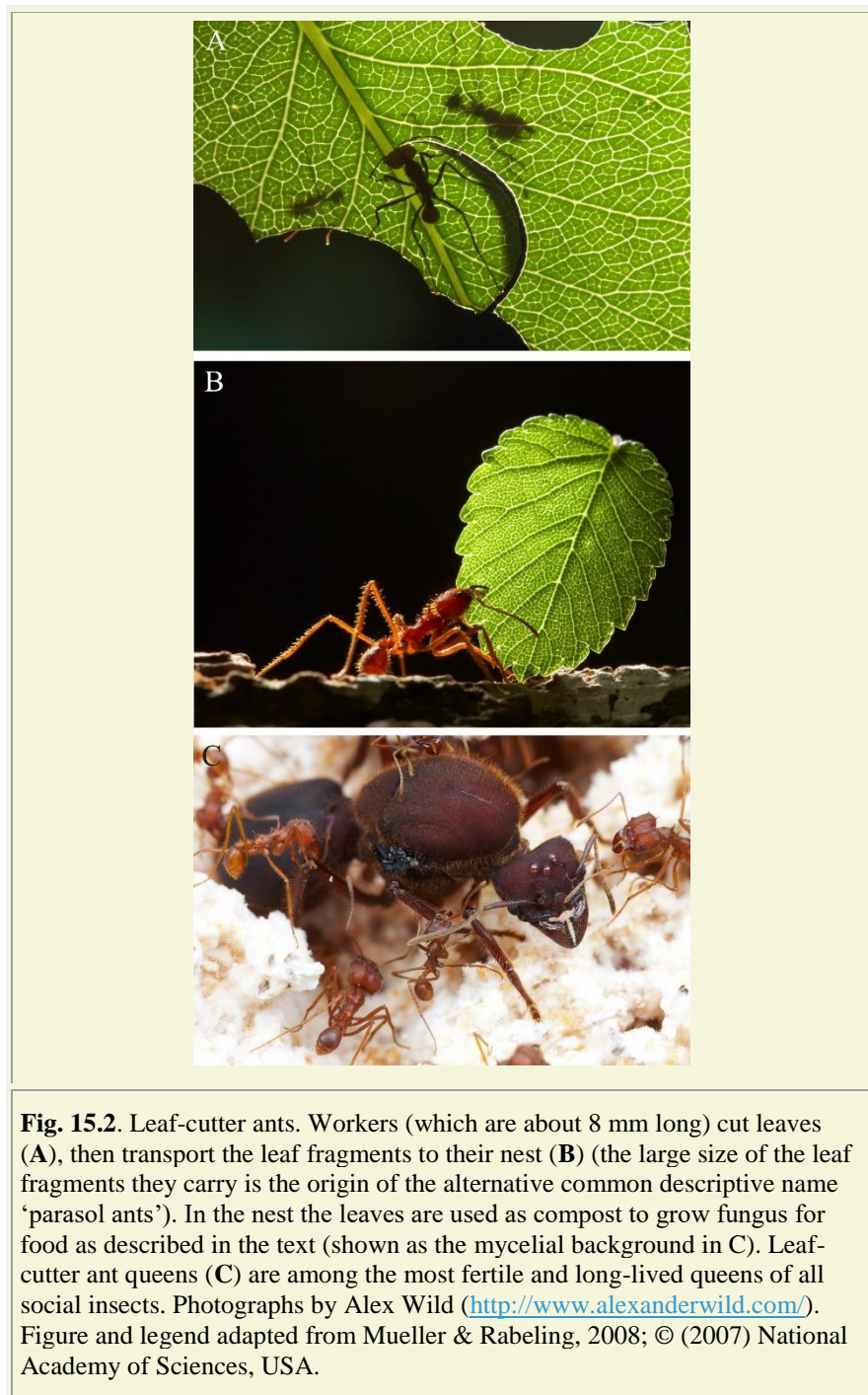


When transcriptomes of four different attine ant species (three species of the genus *Sericomyrmex* and *Apterostigma megacephala*) were specifically searched for genes coding for argininosuccinate synthase and argininosuccinate lyase, they were not found. So, there is strong genomic and transcriptomic evidence that **loss of the ability to produce the amino acid arginine is the reason for the obligate dependence of attine ants on their cultivated fungi**; The evidence also strongly suggests that the loss coincided with the origin of attine ants (Nygaard *et al.*, 2011; Suen *et al.*, 2011; Ješovnik *et al.*, 2016).

The fungus must be carried from nest to nest and this is done by newly-mated females as part of the mating flight. The nest is started off by a single queen with fungus in the infrabuccal pocket (a filtering device located in the oral cavity of all ants). The new queen mixes the **fungus inoculum** she carries with some suitable plant material, and lays eggs on it as soon as the fungus begins to grow. She then lays about fifty eggs each day. The first to hatch become workers who eventually establish a nest with a thousand or so interconnected chambers which might be excavated deep into the forest soil and be able to house a colony of several million individuals. When the first workers emerge, foraging and collection of leaves begins. Foraging may be in nocturnal or diurnal cycles depending on species, habitat and environmental conditions.

The workers cut pieces from leaves on trees and carry them back to the nest (Fig. 15.2A). Because they usually carry the leaves in their mandibles so that the leaf extends over the ant's head (Fig 15.2B), they are also called **parasol ants**. The caste which produces the largest

animals among the several million ants in an average nest is the soldier; a 20 mm long ant which is responsible for protecting the colony and its trails against intruders. The most numerous caste in the colony is the worker caste (these ants are about 8 mm long) which forage in the forest in search of leaves. They can cut leaf pieces bigger than themselves and then **carry them back to the colony**. Leaves are collected along trails within a colony's territory. Trails make it easier to find resources and reduce aggression between neighbouring colonies. Physical trails are lines of cleared ground that increase efficiency of foraging. There are also chemical trails of pheromones that the ants leave to mark the routes to good foraging. Once delivered to the nest, smaller workers, about half the size of the foragers, chew the leaves into smaller pieces and carry it into brood chambers.



Then the smallest ants, only 1.5 mm long, take over. These are the **cultivators** of the fungus garden; the mushroom farmers. They clean the leaf pieces and then inoculate them with fungus mycelium taken from the existing garden. The ants pluck hyphae from existing mycelia and transfer them to new areas of chewed-up leaf substrate in order to expand the garden. The cultivators continuously maintain the fungus garden, but they also tend the larvae and the queen (Fig. 15.2C). Substrate is usually exhausted and dumped after three or four months. Exhausted substrate is deposited into dump chambers in the nest together with other refuse such as dead ants. Refuse can also be taken from garden chambers and emptied outside the nest.

Resources Box

Leaf Cutter Ants video

If you have 8 minutes to spare (and an appropriate player on your device), you might like to view this WMV video showing leaf cutter ants in action

[CLICK HERE to run the video](#)

The fungus cultivated by leaf-cutter ants does not produce spores, but it does have those special hyphal tips (Fig. 15.1) that exude a sort of honey-dew which the cultivator ants collect and feed to the larvae. The fungus concerned in these associations is always an agaric mushroom fungus but not all of them can be found living free in the forest; specifically, the leaf cutter ant fungus is always associated with leaf cutter ant nests. This is a **mutual absolute dependence**.

The demand for leaf material as the colony grows is enormous. In the tropical rain forests of Central and South America, leaf-cutter ants are the dominant herbivores. That 'dominant' label includes the humans of the forest. Around fifty agricultural and horticultural crops and about half that number of pasture plants is attacked. None of this is new, of course. In the last quarter of the nineteenth century leaf cutting ants were described as: '... **one of the greatest scourges of tropical America...**' and early Brazilian farmers were so frustrated in their battles against leafcutter ants that they concluded 'Brazil must kill the ants or the ants will kill Brazil' (Mueller & Rabeling, 2008).

It has been calculated that leaf-cutting ants harvest 17% of total leaf production of the tropical rain forest. Nests located in pastures can reduce the number of head of cattle the pasture can carry by 10 to 30%. Statistics like this reveal how leaf cutting ants can become dominant exploiters of living vegetation and how they impact human agriculture. Leaf-cutting ants compete successfully with humans for plant material and are, therefore, counted as **important pests**. Because of their foraging activities, leaf-cutting ants can cause production losses in cacao and citrus fruit of 20 to 30%. Losses caused by leaf-cutting ants (assuming no control measures are used) could exceed \$US 1 billion per year and this justifies their being described as a '**dominant herbivore**' (Boulogne *et al.*, 2014).

The combination of a top-of-the-range social insect with a top-of-the-range fungal plant-litter degrader seems to be the key to this success. The social insect has the organisational ability to collect food material from a wide radius around its nest; but the extremely versatile biodegradation capability of the fungus enables the insect to collect just about anything that's available.

The total number of species of trees per hectare in most plant communities increases from the poles to the equator. For example, coniferous forest in Northern Canada will have 1 to 5 species ha⁻¹, deciduous forest in North America, 10 to 30, but tropical rain forest in South America has 40 to 100 tree species ha⁻¹.

The tropical rain forest has enormous chemical and physical diversity in its plants and this presents a major problem to herbivores. Most plant eaters have a narrow diet tolerance because evolution has equipped them with only a limited range of digestive enzymes. Plant-eating insects usually only eat one plant. The leaf-cutting ants of the tropical rain forest have, on the other hand, a **very wide breadth of diet**. These ant colonies are able to harvest 50 to 80% of the plant species around their nests. This is almost entirely due to the broad range degradative abilities of the fungus they cultivate.

This is an **obligate symbiotic association** between ant and fungus. Ant faeces contain nitrogen sources (allantoin, allantoic acid, ammonia and over 20 amino acids), which supplement the compost and are used by the fungus. A proteolytic enzyme produced by the fungus is acquired, accumulated and transported by ants and deposited in faeces; it hydrolyses leaf proteins.

