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Chapter 4 Joining forces - fungal co-operative ventures

Fungi have co-existed with animals and plants throughout the whole of the evolutionary time since these three groups of higher organisms originated. Living close together for a long time can cause neighbours to be at each other's throats or in each others pockets. Different fungi have followed both of these routes. We have already seen how the 'at each other's throats' metaphor emerges in fungal diseases and toxins which enable fungi to get some advantage over animal and plant adversaries. But there are several fungi which have taken the opposite route by treating plants and animals as partners in mutually beneficial relationships. Scientists call such arrangements symbiosis or describe them as mutualistic associations. The organisms concerned (often two but sometimes three) live in such close proximity to each other that their cells may intermingle and may even contribute to the formation of joint tissues. In these associations the partners each gain something from the partnership so that the partnership is more successful than either organism alone. Fungi are involved in some very ancient mutualistic associations. Lichens - basically combinations of fungi and algae - can be found in some of the most inhospitable environments. Another association is a mycorrhiza which is a fungal infection of plant roots. At least ninety-five percent of land plants rely on them, especially for mineral nutrients, and in return the fungus gets some sugar formed by the plant in photosynthesis. There's more to it than that, though. In high mountain areas when conditions turn stressful the fungus supplies the plant with sugars and other nutrients it gets from its abilities to degrade waste materials in the soil. By sharing the products of these activities with its plant host it keeps its host alive until the sun shines again! In lush lowland forests the soil is so full of mycorrhizal fungi that they connect the trees together so trees and their seedlings can exchange food and messages. And then there's leaf-cutting ants. Chewing the leaves up to make compost to grow one particular fungus. A fungus which produces a nutrient fluid the ant grubs need to drink. A couch-potato of a fungus that sits there while the ants charge around the forest collecting leaves for it - who's using who?

Lichens form a large and very widespread group of organisms that are associations between fungi and photosynthetic organisms. The most ancient associations, in evolutionary terms, are between fungi and blue-green algae. Blue-green algae are better called cyanobacteria because they *are* bacteria rather than algae, but they do have chlorophyll and can make their own food by photosynthesis. Cyanobacteria were the first organisms to release oxygen into the Earth's atmosphere so they started, probably three billion years ago, the revolution from which the atmosphere we rely on today emerged. Their chemical activities in the shallow seas of those ancient days precipitated insoluble calcium salts into the shapes of their colonies and these (now called stromatolites) became the first fossils on the planet.

As the more advanced plants, specifically algae, evolved the fungi (mostly relatives of 'cup fungi' which are called ascomycetes) developed the partnerships that are now lichens. Lichens have always been thought of as classic examples of symbiosis, able to live in places that are inaccessible to other organisms because of their unique partnership. The fungus takes in water and uses its externalized enzymes to extract nutrients from the soil, and even from the rocks themselves. The algal partner uses photosynthesis to provide carbohydrates to the partnership. More recent studies question about just how truly 'mutually beneficial' the relationship might be, however. Most of the body of the lichen is fungus; the algal partner constitutes only five to ten percent of the total biomass. Also, although some of the algal partners can grow alone without the fungus, most of the fungi can live only in the association with the alga. Indeed, microscopic examination shows that fungal cells actually penetrate the algal cells in a way similar to pathogenic fungi. The feeling now is that the fungus in the lichen is really parasitizing the alga, and using the products of algal photosynthesis to feed the fungus. If the alga likes it, fine; if it doesn't, tough.

Whether it is a mutually beneficial association or a case of the fungi taking algal hostages, there are about twenty thousand species of these unique entities in the living world. They vary in size, shape and colour. Some are flat and firmly attached to the surfaces they grow on (like those yellowy-brown disks that are scattered over building stones in country-clean areas. But others are scaly, leafy, bushy, or hang in strands from their supports. For reproduction several lichens produce flakes (they're called soredia) as a powdery mass on the upper surface of the lichen, or fragile upright columns (called isidia). Either way the fragments include both fungus and alga and are easily dislodged to be blown about in the breeze like spores. They can begin a new colony if they land in a suitable place.

Lichens have a remarkable ability to thrive where no other organisms can exist. They can tolerate temperature extremes from the heat of deserts to the cold of Arctic and Antarctic wastes. They survive drought by extracting moisture from mists and fog. Understandably, lichen growth can be on the slow side. Maybe two inches per thousand years in the Arctic but in less extreme environments (like on the roof of your country cottage) they may grow as much as an inch or two in ten years! Some large colonies have been estimated to be around five thousand years old. Lichen dependence on the atmosphere and rainfall makes them highly sensitive to atmospheric pollution. Acidic rain and sulfur dioxide kill many lichens, so cities in industrialized countries may have few lichens because of the poor air quality. If you do have lichens growing on your stonework it's probably good for your lungs!