Cellulases produced by the fungus digest cellulose and the products are converted to the fungal carbohydrates that the ants harvest (like other animals, the ants can digest glycogen, but they cannot digest cellulose). So the fungus enables the ants to use plant materials as nutrients, **but** the ants provide the fungus with a far wider range of plant materials than any plant pathogenic fungus can attack. Some plants do protect themselves by producing deterrents which inhibit cutting, pick-up or feeding. These protectants include toughness, production of sticky latex, and a wide range of defensive chemicals.

There's a certain irony in the fact that the tropical rain forest is lush and green because all those mycorrhizal fungi in the roots of the trees give the plants that extra something that enables them to grow with tropical exuberance (see [Chapter 13](#)). And then along comes a six-legged army of harvesters to cut down all those lush green leaves. To do what? To feed another fungus, that's what!

Schultz & Brady (2008) analysed molecular phylogenies covering the entire fungus-growing ant tribe (Attini); their data are fossil-calibrated (so they have a reliable time-scale) and involve multiple-genes (so they are a reliable sample of the genomes). They showed that ant agriculture originated with the cultivation of several species of fungi over **50 million years ago in the early Eocene**, the second epoch of the Paleogene Period in the Cenozoic Era (Schultz & Brady, 2008) (see Fig. 2.6 for a geological timescale). Ješovnik *et al.* (2016) reported slightly older dates than these, so the best estimate is that about 55-65 million years ago ant agriculture, as practised by the fungus-farming 'attine' ants, **arose in the wet rainforests of South America**. In contrast, the transition from lower to higher agriculture (see bullet points below) is most likely to have occurred in a seasonally dry habitat, which would have been inhospitable to the independent free-living growth of the fungus cultivated by attine ants. In other words, dry habitats provided selection pressure favouring fungal cultivars specialised for growth in the ant gardens (Branstetter *et al.*, 2017).

Most of the fungi involved in ant-fungus associations (generally described as 'leucocoprineaceous fungi') belong to the tribe Leucocoprineae, which forms a large monophyletic clade in the Agaricales (Basidiomycota) made up mainly of the genera *Leucoagaricus* and *Leucocoprinus* (Mehdiabadi *et al.*, 2012). Approximately 60 million years ago the Attine ants split into Paleoattine and Neoattine sister clades and during the past 30 million years, three major ant agricultural systems have diverged from the original Paleoattine system, each involving distinct leucocoprineaceous fungal cultivars. Schultz & Brady (2008) categorised ant agriculture into five different types as follows:

- **Lower agriculture**, practiced by 76 species of ants in the majority of attine genera, including the most primitive, which cultivate a wide range of fungal species; these are the Paleoattine ants, and all the fungi involved in this category are still able to grow in nature independently of ant garden cultivation.

- **Coral fungus agriculture**, which evolved about 15 million years ago, is employed by a few ants in the ant genus *Apterostigma* that cultivate a clade of coral fungi (family Pterulaceae; which is a small, mainly tropical, family of wood and leaf-decomposing fungi) closely related to the fungal genera *Pterula* and *Deflexula* (Munkacsi *et al.*, 2004).
- **Yeast agriculture** evolved about 20 million years ago and is used by a few species in the ant genus *Cyphomyrmex* that cultivate a distinct clade of dimorphic leucocoprineaceous fungi that grow wild as mycelia but as yeast morphs when associated with ants, the gardens being clusters of small, irregularly shaped nodules about 0.5 mm diameter composed of single-celled yeasts (even though the yeast mode of growth is unusual in the Agaricales).
- **Generalised higher agriculture**, utilised by species in the genera of non-leaf-cutting ‘higher attine’ ants (*Sericomyrmex* and *Trachymyrmex*), which cultivate another distinct clade of leucocoprineaceous fungi so adapted to garden life that they are not found outside the ant nest (though they can fruit from nests reared in the laboratory).
- **Leaf-cutter agriculture**, evolved from within the higher agriculture ants between 8 and 10 million years ago, and is practiced by species of ecologically dominant ants in the genera *Atta* and *Acromyrmex* that cultivate a single highly derived species of fungus, which has been identified as *Leucoagaricus gongylophorus* (Pagnocca *et al.*, 2001).

An interesting feature of molecular studies is that the phylogenetic relationships of the attine ants are mirrored in the phylogenetic patterns among their fungal cultivars (they are said to be **congruent**) and the congruence might even extend to a fungal parasite of the gardens; Currie *et al.* (2003) illustrated the relationship as shown in Fig. 15.3. The evolution of mutualist partners can take two extremes (or something between):

- Co-evolution may be tightly integrated, that is, an evolutionary change in one partner results in a simultaneous evolutionary change in the other.
- At the other extreme, frequent switching between partners can weaken the associations between any given pair of partners, causing a diffuse association between species.

Extensive phylogenetic data indicate that these ant-fungus partnerships have been stable over evolutionary time spans of several millions of years. This historical pattern of long-term ant-fungus fidelity for several million years provides the opportunity for coevolution, in which evolutionary modification in an ant host can prompt modification in its fungal symbiont, and *vice versa*. Overall it seems that attine ants and their fungal partners represent the full spectrum from ‘one-to-one’ coevolution among the leaf-cutter agriculture group that exhibit coevolved modifications, to something more like diffuse (‘one-to-many’) coevolution in the (more ancient) lower agriculture groups; with a narrowly diffuse (‘one-to-few’) coevolution in the *Cyphomyrmex* ant clade (Mehdiabadi *et al.*, 2012).

As Fig. 15.3 indicates, the association may be even more complex than described so far. There is a common (fungal) parasite of the fungus garden, called *Escovopsis*, and this too has been claimed to show co-evolution with the ants and their cultivars of fungi in a tripartite evolutionary model (Currie *et al.*, 2003). However, analysis of a much greater range of specimens has found that gardens from different clades of ants can sometimes be infected by closely related strains of *Escovopsis*, suggesting that the alleged tripartite co-evolution may be spurious.

It seems that *Escovopsis* shows much greater diversity than previously thought and has a correspondingly more complex evolutionary history (Meirelles *et al.*, 2015). *Escovopsis* (Ascomycota: anamorphic Hypocreales) is a known parasite of free-living fungi, the ancestors of

which were probably associated accidentally with the ancient fungal cultivars. *Escovopsis* has evolved and has now emerged as a **specialised parasite of ant garden fungal cultivars**. Interestingly, *Escovopsis* is a parasite of **filamentous hyphae**; **it does not infect yeast gardens**. Avoidance of parasitism by *Escovopsis* may have been part of the selection pressure favouring yeast growth in the ant-yeast agriculture mutualisms.

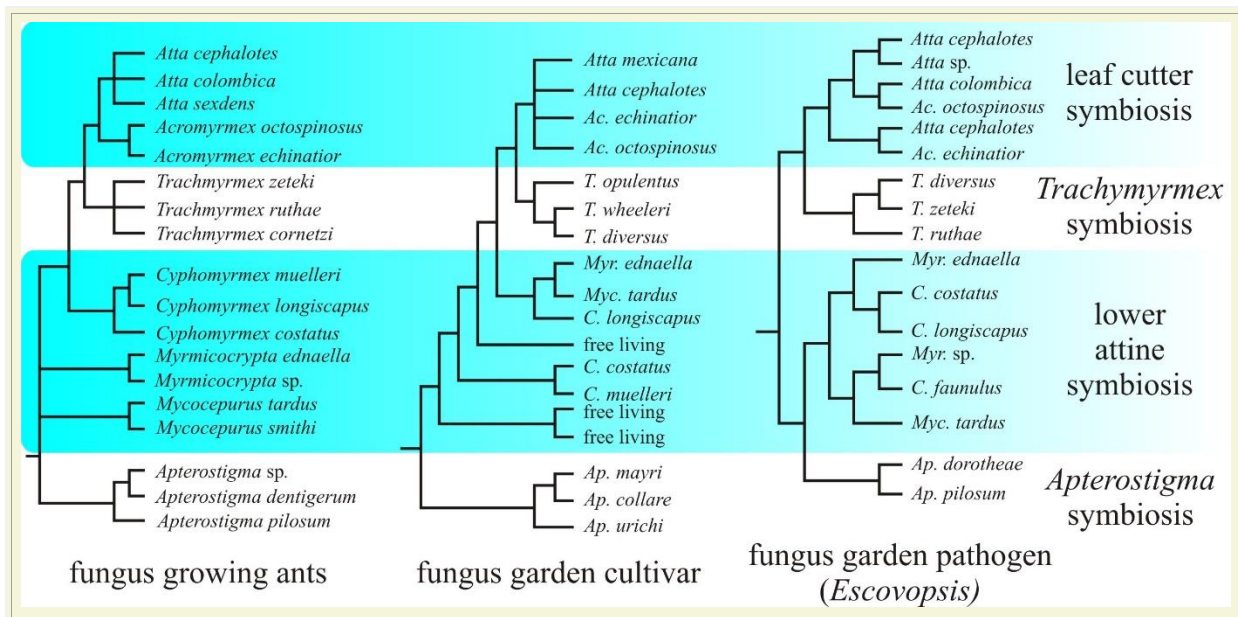


Fig. 15.3. Possible phylogenetic trees of attine ants and their associated fungi. This figure shows the phylogenetic reconstruction of the coevolution of fungus-growing ants (based on the genetic sequences of the ants), their garden fungal cultivars (based on the genetic sequences of the fungi isolated from the gardens), and the fungus garden pathogen *Escovopsis* (based on the genetic sequences of *Escovopsis* isolated from the gardens). The different fungus garden cultivar and *Escovopsis* strains in the cladograms are indicated by the name of the host ant species maintaining the garden from which they were isolated. The similarities in the cladograms of this illustration show the **apparent tripartite coevolution of the symbioses**, but this may oversimplify a complex *Escovopsis* population (see text).

The highly significant similarity displayed here is that all three phylogenies separate into four major lineages, representing four major evolutionary innovations: (i) the initial lower attine symbiosis between Leucocoprinaceous fungi and the most primitive attine ant species; (ii) the *Apterostigma* symbiosis, involving Tricholomataceous fungi and a clade of ants derived within the genus *Apterostigma*; (iii) the symbiosis, incorporating derived Leucocoprinaceous fungi and ants in the genus *Trachymyrmex*; and (iv) the leaf cutter symbiosis, involving highly derived Leucocoprinaceous fungi and the well-known leaf cutting ant species. Illustration redrawn and modified from Currie *et al.*, 2003.

Exact correspondence in branching patterns is not expected for a number of reasons. Overall it seems that attine ants and their fungal partners are sufficiently diverse to represent the full spectrum from ‘one-to-one’ coevolution among the leaf-cutter agriculture group that exhibit coevolved modifications, to something more like diffuse (‘one-to-many’) coevolution in the (more ancient) lower agriculture groups; with a narrowly diffuse (‘one-to-few’) coevolution in the *Cyphomyrmex* ant clade (Mehdiabadi *et al.*, 2012).

The interactions and dependencies go even further than described so far; because **yet another organism** is involved. The ants have developed a symbiotic relationship with a filamentous **actinomycete bacterium** of the genus *Streptomyces*. Areas of the cuticle of fungus-growing ants are coated with what appears to the naked eye to be a powdery, whitish-grey crust which is actually formed from masses of *Streptomyces*. Actinomycetes are common organisms, mostly soil-dwelling, that produce many secondary metabolites with antibacterial or antifungal properties (most of our own clinical antibiotics are actinomycete metabolites, and many are from the genus *Streptomyces*).

The *Streptomyces* associated with fungus-growing ants produces antibiotics that suppress growth of the parasite *Escovopsis*. This actinomycete is associated with all species of fungus-growing ants so far studied, being carried in genus-specific crypts and exocrine glands on the surface of the ant. The bacteria are transmitted vertically (from parent to offspring colonies), and the association of *Streptomyces* with attine ants is both highly evolved and of very ancient origin (Currie, 2001; Currie *et al.*, 2003, 2006; Mueller *et al.*, 2001). Effectively, attine ants use *multidrug therapy* to maintain their fungal cultivars (Barke *et al.*, 2011).

15.3 Termite gardeners of Africa

Leaf cutting ants cultivate their fungus in the Americas, but in the Old World, Africa and Asia, the insect partner which engages in a similar fungus-gardening relationship is a termite. Termites are responsible for the bulk of the **wood degradation** in the tropics. Most of them carry populations of microbes in their guts to digest the plant material and release its nutrients, but termites in the insect subfamily Macrotermitinae have evolved a different strategy. They eat the plant material to get what nutrition they can from it, and then use their faeces as a compost on which they cultivate a fungus. The fungus belongs to the **mushroom genus** *Termitomyces* (Basidiomycota: family Lyophyllaceae) including *Termitomyces titanicus* of West Africa, which produces some of the largest mushrooms you can find, being up to about a metre across the cap. In the termite nest, fungal enzymes digest the more resistant woody plant materials and the fungus becomes a food for the termites.

Termites maintain their fungal cultivar on special structures in the nest, called **fungus combs**, within specially constructed chambers, either inside a nest mound or dispersed in the soil. Workers feeding on dry plant material produce faecal pellets (primary faeces) which are added continuously to the top of the comb, providing fresh substrata into which the fungal mycelium rapidly grows. In a few weeks, the fungus produces vegetative ‘nodule’ structures, which are **aborted mushroom primordia**. These are cropped and consumed by the termite workers who later consume the entire fungus comb, both mycelium and spent compost.

Nests of fungus-growing termites can have volumes of thousands of litres, may persist for decades, and contain millions of sterile workers, which are normally the offspring of a single queen. Different termites produce mounds of different size and shape. Chimney-like termite mounds up to 9 m tall (thirty feet) are common in several parts of the bush in Africa. Inside, the mounds have many chambers and air shafts that ventilate both nest and fungus culture; perhaps the most complex colony and mound structures of any invertebrate group. Thus:

‘...termite mounds are metre-sized structures built by millimetre-sized insects. These structures provide climate-controlled microhabitats that buffer the organisms from strong environmental fluctuations and allow them to exchange energy, information, and matter with the outside world...’ (King *et al.*, 2015).

As we stated above, phylogenomics identifies independent origins of insect agriculture in the three clades of fungus-farming insects: the termites, ants or ambrosia beetles and dates all of them to the Paleogene Period in the Cenozoic Era (66 to 24 million years ago). Fossil fungus gardens, preserved within 25-million-year-old termite nests, have been found in the Rukwa Rift Basin of southwestern Tanzania, and confirm an African Paleogene origin for the termite-fungus symbiosis; perhaps coinciding with Rift initiation and consequential changes in the African landscape (Roberts *et al.*, 2016).

All termite larval stages and most adults eat the fungus. The termite queen, ‘king’ and soldiers are exceptions, being fed on salivary secretions by the workers (Aanen *et al.*, 2002, 2007). The two main symbioses of social insects with fungi, the agricultural symbioses of ants and termites

are similar in many respects, but they differ in others. Mutualism with fungi has allowed both ants and termites to occupy otherwise inaccessible habitats that have abundant resources: the attine ants are dominant herbivores of the New World tropics; fungus growing termites are major decomposers of the Old-World tropics. However, the fungal cultivars of attine ants rarely fruit and are normally propagated clonally and vertically by being carried by dispersing queens (see Section 15.2, above), whereas fungal symbionts of the Macrotermitinae often produce fruiting bodies. In the rainy season the termites may take portions of the culture out of the nest mound to fruit on the ground nearby and abandoned nest mounds also produce mushrooms after the termites have left.

These wild fruit bodies are inferred to be the source of fungal inoculum for new nests; that is, it is assumed that the fungal cultivar is generally a 'horizontal acquisition' because the termite fungal cultivars have a freely-recombining genetic population structure rather than being clonally-related. This implies that new termite colonies will usually start up without a fungus and then acquire the fungal symbiont through the occurrence of its basidiospores (produced by mushrooms growing from other nests) on the plant litter that the workers collect (Aanen *et al.*, 2002). There are, however, two examples in which the fungal cultivar *is* transmitted clonally between nest generations. In the termite species *Macrotermes bellicosus* (via the male termite sexuals) and all species in the genus *Microtermes* (via the female sexuals) the reproductives of one or the other sex ingest asexual spores of the fungus before the nuptial flight and use these as inoculum for the new fungus comb after foundation of their new nest colony (Aanen *et al.*, 2007).

Symbiotic relationships with a wide range of *intestinal microorganisms*, including protists, methanogenic archaea and bacteria, have played a major role in termite evolution. Plant biomass conversion is a multistage cooperation between *Termitomyces* and gut bacteria, with termite farmers mainly providing the gut compartments in which this fermentation can occur.

Termitomyces has ability to digest lignocellulose and gut microbes of worker termites primarily contribute enzymes for final digestion of oligosaccharides. Termite gut microbes are most important during the second passage of comb material through the termite gut, after a first gut passage where the crude plant substrate is inoculated with *Termitomyces* asexual spores so that initial fungal growth and lignocellulose decomposition can proceed with high efficiency. All termites rely on gut symbionts to decompose organic matter but the single subfamily Macrotermitinae evolved a mutualistic ectosymbiosis with *Termitomyces* fungi to digest lignocellulose of woody substrates (Varma *et al.*, 1994; Bignell, 2000; Poulsen *et al.*, 2014).

The Macrotermitinae comprises 11 genera and 330 species; 10 of the 11 genera are found in Africa, 5 genera occur in Asia (one of these exclusively) and 2 genera occur in Madagascar. Approximately 40 species of the *Termitomyces* symbiont have been described. Molecular phylogenetic analyses of termites and their associated fungi show that the symbiosis had a single origin in Africa. These data are also consistent with horizontal transmission of fungal symbionts in both the ancestral state of the mutualism and most of the extant taxa. Clonal vertical transmission of fungi in *Microtermes* and *Macrotermes bellicosus* (mentioned above) had two independent origins. Despite these features there was a significant congruence between the termite and fungal phylogenies, probably because mutualistic interactions show high specificity; meaning that **different genera of termites tend to rear different clades of *Termitomyces*** (Aanen *et al.*, 2002, 2007).

Fungus-growing termites are **pests because they attack wooden structures**; by eating through the wood they leave a maze of galleries that destroy the strength of the timber. Of the more than 2300 species of termite in the world, 183 species are known to damage buildings. Termite damage and control costs are estimated at US\$5 billion annually in the United States alone (Varma *et al.*, 1994; Su & Scheffrahn, 1998); and see the following URL:

[<https://www.americanpest.net/blog/post/the-real-facts-about-termite-damage>].

Insecticides and fungicides can help to control this pest. The chitin synthesis inhibitor, **hexaflumuron** (1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy) phenyl]-3-(2,6-difluorobenzoyl) urea), has proved particularly effective as a slow-acting bait. Termite colonies can be eliminated using less than 1 g of hexaflumuron, which is described in pesticide listings as a systemic (stomach-acting) insecticide, but it will also target chitin synthesis in the fungus and poison the nest that way as well (Su & Scheffrahn, 1998). Hexaflumuron is registered with the United States Environmental Protection Agency as a **reduced-risk pesticide** (one believed to pose less risk to human health and the environment than existing alternatives).