The nutritional value of lichens is similar to cereal seeds, though they do not make major contributions to human food. In the harsh areas where lichens grow, native peoples use them as food supplements. One lichen, which occurs in the deserts of the Middle East, may have been the manna that fell from heaven to rescue the Children of Israel from starvation in the Old Testament story. '...and in the morning the dew lay round about the host. And when the dew that lay was gone up, behold upon the face of the wilderness there lay a small round thing, as small as the hoar frost on the ground. And when the children of Israel saw it they said one to another, It is manna: for they wist not what it was. And Moses said unto them, This is the bread which the Lord hath given you to eat..' [Exodus, 16, v. 13-15].

Lichens are much more important as animal food, especially in the Arctic, where lichens can form as much as ninety-five percent of the diet of reindeer. In less harsh conditions, most mammals of these wildernesses will supplement their normal winter diets with lichens. And sheep in Libya graze a lichen that grows on the desert rocks. Grinding their teeth away as they chew it off! The extreme life style of lichens leads them to produce several exotic chemicals, some of which may be useful to humans. There are antibiotics, essential oils for perfumery and dyes for textiles. And there are probably many others awaiting discovery and exploitation.

The lichen partnership contributed to the earliest steps in evolution of the Earth's environment, but the partnership that contributed by far the most must be the mycorrhizal association between fungi and the higher plants. In this relationship the roots of the plant are infected by a fungus. But the rest of the fungus continues to grow through the soil, digesting and absorbing nutrients and water and sharing these with the plant. This was discovered by a German botanist called Frank in 1885. He revealed this curious relationship between fungi and the smaller roots of higher plants. He claimed that it resulted in a compound structure composed of both plant root tissue and fungus mycelium and called the compound structure a Pilzwurzel, or fungus-root. The name has now been translated into a mixture of Greek and Latin to become 'mycorrhiza', but it still means fungus-root! Frank suggested that the mycorrhiza might be of fundamental importance in the nutrition of trees. Later studies have shown that just about all of Frank's interpretations were correct. Mycorrhizas are indeed, discrete organs similar to lichens in that they are composed of two partners: a fungus and a plant root.

It seems that plants roots are just not up to the job of supplying the plant with everything it needs. Certainly, all plant roots are much bigger and more unwieldy than fungal hyphae and those ‘root-hairs’ you learned about at school occur only at the apex of the finest roots. There are far more mycorrhizas. So the mycorrhizas enable the plant to connect with a vast network of fine hair-like fungal cells which, like fungi everywhere, are exploring and seeking out fresh nutrients. The external mycorrhizal network is made up of such a large number of thin cells that it has an enormous surface area and equally enormous capacity to absorb things from the surroundings. These are the advantages over the root alone - active exploration and a large surface area for absorption. Add the facts that the external parts of a mycorrhiza form an interconnected network through which nutrients are readily transported and you have the ideal nutrient search, capture and delivery device for the plant to use. But this is a mutualistic relationship, so the plant pays for the privilege of using this fungal device by sharing up to twenty-five percent of the products of its photosynthesis with the fungus. Despite this ‘tax’ on its activities, the plant grows better than it would without the mycorrhiza. Each partner benefits from the special abilities of the other and the two together make out better than either would if left to grow on its own.

Most terrestrial plants have mycorrhizal relationships. The arrangement has become the rule rather than an exception. And although most fungi are not mycorrhizal, there are common and important examples from all the major types of fungi. The mycorrhizal fungi we are most likely to meet are the mushrooms we see in wooded areas. Names already mentioned, like *Amanita* and *Boletus* are mycorrhizal partners with trees and other forest plants, as are chanterelles, and truffles too, although truffles are not mushrooms, of course.