15.4 Agriculture in beetles

The final example of an intimate interdependent association between an insect and a fungus concerns wood-boring beetles in the weevil subfamilies Scolytinae and Platypodinae, which are unusual in that the adults burrow into the trunks of living trees which have been under some sort of stress (drought, air pollution, etc.) for feeding and egg-laying; they may also be found in trees that have been recently cut or blown down. Rather than create fungal gardens underground like ants and termites, these female beetles dig tunnels and galleries into wood (Fig. 4), laying eggs on the tunnel wall and inoculating the wood with fungal material carried from a previous nest. They bring the fungi to their new host tree in one of several glandular small pits on the surface of their body, called **mycangia** or **mycetangia**.

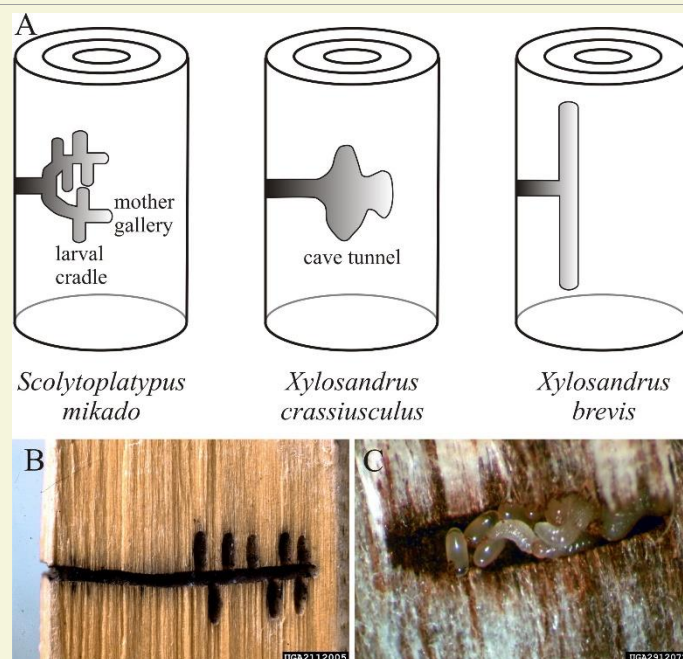


Fig. 15.4. Ambrosia beetles are pests which damage timber. **A**, across the top, shows a set of diagrams of gallery structures of typical ambrosia beetles, with the names of beetles forming that type of gallery indicated below the diagrams. **B**, galleries of the striped ambrosia beetle (*Trypodendron lineatum*) in timber of Norway spruce (*Picea abies*) (photograph is image number 2112005 by Petr Kapitola, State Phytosanitary Administration, Department of Pest Risk Analyses, Czechia (<http://www.forestryimages.org/browse/autimages.cfm?aut=11449>)). **C**, eggs and larvae of the granulate ambrosia beetle (*Xylosandrus crassiusculus*) (image number 2912072 by Will Hudson, Department of Entomology, University of Georgia, USA (<http://www.forestryimages.org/browse/autimages.cfm?aut=153>)). Photographs B and C from Forestry Images (<https://www.forestryimages.org/>), a joint project of The Bugwood Network and USDA Forest Service; reproduced with permission.

Ambrosia beetles are completely dependent on their fungal symbionts and have deep and complicated pouch-like mycangia. These specialised structures on the body of the animal are adapted for the transport of symbiotic fungal spores and mycelia in pure, often yeast-like, cultures, secreting substances to support fungal spores and perhaps to nourish mycelium during transport. Once introduced, the fungi grow in the wood as mycelia and these help the beetle to evade tree defences as well as serving directly as food (Farrell *et al.*, 2001). They are found in many xylophagous ('wood-eating') insects, although, despite this name, the beetles derive most of their nutrition from digestion of the fungi growing on the wood (Filipiak, 2018). In some cases, as in ambrosia beetles, the fungi are the sole food, and the excavations in the wood are only to create suitable microenvironments for the fungus to grow. In other cases (for example, the pine beetle, *Dendroctonus frontalis*) the fungus is carried by mites that ride on the beetles.

By the time the eggs hatch, the fungus will have grown over the tunnel walls, using its enzymes to digest constituents of the wood. This fungus 'lawn' (called, rather fancifully, '**ambrosia**') provides the developing young larvae with a **readily digested food**. Ambrosia beetle larvae do little tunnelling; instead they feed together in chambers on asexually produced fungal conidia induced in cultures kept pure by the parents. Eventually, the larvae pupate and subsequently emerge as adults with a supply of fungus in their mycangia. Because they are 'gratuitous' food for the larvae, the fungi have become known as ambrosia fungi and the insects as ambrosia beetles.

Ambrosia fungi are **derived from plant pathogens** in the group known as ophiostomatoid fungi (that is, they are related to, or belong to, the genus *Ophiostoma* (order Ophiostomatales, subclass Sordariomycetidae, and phylum Ascomycota); the fungal cultivars are now classified to the genera *Ambrosiella* and *Raffaelea*. Other beetles in this group are known as **bark beetles** and are associated with free-living, pathogenic ophiostomatoid fungi that aid beetle attack of phloem of trees with resinous defences. In these cases the beetle is acting as a vector for the disease fungus; we have already described one such association in our discussion of Dutch Elm Disease (see the section entitled *Ophiostoma (Ceratocystis) novo-ulmi (Dutch Elm disease or DED)* (Ascomycota) in Chapter 14; [CLICK HERE](#) to view the page).

Ambrosia beetles total about 3400 species, and many have been found in 30 million-year-old amber, suggesting that the origins of this association occurred in the Tertiary, up to 60 million years ago, roughly contemporaneous with the origin of the attine ant symbiosis. However, in contrast to the single instance of origin of the attine ant symbiosis, there are at least **seven independent instances** of the evolution of the ambrosia fungus gardening association. Adoption of the fungus-gardening habit allows ambrosia beetles to adopt a 'generalist strategy' because the digestive abilities of the fungus widen the range of tree types they can use, wood boring by adults transports the fungi deep within trees, and fungal gardening by the beetle enhances their joint ability to use the biomass-rich resource that the forest trees represent (Farrell *et al.*, 2001).

Ophiostomatoid fungi emerged about 200 million years ago, soon after the appearance of conifers, which are the principal hosts of pathogenic *Ophiostoma*. This considerably predates the earliest possible origin of the ambrosia beetle-fungus association, 50 million years ago.

Raffaelea and *Ambrosiella*, the two genera of ambrosia fungi, are both polyphyletic and each arose at least five times from the ophiostomatoid clade that includes the obligate pathogens *Ophiostoma* and *Ceratocystis*. Phylogenetic analysis estimated the oldest origin of fungus farming near to 50 million years ago, long after the origin of the Scolytinae subfamily (100-120 million years ago). Younger origins were dated to 21 million years ago. ***Origins of fungus farming corresponded mainly with two periods of global warming in the Cenozoic era***, which were characterised by broadly distributed tropical forests. Hence, it seems likely that warm

climates and expanding tropical angiosperm forests played critical roles in the successful radiation of different fungus farming groups (Jordal & Cognato, 2012).

Today, invasions of non-native bark and ambrosia beetles are a threat to forests worldwide, and the climatic impact implied by the evolutionary story told in the previous paragraph seems likely to be played out again. Study of invasion patterns in the USA reveal differences between bark beetles and ambrosia beetles depending on their differing ecology. Bark beetles are less dependent on climate, which allows them to colonise more areas within the USA, while non-native ambrosia beetles, are dependent on higher rainfall and warmer temperatures (Rassati *et al.*, 2016). It is the sudden appearance of pathogenicity in insect-fungus symbioses that is the ***new and currently uncontrollable threat to forest ecosystems***, as well as fruit and timber industries, around the globe. Increasingly, perhaps triggered by climate change, some invasive bark and ambrosia beetle/fungus symbioses are shifting from non-pathogenic saprotrophy in their native ranges to a prolific tree-killing in invaded ranges; new and significant diseases are emerging in the world's forests (Hulcr & Dunn, 2011; Keskitalo *et al.*, 2018; Pasanen *et al.*, 2018).

15.5 Anaerobic fungi and the rise of the ruminants

We point out above Schultz & Brady's (2008) statement that agriculture is a specialised symbiosis that has evolved in the four animal groups: ants, termites, bark beetles, and humans. So far, we have dealt with ants, termites and bark beetles, and though we do not intend to deal at any length with human agriculture most of the rest of our topics do have some relevance to farms and agriculture. In this section we will expand on the symbiotic **association between chytrid fungi and ruminant mammals**.

This is crucial to human agriculture because the diet of grazing farm animals, consisting predominantly of plant structural carbohydrates such as lignocellulose, cellulose and hemicellulose, can only be digested because the animals have evolved to rely on symbiotic microorganisms in their alimentary tracts to hydrolyse these compounds under the anaerobic conditions that prevail in the gastrointestinal tract.

Ruminants are well adapted to achieve maximum digestion of the otherwise indigestible fibrous components of plant foods within the rumen. The most characteristic behaviour pattern of all ruminants is the regurgitation, rechewing and reswallowing of partially fermented food from the foregut, which is termed **rumination**. Foregut fermenting mammals also produce two enzymes, stomach lysozyme and pancreatic ribonuclease which accompany and are adaptations to this mode of digestion. The microbial community of the ruminant gastrointestinal tract contains the full range of microbes: bacteria and bacteriophage, archaea, ciliate protozoa, and anaerobic fungi; and all these at characteristically high species diversity and population densities, and exhibiting complex interactions (Mackie, 2002; Kumar *et al.*, 2015).

We will concentrate on just one component of this complex community: the anaerobic chytrid fungi. These fungi are not exclusively found in the rumen of the animals, but throughout the entire digestive tract. Further, anaerobic fungi have also been recovered from the faeces of ruminants, suggesting that the fungi have the ability to enter a resistant stage enabling them to survive desiccation and the oxygenated atmosphere.

Through to the middle of the twentieth century it was commonly assumed that all fungi required oxygen to survive, which led to the view that the microbial population of the rumen consisted primarily of anaerobic bacteria and flagellate protozoa. This view held until 1975, when the rumen 'flagellate', *Neocallimastix frontalis* was properly described as a ***Chytridiomycete fungus*** even though it was ***a strict anaerobe*** (Orpin, 1975; Gruninger *et al.*, 2014).

Chytrids are an ancient group of true fungi (see [Section 3.2](#)). They usually produce uniflagellate zoospores, but some of the obligately anaerobic chytrids produce bi- or multiflagellated zoospores. All chytrid flagella are of the whiplash type, lacking hairs or scales, and are **located posteriorly on the zoospore**. Chytrids are usually described as aquatic organisms, but in fact they are equally abundant in terrestrial environments and have even been isolated from sand from arid canyons in Arizona, and from the permafrost in the Arctic. Chytrids are saprotrophs or parasites and their real importance lies in their ecological role as decomposers able to digest complex polymers such as cellulose, hemicellulose, chitin, and keratin, as well as some of the most recalcitrant materials in the biological world such as lignin and sporopollenin (a complex, highly cross-linked polymer found in the outer wall layers of pollen grains and some fungal spores). As parasites, they exist on/in a wide range of hosts including algae, other fungi, plants, mosses, insects and invertebrates; the first chytrid parasite of a vertebrate, *Batrachochytrium dendrobatidis*, has been found parasitising and killing amphibians ([Section 16.7](#)). Chytrids are found throughout the world, the majority (80%) in temperate regions of the world, although this relative abundance of chytrids is most likely due to biased collection from these regions; that is chytrids have been under-collected in tropical and polar regions (Shearer *et al.*, 2007; Fliegerova *et al.*, 2015).

Although they are morphologically similar to other chytrids, differences are sufficient for the anaerobic chytrids to be placed in their own phylum, which is called **Neocallimastigomycota** (see the section entitled *Neocallimastigomycota* in [Section 3.3](#) and the photographs of *Neocallimastix* in [Section 3.2](#); [CLICK HERE](#) to view this page). They appear to be among the most primitive of all the fungal phyla; the Neocallimastigomycota being the earliest diverging lineage as a sister group to the rest of the Chytridiomycota. Evidently, this fungal lineage originated long, long before its current hosts, ruminant herbivores, appeared on the geological scene.

Six genera have been described among rumen fungi, on the basis of number of flagella and sporangial characters, and all are placed in the Order Neocallimastigales. No sexual stage is known. Anaerobic chytrids may be monocentric (having one centre of growth, producing either a single or several sporangia) or polycentric (having several centres of growth), and the sporangia may have filamentous or bulbous rhizoids and produce multiflagellate or uniflagellate zoospores. The six genera described are: *Neocallimastix*, *Piromyces*, *Caecomyces*, *Anaeromyces*, *Orpinomyces* and *Cyllamyces* (Ho & Barr, 1995, Ozkose *et al.*, 2001). *Neocallimastix frontalis* was the original isolate from the domestic cow and *Piromyces* spp. have been isolated from horses and elephants.

Rumen chytrids are the **primary invaders** of freshly ingested plant material in the rumen and, overall, the rumen chytrid biomass can amount to about 20% of the total rumen microbial biomass (Rezaeian, Beakes & Parker, 2004a & b). Zoospores alight on plant fragments and encyst, forming a thallus with a well-developed rhizoidal system that penetrates the plant material to extract energy by fermentation of its carbohydrate and other polymers within the animal's rumen and intestine. The nucleus of the zoospore is retained in the cyst as it develops into a sporangium, which is cut off from the anucleate rhizomycelium by a septum. Protoplasm in the sporangium is cleaved into uninucleate zoospore initials; eventually, zoospores (with up to 16 flagella per zoospore in *Neocallimastix frontalis*) are formed in the sporangium and eventually released from the apex of the sporangium into the surrounding fluid. *N. frontalis* is obligately anaerobic and grows on fragments of grass in the rumen of cattle, sheep (see illustrations in Rezaeian *et al.*, 2004a & b) and other herbivorous animals including water buffalo, goat and deer.

When grown in culture *N. frontalis* forms an extensive **rhizomycelium**. The fungal mode of growth is what makes the role of the chytrids so crucial. The filamentous rhizoids extend into the

plant material secreting the array of enzymes needed to degrade cellulose (animals do not produce their own cellulose-degrading enzymes) and other polymers of the fragments of plant material that make up the herbivore's food.

The rumen is a dynamic habitat, nutrient rich and oxygen poor. The pH is continuously modified by the host's diet, the metabolic activity of the resident microorganisms and by the tissues of the host. Anaerobic fungi are deficient in mitochondria, and so unable to produce energy by aerobic respiration. Instead, they possess hydrogenosomes that allow a mixed acid fermentation of carbohydrate to be carried out. As a result of mixed acid fermentation hexose and pentose sugars are converted to formate, acetate, lactate, and ethanol which the organelle converts to **energy** in the form of ATP, CO₂ and **hydrogen** by producing pyruvate oxidoreductase and hydrogenase.

Subsequently, **methanogenic bacteria** convert the excess H₂ into methane, which is expelled from both ends of the animal. The fungi also produce a wide range of digestive enzymes, giving them broad substrate specificity, and enabling the fungi to transform the core structural polymers of plant cell walls into a variety of simple oligosaccharides, disaccharides, monosaccharides, amino acids, fatty acids, etc., which enter mixed acid fermentation to create energy resources, and other aspects of anabolism that contribute to cell growth, reproduction and population growth. Microbial growth is eventually passed onto the host's stomach where digestion by the animal makes it available as a source of nutrients and energy (Trinci *et al.*, 1994; van der Giezen, 2002; Puniya *et al.*, 2015).

The microorganisms within the rumen form both co-operative and competitive interactions, producing a **complex ecosystem**. Some of the interactions are purely competitive. Ciliate protozoa are a major component of the rumen microflora. Ciliates ingest fungal zoospores as well as bacteria, and their predation of the fungal population can reduce overall cellulolytic activity. Bacteria like *Ruminococcus albus* reduce the ability of *Neocallimastix frontalis* to digest the xylan of barley straw, maize stem and wheat straw, compared to fungal monocultures. The bacteria secrete extracellular factors that inhibit fungal xylanases and cellulases. Some species of fungi produce inhibitors effective against bacteria. These characteristics seem to be simply an expression of the competition between the organisms. However, the overall degradation of plant material is greater when the fungi and bacteria are working together than when they are working individually.

Some of the interactions extend the mutualisms; for example the methanogenic bacteria are the primary hydrogenotrophs in the rumen ecosystem and their activity enables the chytrids to work more efficiently. Even low levels of free hydrogen inhibit the action of hydrogenase, yet this enzyme is crucial to fungal metabolism, so accumulation of hydrogen results in a decrease in carbon flow and an increase in inhibitory products such as ethanol and lactate. The methanogenic bacteria use the hydrogen in the rumen, releasing the hydrogenase enzyme from inhibition. The result of methanogenic bacterial activity is an increase in carbon flow through the chytrids, and, incidentally, increased production of H₂.

Consequently, the **methanogenic bacteria and fungi are synergistic**; together they carry out a more efficient fermentation process, consequently releasing a higher biomass yield from the food, generating a larger microbial community, and greater benefit to the host. So, we now have a **tripartite mutualism**: mammal-chytrid-methanogen. If we add the farmer who manages the pastureland for his cattle, the butcher who prepares the meat, the restaurateur who turns the meat into a meal, and the diner who eats that meal, the range of mutual dependencies increase even further.

A newly born ruminant does not possess this microbiota; instead it must acquire the anaerobic fungi, bacteria and protozoa that would normally inhabit a mature animal. This colonisation is achieved rapidly, before the rumen becomes functional. It is brought about by accidental

exposure to faeces in its pasture that contain resistant stages of the chytrids; also, fungi are present throughout the alimentary canal of ruminants, including the mouth and throat, suggesting that saliva is a likely vehicle for inoculation through licking and grooming of the infants by their mother, and interactions with other juveniles. Air samples have also been found to contain several species of anaerobic fungi, suggesting the possibility that aerosols act as another route for transmission (especially likely in large herds or flocks of individuals such as occur in intensive farming).

Additional Resources

A page of *Additional notes about the metabolism of anaerobic chytrids* is attached as an appendix to this PDF

High-efficiency fermentation is achieved by larger mammals in two different ways.

- The first, termed **hindgut fermentation** occurs in non-ruminant herbivores, which possess an enlarged area of the hindgut, usually the caecum, where fermentation takes place long after the initial gastric digestion in the stomach.
- The second, referred to as **foregut fermentation** applies in ruminants; these large animals provide accommodation for their microbial partners in a stomach which is modified into four chambers. The three forestomachs, sometimes considered to be elaborations of the oesophagus though other authorities consider them derivatives of the stomach comprise: the rumen (by far the largest), reticulum and omasum. The true stomach or abomasum then follows; this is the only site in the digestive tract that produces acid and digestive enzymes (pepsin and rennin).