Some mycorrhizal fungi form a mat of fungal tissue around the root, the fungal cells penetrating between the cells of the plant root, but never actually crossing the plant walls. These are called ‘ectomycorrhizas’ and they are common on conifer trees as well as some deciduous plants. In another mycorrhizal partnership (called endomycorrhizas) the fungal cells penetrate plant cell walls. Inside the plant cells they branch a lot to make structures that absorb materials from the plant cells. These endomycorrhizas are made by the most primitive fungi and can even be identified in the most ancient of plant fossils. So these could be the first mycorrhizas. The ones that helped the first plants to invade the land. Today they occur on many deciduous trees, shrubs and crop plants. By greatly increasing the absorbing surface of a host plant’s root system, mycorrhizas improve the plant’s ability to withstand drought and other extremes, like temperatures and acidity. A mycorrhizal plant also has an improved supply of mineral nutrients and some protection from pathogens in the soil. The importance of mycorrhizas is most clearly seen in new plantings. Pines are completely dependent on mycorrhizal infection for normal development. When Pine is planted in recently cleared ground where the tree has not grown before it is necessary to supply suitable mycorrhizal fungi. The ground can be inoculated with roots, with humus obtained from beneath established pines, or with pure cultures of the proper fungus produced in the laboratory. When this is done vigorous growth is seen in seedlings placed nearest to the point where inoculation was made. Infection spreads rapidly from plant to plant after the mycorrhizal fungus is established in the soil. Healthy plants have mycorrhizas; where these structures are lacking or poorly developed the plants will be stunted. Seedlings with mycorrhizas weigh more, have a smaller root-shoot ratio, and contain greater quantities of nitrogen, phosphorus, and potassium than seedlings lacking mycorrhizas. In stressful sites tree seedlings that are not infected show symptoms of nitrogen and phosphorus deficiency and eventually die. Every afforestation project is doomed to failure unless a suitable mycorrhizal fungus is introduced along with the trees. A high degree of specificity exists in the establishment of the mycorrhizal relationship in some cases - to the extent that the distribution of certain plants might be governed in part by the distribution of fungi capable of forming mycorrhizas with them. In other cases there is little or no specificity. At the extreme end of the mycorrhizal spectrum are associations between fungi and plants which lack chlorophyll. In these nongreen plants (of which Indian pipe (*Monotropa*) is an example) the entire root system is involved in a mycorrhizal relationship. Since the plant is unable to photosynthesize, it is completely dependent on the fungus associated with its roots for supplies of all nutrients - including all of its energy-containing carbon compounds derived from the fungal degradation of substrates in the soil. In this extreme case the plant gives little, if anything, to the partnership and is actually a parasite on the fungus infesting its root system.

Aside from pathogens or mycorrhizas some, maybe many, plants harbor other fungi that affect their growth. These fungi are called ‘endophytes’ because they exist within their host plants. Of course, parasites also do this, but parasites damage the host whilst endophytes are at least harmless and may be beneficial. They may be harmless or beneficial to their host, but their benign nature is not universal. Endophytes became a hot research topic when it was found that some which live entirely within grasses are responsible for the toxicity of grasses to livestock. The particular grass concerned is called tall fescue. This is a hardy, pest-free and drought-tolerant grass that was introduced to the US as a forage grass from Europe in the early 1900s. The US plantings contained an endophyte, however, which produced toxins related to those of the ergot fungus. So animals fed on tall fescue suffered illnesses similar to the ergotism of the Middle Ages. Pregnancies were disturbed, so foals and calves were lost, and some cattle lost limbs and suffered gangrene. And all were more sensitive to other diseases because of a general reduction in vitality. The ergot alkaloids the endophyte produces in tall fescue are bad for the livestock which graze on the plant, but they improve the health of the plant by protecting against attack by grazing insects - not to mention cattle and horses! The endophyte-infected tall fescue must be avoided in livestock feed, but it is actively encouraged in fescue seeds intended for amenity sites, especially golf courses. Because the infected grass produces its own pesticide, use of chemical pesticides in those locations can be reduced significantly.

There are numerous other endophytic fungi. A functional relation to the host is not always obvious, and some may be simple passengers; living in the inner spaces of the plant in much the same way as they would live in any other moist, secluded place. But it seems likely that some intriguing stories await revelation. Like the endophyte in oak leaves that remains dormant until a sedentary insect activates it by chewing on the leaf. The fungus responds to the insect attack by becoming a pathogen, killing a zone of the leaf surrounding the insect so that the insect dies for lack of live leaf tissue to feed on. With the insect pest taken out of the story, the fungus returns to being harmless and the oak’s leaves can photosynthesize in peace!

Fungi are not nasty to all insects, though. Fungi produce chemicals to attract insects to particular smells and tastes, so that the insects can carry fungal spores around. On the other hand, insects feed on fungal fruit-bodies like mushrooms. But there are a few other examples of insects and fungi evolving together to become even more intimately involved.