In the new-born calf, the abomasum makes up about 80% of the total stomach volume, while in the mature cow it amounts to only 10%. During the first weeks of life, when the animals are still suckling milk, the rumen is not functional: the suckled milk does not pass through it due to closure of the oesophageal groove by reflex action. Its relative proportions are considerably smaller than in the adult and some of its rumen wall villi, which serve for absorption of nutritional components, have not yet developed. Changes in the structural and physiological properties of the rumen with age are associated with development of the rumen microorganisms, as their fermentation products are important for the development of the wall villi. The rumen of new-born animals is rapidly colonised by aerobic and facultatively anaerobic microbial taxa close to birth, which are gradually replaced by exclusively anaerobic microbes (Jami *et al.*, 2013). In the mature dairy cow, total volume of the stomach is about 130 litres (human monogastric stomachs generally have a volume of about one litre) and in the cow these organs, collectively, occupy almost 75% of the abdominal cavity.

Taxonomically, a ruminant is a mammal of the order **Artiodactyla** (even-toed ungulates); the anatomical features just described are exemplified by cattle, sheep, goats, giraffes, bison, yaks, water buffalo, deer, wildebeest, and various antelopes. All of these are placed in the suborder **Ruminantia**. Other animals, also generally called ruminants, have slightly different forestomach anatomy; camels, llamas, alpacas, vicunas have a reduced omasum and are occasionally referred to as pseudoruminants or as having ‘three stomachs’ rather than four. These are placed in the suborder **Tylopoda**. This drastic adaptation of the alimentary canal is the ‘evolutionary investment’ that the animals have made in this mutualism. It provides the microbes with a steady supply of freshly-cropped plant material and a warm safe habitat in which they can digest the supplied food matter; in return the animal gets a high-efficiency plant cell wall digester.

Piromyces and *Caecomyces* have been isolated from the horse and donkey (both of which are in the genus *Equus* in the order **Perissodactyla** or odd-toed ungulates), and Indian elephant (order

Proboscidea). These animals are examples of the non-ruminant herbivores in which **hindgut fermentation** occurs. Hindgut fermentation provides the host with sources of energy and a range of nutrients that the microbes extract and make available from the digested plant materials, but because it takes place downstream of the stomach the lack of subsequent digestive processes means that the benefit is limited.

Hindgut fermentation is effectively a ‘downstream recovery’ process; it offers an evolutionary advantage to the animal by scavenging some of the nutritional value from the food that would otherwise be lost. But it is a relatively low-efficiency system: for example, elephants spend 16 hours a day collecting plants for food (about half is grasses, and they browse for other leaves, shoots, roots, fruits, etc.), but 60% of that food leaves the elephant’s body undigested. In comparison, the ruminant strategy offers a high efficiency nutrient extraction process since fermentation occurs for an extended period of time because of rumination, and because the products of fermentation enter the stomach where the digestive fluids of the host animal can digest both fermented plant materials **and** the extremely large populations of microbes.

The symbiotic relationship between Artiodactyla and chytrids enabled the animals to incorporate difficult-to-digest grasses into their diet, and the efficiency of ruminant digestion together with expansion of the grasslands gave the Artiodactyls the opportunity to become the **dominant terrestrial herbivores** throughout the world in the most recent epochs. However, the story starts long before the evolution of grasses. It is interesting that in fungal phylogenies the Neocallimastigomycota emerge as the earliest diverging lineage of the chytrid fungi (James *et al.*, 2006). We take this to mean that these fungi have existed on Earth, presumably as saprotrophs in anaerobic niches like muds and stagnant pools, since before herbivorous animals of any sort emerged.

Fossilised flagellated fungi have been reported from the Precambrian, but the identification of the material is disputed. On the other hand, chytrids are ‘...probably the most common microbial element...’ (Taylor *et al.*, 2004) in the Devonian Rhynie Chert, which is 400 million years old. Several arthropod groups, including mites and collembolans are also well represented in the Rhynie Chert even though it is best known for its plant communities. Consequently, this excellently preserved fossil record demonstrates that the chytrids and other fungal classes, and their associations with plants and microarthropods of the day were well established by about the middle of the Paleozoic era ([Fig. 2.7 in Chapter 2](#)) (Taylor *et al.*, 2015; Edwards *et al.*, 2018; Krings *et al.*, 2018).

From that time onwards the fungi were abundant, so any browsing animal that feasted on the community of plants represented in the Rhynie Chert would have got a mouthful of saprotrophic microfungi along with their salad. The first mammalian herbivores were most probably fruit and seed eaters (**frugivores**) because the starch, protein and fats stored in fruits and seeds can be more easily digested than the plant fibres in foliage. It is argued that the evolution of large size was a prerequisite for the exploitation of leaves because of the need for a long residence time in the gut for fermentation to extract sufficient nutrients from foliage and herbage (Mackie, 2002). That includes the dinosaur megaherbivores (Sauropods), the dominant herbivores throughout the Jurassic, which could only browse on pre-angiosperm plants such as gymnosperms, ferns and fern allies for food (Hummel *et al.*, 2008).

It is also argued that the evolution of herbivores (and the microbiota of their guts that digested the plant food) drove the evolution of plant defences against herbivores through the animal’s feeding choices (Poelman & Kessler 2016). True grazing animals appeared much later in the Miocene (around 20 million years ago; [Fig. 2.7 in Chapter 2](#)) with the radiation of grassland-forming grasses of the plant family Poaceae (but see below). Plant-eating mammals during the late Cretaceous and early Palaeocene (say, 80 million years ago) were physically small frugivores; mammals did not become **herbivores** until the Middle Palaeocene (60 million years

ago). Herbivore browsers first appeared in the Middle Palaeocene, but they did not become major components of the fauna until the late Eocene (40 million years ago; [Fig. 2.7 in Chapter 2](#)).

It is envisaged that the earliest herbivores were large, ground dwelling mammals, reaching their dietary specialisation by evolution from large, ground dwelling frugivores or by a major size increase from small insectivorous ancestors (Mackie, 2002). It is also argued that hindgut fermentation must have developed first, with foregut fermentation emerging after this initial adaptation of the hindgut, and this seems to reflect the evolutionary appearance of Perissodactyls first, followed by Artiodactyls.

Grass-dominated ecosystems, including steppes, temperate grasslands, and tropical–subtropical savannas, play a central role in the modern world, occupying about 25% of the Earth’s land surface; these ecosystems evolved during the Cenozoic. Grasses are thought to have initially evolved 60 million years ago; the first to appear used the C₃ photosynthetic pathway, but the grasses that dominate the semi-arid savanna are the C₄ grasses (Strömberg, 2011; Oliveras & Malhi, 2016).

C₃ photosynthesis is the typical photosynthetic pathway that most plants use; C₄ plants can photosynthesise more efficiently in the higher temperatures and sunlight encountered by savanna grasses because they use water more effectively and have biochemical and anatomical adaptations to reduce photorespiration. There is a good argument for the evolution of the Artiodactyls being driven by the development and expansion of savanna and steppe grasslands in Africa and Eurasia (Cerling, 1992; Bobe & Behrensmeyer, 2004).

The appearance of grasslands in Africa and Eurasia during the Eocene epoch, and subsequent spread during the Miocene saw the Artiodactyls begin to dominate over the Perissodactyls. A credible hypothesis for the **evolution of rumination** in artiodactyls is that it represented a joint adaptation to increasing aridity of the local environment due to climatic cooling and drying.

The Eocene climate was humid and tropical and is likely to have favoured browsers and frugivores (and hindgut fermentation). With the onset of the Oligocene, the climate became generally cooler and drier, and this trend persisted throughout the Tertiary. This increasing aridity, coupled with high sunlight exposure in the equatorial zone, would have favoured the C₄ grasses, and as they became the dominant vegetation the emphasis in herbivore evolution would be to increase the efficiency of the fermentation of the more fibrous plant material. Selection pressure, in other words, against hindgut fermentation and in favour of foregut fermentation and rumination (Mackie, 2002; Bobe & Behrensmeyer, 2004).

The expansion of grassland at the expense of Miocene forests created conditions favourable for the evolution of Artiodactyla that could survive aridity and exploit grassland vegetation; changes in the environment drive major evolutionary events, and in this case major changes in bovid abundance and diversity were caused by dramatic **climatic changes** affecting the entire ecosystem (Bobe, 2006; Bobe & Eck, 2001; Franz-Odenaal, Lee-Thorp & Chinsamy, 2002). The artiodactyls became the most abundant and successful order of current and fossil herbivores, with about 190 species living today.

An added twist to the story is that the emergence of the genus *Homo* in the Pliocene of East Africa also appears to be broadly correlated in time with the advent of these same climatic changes and the introduction of the ecosystems they brought about (Bobe & Behrensmeyer, 2004). Grasslands currently represent 25% of the vegetation cover of planet Earth; and this family of plants (Poaceae) is the most important of all plant families to human economy as it includes our staple food cereal grains.

- The grasses owe their success to the environmental pressure to which plants responded during the evolution of the savanna grasslands of East Africa.

- The artiodactyls owe their success wholly to their symbiotic relationship with the rumen chytrids.
- Humans found their staple cereal foods among the Poaceae and their main food animals among the ruminants.
- **And is it too much to add the claim that it was all made possible by the fungi?**

15.6 Nematode-trapping fungi

Fungi are pathogenic, parasitic or symbiotic with a range of different animals, but their relationship with soil nematodes goes a step beyond parasitism and into **predation**. There are about 700 species of taxonomically diverse fungi that are able to attack living nematodes (eelworms), which are active animals about 0.1 to 1.0 mm long.

Among these nematophagous fungi, only a few species are obligate parasites of nematodes; the majority are facultative saprotrophs. *Nematophagous fungi* fall into four general groups:

1. fungi that use specialised structures that trap and then invade eelworms;
2. fungi that invade eelworms after first immobilising them with toxins;
3. obligate endoparasitic fungi that invade eelworms following spore germination;
4. opportunistic saprotrophic fungi that colonise nematode eggs, females, or cysts.

Nematophagous fungi are natural enemies of nematodes in soil ecosystems and have potential as biocontrol agents against plant- and animal-parasitic nematodes (Jiang *et al.*, 2017).

Over 200 species of fungi (zygomycetes, Basidiomycota, and Ascomycota) catch free-living nematodes in the soil **using traps produced by the fungal mycelium** that adhere to the worm, then penetrate, kill, and digest the tissue of the nematode. The most widespread **predatory fungi** are in the family of Orbiliaceae (Ascomycota). Five kinds of trapping device have been recognised (Figs 15.5 and 15.6); the first four of those listed below capture nematodes using an adhesive layer covering part or the entire surface of the hyphal structure (Yang *et al.* 2007; Su *et al.*, 2017):

- **Adhesive network (AN)**, the most widely distributed trap, is formed by lateral branches from a vegetative hypha, looping around to fuse with the parent hypha, developing a network of loops with an aperture diameter of about 20 μm (Fig. 15.5A); this is a three-dimensional network (see Fig. 15.6A) which entangles the prey (Fig. 15.6B) and can be formed by germinating conidia (Fig. 15.6C).
- **Adhesive knob (AK)** is a morphologically distinct inflated cell that is either a short ('sessile') or long ('stalked') hyphal branch, which are usually closely spaced along the hypha (Fig. 15.5B).
- **Nonconstricting rings (NCR)** always occur alongside AK and are produced when lateral branches from a vegetative hypha loop and inflate, forming a three-celled ring on a supporting stalk (Fig. 15.5B).
- **Adhesive column (AC)** is a short erect hyphal branch consisting of a few swollen cells (Fig. 15.5C).
- **Constricting ring (CR)**, is also a looped hyphal branch of (usually) three cells, but it is the most sophisticated trapping device (Fig. 15.5D) and captures prey actively. When a nematode enters a constricting ring the three ring cells are triggered to swell inwards within 1 to 2 seconds and firmly lasso the victim; the cells inflate to maximum size, which is an approximate threefold increase in cell volume, within 0.1 second, with the swelling of the ring cells being strictly inward (Fig. 15.7).

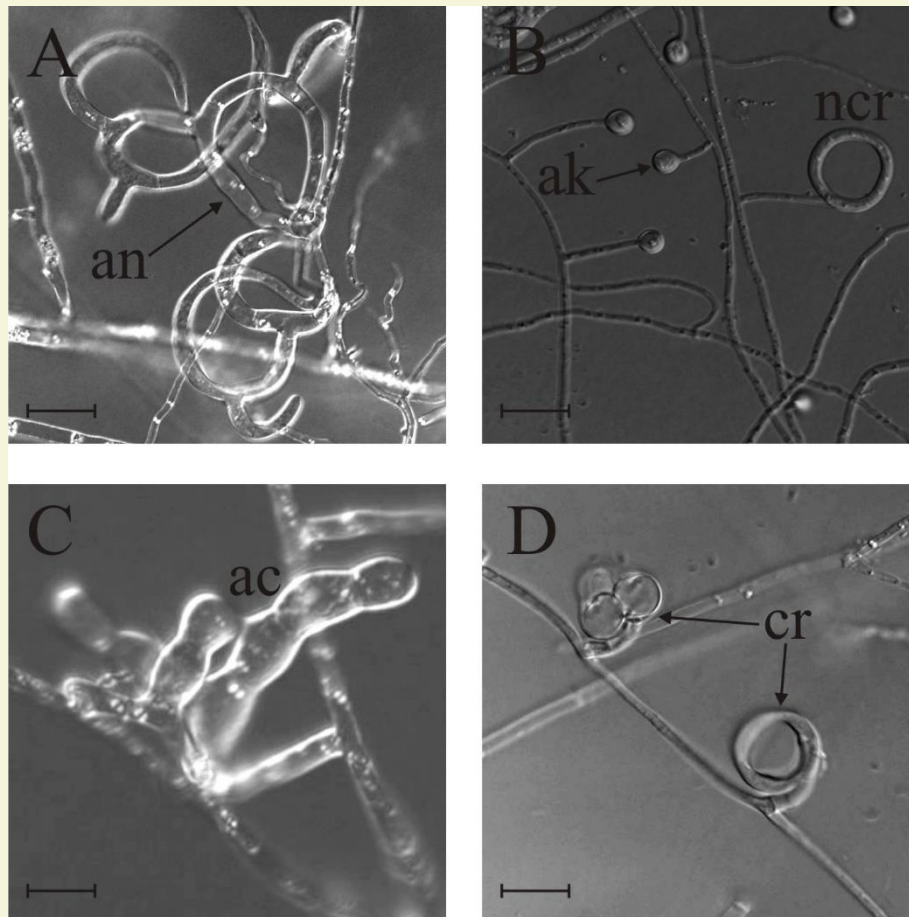


Fig. 15.5. Natural nematode-trapping devices. **A**, adhesive network (**an**), the most widely distributed trap. **B**, adhesive knob (**ak**) with nonconstricting rings **ncr**. **C**, adhesive column (**ac**) is a short erect branch consisting of a few swollen cells produced on a hypha. **D**, constricting ring (**cr**), the most sophisticated trapping device, captures prey actively; when a nematode enters a constricting ring, the three ring cells are triggered to swell rapidly inwards and firmly lasso the victim within 1 to 2 second. The ring at upper left in panel D has been triggered, that at bottom right is an unsprung trap. Scale bars = 10 μ m. Modified from Yang *et al.*, 2007 using images kindly supplied by Prof. Xingzhong Liu and Dr Ence Yang, Institute of Microbiology, Beijing, China. Original images © (2007) National Academy of Sciences, USA.

If you've not already done so, we recommend that you check out the *Life in the soil movies* showing soil nematodes and nematode-trapping fungi; hyperlinks are in the following [Resources Box](#).

Resources Box

Life in the soil

Thomas E. Loynachan, Emeritus Professor of Agronomy and Microbiology at Iowa State University, created a set of 16 short digital videos showing the scope of life in the soil. [CLICK HERE](#) to visit a page providing access to these.

Life in the soil on YouTube

Deep Down & Dirty: the Science of Soil. A close-up of creatures living beneath the soil, made by the British Broadcasting Corporation: <https://www.youtube.com/watch?v=gYXoXiQ3vC0>

The Living Soil Beneath Our Feet. Made by the California Academy of Sciences: <https://www.youtube.com/watch?v=MIREaT9hFCw>

Phylogenetic analysis suggests that the trapping structures fall into two lineages: one based on constricting rings and the other using adhesive traps. Only the traps, not the rest of the mycelium, have adhesive layers, and traps have thicker cell walls than vegetative hyphae and their cells contain electron dense bodies. There is a recognition event between lectins (sugar-binding proteins) in the cell wall of the fungus and one or more carbohydrates on the surface of the nematode; among which are the *ascarosides*. These are **lipophilic glycosides of the dideoxy sugar ascarylose**, which serve essential functions in regulating nematode development and behaviour, so they are highly specific to the desired prey of the fungus.

Nematophagous fungi, natural predators of soil-dwelling nematodes, can detect and respond to their prey's own ascaroside pheromones (Hsueh *et al.*, 2012). An interesting addendum to this story is that **some bacteria can mobilise nematode-trapping fungi to kill nematodes**. In their soil habitat, bacteria are consumed by bacterivorous nematodes; however, some of these bacteria release urea, which triggers a lifestyle switch in the fungus *Arthrobotrys oligospora* from saprotrophic to its nematode-predatory, nematode trapping, form; it seems to be ammonia that the fungus produces from the urea that functions as the signal to form traps. This bacterial defensive mechanism significantly promotes the elimination of nematodes by *A. oligospora* (Wang *et al.*, 2014).

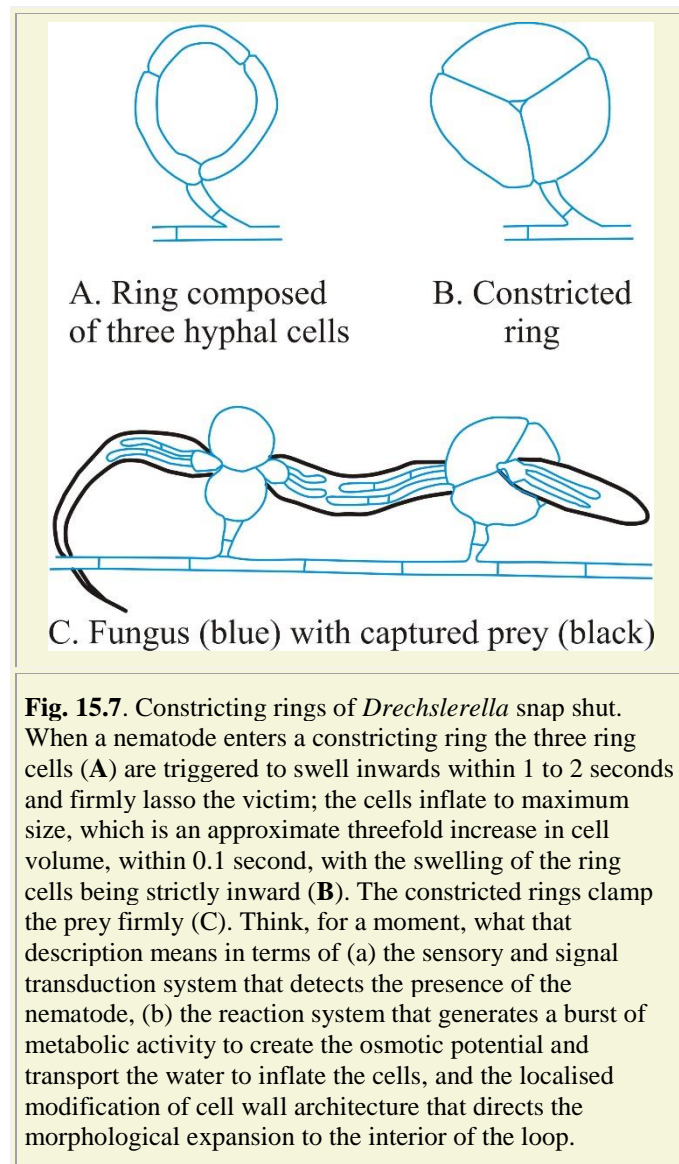


Fig. 15.6. Nematode-trapping devices. **A.** Scanning electron micrograph of typical adhesive network trap of *Arthrobotrys oligospora*, bar = 10 μ m. **B.** Light micrograph of a nematode captured in an adhesive network trap of *A. oligospora*, bar = 20 μ m. **C.** Light micrograph of conidial traps of *A. oligospora*, induced by the inclusion of peptides in the agar used to germinate the spores, bar = 20 μ m. From Nordbring-Hertz, 2004; reproduced with permission of Elsevier.