The first example is the leaf-cutter ant which cultivates fungi in its nest as an ongoing food supply. There are several sorts of ant in Central and South America that are known as leaf-cutter ants. The workers cut pieces from leaves on trees and carry them back to the nest. Because they usually carry the leaves in their mandibles so that the leaf extends over the ant’s head, 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 twenty millimeters 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 eight millimeters 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. 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 one and a half millimeters long take over. These are the cultivators of the fungus garden. They clean the leaf pieces and then inoculate them with fungus mycelium taken from the existing garden. The cultivators then continue to maintain the fungus garden, but they also tend the larvae. The fungus cultivated by leaf-cutter ants does not produce spores but it does have special cells that exude a sort of honey-dew which the cultivator ants collect and feed to the larvae. The fungus concerned is related to the mushroom-producing fungi but it has never been found living free in the forest; the leaf cutter ant fungus is always associated with leaf cutter ant nests. This is a mutual dependence. Although the mature ants can get nutrients from the plant sap and tissue of the leaves, so that the fungus is merely a diet supplement, the larvae depend entirely on the fungus to digest the leaves, and supplement the protein and vitamin content of the leaf material.

Since the fungus does not have an existence separate from the ants it must be carried from the parental nest by newly-mated females as part of the mating flight. 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 five meters down into the forest soil and be able to house a colony of five to seven million individuals. The demand for leaf material as the colony grows to this sort of size is enormous. In the tropical rain forests of Central and South America, leaf-cutter ants are the dominant herbivores. That ‘dominant’ includes the humans of the forest. Leaf-cutting ants compete successfully with humans for plant material and are, therefore, counted as important pests. Potential losses each year (assuming no control measures are used) could exceed one thousand million US-dollars. Around fifty agricultural and horticultural crops and about half that number of pasture plants are 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...’ It’s been calculated that in tropical rain forest leaf-cutting ants harvest seventeen percent of total leaf production. Nests located in pastures can reduce the number of head of cattle the pasture can carry by ten to thirty percent. Statistics like these reveal how leaf cutting ants can become dominant exploiters of living vegetation. Amply justifying the description ‘dominant herbivore’. 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 organizational ability to collect food material from a wide radius around its nest; but the extremely versatile biodegradation capabilities 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 one to five species per hectare, deciduous forest in North America, ten to thirty, but tropical rain forest in South America has forty to one hundred species per hectare. The tropical rain forest has enormous chemical and physical diversity in its plants and this presents a major problem to most 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 fifty to eighty percent of the plant species around their nests. This is almost entirely due to the broad range degradative abilities of the fungus they cultivate. Some plants do protect themselves by producing deterrents which inhibit cutting, pick-up or feeding. These include toughness, production of sticky latex, and a wide range of defensive chemicals. These natural defenses have recently been subject to intense study in the hope that they might suggest ways in which the insects might be controlled in the field. 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 gives the plant that extra something that enables it to grow with tropical exuberance. 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! Maybe it's not as simple as irony. Maybe we're missing the plot here!

Leaf cutting ants cultivate their fungus in South America, but in Africa the insect partner in a similar relationship is a termite. Termites are responsible for the bulk of the wood degradation in the tropics. Most of them carry populations of protozoans in their guts to digest the plant material and release its nutrients. Termites in the family Microtermitinae have evolved a different strategy. They eat the plant material to get what nutrition they can from it, and then use their feces as a compost on which they cultivate a fungus from the mushroom genus *Termitomyces*. The wider range of enzymes the fungus can produce digests the more resistant woody plant materials and the fungus becomes a food for the termites.

Some fungus-cultivating termites build mounds consisting of fecal-compost and fungus above their underground nests. Different termites produce mounds of different size and shape. Chimney-like termite mounds up to thirty feet tall 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. All termite larval stages and most adults eat the fungus. The termite king, queen, and soldiers are exceptions, being fed on salivary secretions exuded by the workers. The fungus cultivated by the termites is one that produces mushroom fruit bodies, but not in an active mound. In some way the termites prevent the mushrooms from forming in the mound. In the rainy season the termites may take portions of the culture out of the mound to fruit on the ground nearby. Abandoned mounds also produce mushrooms after the termites have left, and these are some of the largest mushrooms you can find, being up to about a meter across the cap.

Fungus-growing termites are pests because they attack wooden structures. Eating through the wood they leave a maze of galleries that destroy the strength of the timber. Insecticides and fungicides can help to control this pest.

The final example of an intimate inter-dependent association between an insect and a fungus concerns a wood-boring beetle in the family Scolytidae. These are usually found inhabiting the trunks of living trees which have been under some sort of stress (drought, air pollution, etc.). They may also be found in trees that have been recently cut or blown down. Female beetles bore into the tree, laying eggs on the tunnel wall and inoculating the wood with fungal material she has carried from a previous nest. The beetle has a specialized body cavity (called a mycetangia) in which the fungus spores and mycelium is transported. 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 of the gods'. Eventually, the larvae pupate and subsequently emerge as adults with a supply of fungus in their mycetangia. Because they supply 'gratuitous' food for the larvae, the fungi have become known as ambrosia fungi and the insects as ambrosia beetles.