Recognition of the prey results in reorganisation of the **adhesive surface polymer** on the fungus and adhesion of the nematode to the fungus. It also triggers the growth of hyphal branches into the nematode to initiate its digestion. Interaction between predator (e.g. *Arthrobotrys oligospora*) and prey (nematode) shows no species specificity. **Hyphae penetrate the nematode within 1 hour of capture**. These hyphae **digest the nematode** (Li *et al.*, 2005; Yang *et al.* 2007; Su *et al.*, 2017).

The capture organs in some predatory nematode-destroying fungi are constitutive, others are inducible. Formation of traps by *Arthrobotrys oligospora* is induced by presence of nematodes or peptides secreted by them; in the absence of nematodes the fungus grows saprotrophically. It is thought that the nematode-trapping habit is a way that saprotrophic soil fungi compensate for the poor nitrogen content of their substrates. Trap formation and nematophagous activity of *Arthrobotrys oligospora* were observed *in vitro* only where conidia were inoculated on nutrient poor water agar, low-nitrogen medium or a medium containing no amino-acids or vitamins.

Trapping devices remain inducible after many years of culture as saprotrophs on artificial media, demonstrating that these highly differentiated trapping structures remain crucial to the survival and virulence of their producer.



Arthrobotrys is characterised by adhesive networks and unstalked adhesive knobs that grow out to form networks; *Dactylellina* by stalked adhesive knobs and non-constricting rings, and unstalked adhesive knobs that grow out to form loops adhesive knobs; and *Drechslerella* by constricting-rings.

Predatory fungi appear to have been **derived from nonpredatory members of the Orbiliaceae** (Ascomycota), although this is a lifestyle that has appeared several times and in different fungal phyla. For example, the **oyster mushroom** (*Pleurotus ostreatus*), which is in the Basidiomycota, is a **nematode-trapping fungus** that uses adhesive hyphal branches. We recently received this e-mail from Mr John L. Taylor of the North West Fungus Group:

‘While I have read about *Pleurotus* species being able to capture nematode worms, I imagined this skill to be limited to hyphae within the woody host material. However, I needed to check a mis-identified Oyster Mushroom, *P. ostreatus*, so sectioned across cap and 5 gills, finding 15 dead nematodes curled in 2 spaces in the cap tissue. One easily visible had a single cup-shaped hyphal attachment (said to present a nematode toxin), and hyphal growth branching into the nematode from the point of attachment, suggesting ingestion. While this mushroom was structurally sound, it

was visually past its edible condition, so those who eat fresh Oyster Mushroom should not be deterred!’

In the orbiliaceous fungi the adhesive knob is considered to be the **ancestral trapping device** from which constricting rings and networks were derived via two pathways:

- one in which adhesive knobs developed first into adhesive two-dimensional networks, then three-dimensional networks;
- a second pathway in which adhesive was lost and nonconstricting rings developed inflatable cells to form constricting rings (Li *et al.*, 2005; Yang *et al.* 2007; Su *et al.*, 2017).

Nematode-trapping fungi have been found fossilised in amber dated at 100 million years old. The fossil fungi used hyphal rings as trapping devices and are preserved together with their nematode prey. Evidently the predaceous habit of these fungi was well represented in the Cretaceous (the age of the dinosaurs) (Schmidt, Dorfelt & Perrichot, 2007).

Soil nematodes are very abundant in all soils (commonly millions per square metre) and species diverse (commonly more than 30 taxa). They feed on a wide range of soil organisms within the rhizosphere of agricultural crops and several eelworms are parasitic. Some are important pests of crop plants and farm animals and nematode-trapping fungi may have use in **biological control** (Yeates & Bongers, 1999; Moosavi & Zare, 2012; Moura & Franzener, 2017).

For example, the nematode-trapping fungus *Nematophthora gynophila* can be used to control the cereal nematode, *Heterodera avenae*, which feeds on the host roots damaging the roots and reducing water uptake. Nematodes that are gastrointestinal parasites of farm animals can be controlled with the nematode-trapping *Duddingtonia flagrans*. The resting spores (chlamydospores) can be included in animal feed and survive passage through the animal. Subsequently, the fungus grows in the animal dung where it traps and destroys the parasitic nematode, so reducing pasture infectivity and the worm burden of grazing animals, especially young cattle, sheep and goats. Three months treatment can reduce the worm burden by 90% (Graminha *et al.*, 2005; Larsen, 2006).

Chapter 15.7 References and further reading

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The following appendix item is attached:

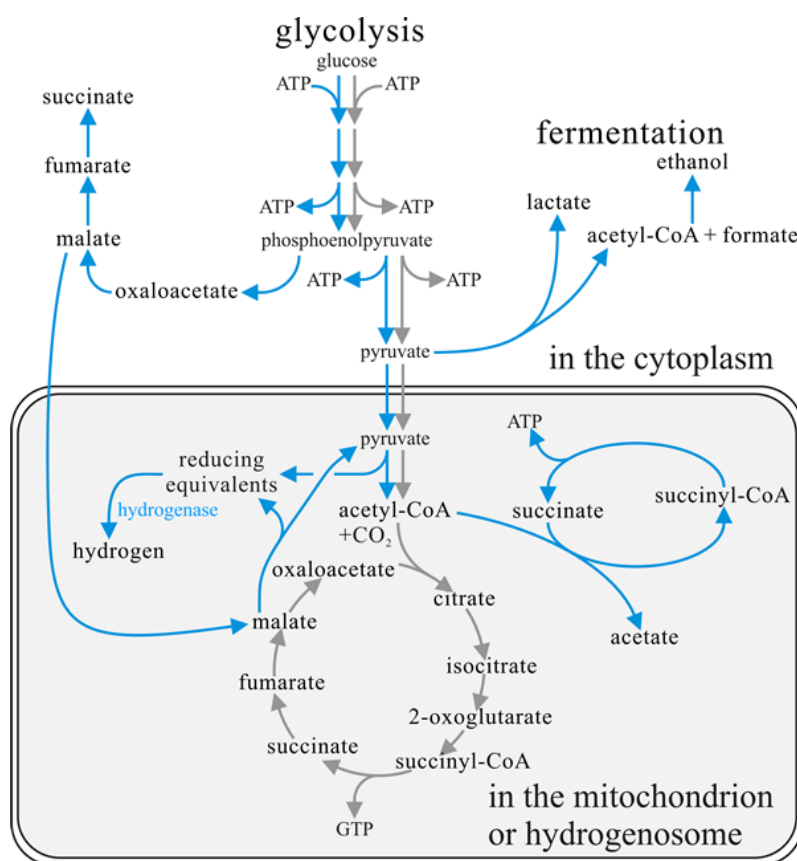
Additional notes about the metabolism of anaerobic chytrids

Additional notes about the metabolism of anaerobic chytrids

Obligately anaerobic chytrids have a crucial role in the primary colonization and enzymic degradation of lignocellulose in plants eaten by herbivores (and therefore crucial to the evolution of herbivores and to the prosperity of animal husbandry since humans first domesticated animals).

These chytrids are potent producers of enzymes needed to degrade cellulose. Their own carbon metabolism relies on fermentation of glucose to acetate, lactate, ethanol and hydrogen.

They possess an organelle called a hydrogenosome that generates ATP and appears to be a degenerate mitochondrion lacking a genome (see van der Giezen, 2002).



Generalised pathway of aerobic carbohydrate degradation in eukaryotes compared to that of the anaerobic fungus *Neocallimastix frontalis*. Aerobic degradation is indicated by grey arrows and the *Neocallimastix* anaerobic route by red arrows. In the mitochondrial pathway pyruvate is reductively decarboxylated to acetyl-CoA by the pyruvate dehydrogenase complex. In the hydrogenosome pyruvate:ferredoxin oxidoreductase catalyses the oxidative decarboxylation of pyruvate to acetyl-CoA and CO₂ producing reducing equivalents (chemicals that transfer the equivalent of one electron in redox reactions). Modified from van der Giezen, 2002.

van der Giezen, M. (2002). Strange fungi with even stranger insides. *Mycologist*, **16**: 129-131. DOI: <https://doi.org/10.1017/s0269915x02003051>.

For an overall discussion of the biology of rumen chytrids consult this paper:

Trinci, A.P.J., Davies, D.R., Gull, K., Lawrence, M.I., Bonde Nielsen, B., Rickers, A. & Theodorou, M.K. (1994). Anaerobic fungi in herbivorous animals. *Mycological Research*, **98**: 129-152. DOI: [https://doi.org/10.1016/S0953-7562\(09\)80178-0](https://doi.org/10.1016/S0953-7562(09)80178-0).