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Introduction

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1.1 The symbiosis

Lichens are by definition symbiotic organisms, usually composed of a fungal partner, the mycobiont (Chapter 3), and one or more photosynthetic partners, the photobiont (Chapter 2), which is most often either a green alga or cyanobacterium. Although the dual nature of most lichens is now widely recognized, it is less commonly known that some lichens are symbioses involving three (tripartite lichens) or more partners. The potential relationships of mycobionts and photobionts may in fact be quite complex (Chapter 4), and a rigorous classification of many types of relationships was developed by Rambold and Triebel (1992). In general, lichens exist as discrete thalli and are implicitly treated as individuals in many studies (but see Chapter 13), even though they may be a symbiotic entity involving three kingdoms! From a genetic and evolutionary perspective, lichens can certainly not be regarded as individuals and this fact has major implications for many areas of investigation, such as developmental and reproductive studies (Chapter 5).

The nature of the lichen symbiosis is widely debated and deserves further investigation. Most general textbooks and many researchers refer to lichens as a classical case of mutualism, where all the partners gain benefits from the association. Alternatively, lichens are regarded as an example of controlled parasitism, because the fungus seems to obtain most of the benefits and the photobiont may grow more slowly in the lichenized state than when free-living (Ahmadjian 1993). In fact, the relationships may be much more complex, especially when additional lichenicolous fungi (Lawrey and Diederich 2003) occur on/in lichens. These are different fungi from the dominant mycobiont, and they may have a parasitic, commensalistic, mutualistic or saprophytic/saprobic

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relationship to the lichen (Rambold and Triebel 1992). Parasitic symbiotic fungi may cause extensive damage, resulting in localized necrotic patches or in complete death of the thallus. On the other hand, commensalistic symbiotic fungi apparently share the photosynthetically derived products from the photobiont with the mycobiont of the existing symbiosis. Such secondary fungi are assumed not to benefit their hosts, and they do not appear to damage them, although they may lead to the formation of gall-like growths, the morphology and physiology of which are, as yet, little understood. A few cases are being discovered where the secondary parasitizing fungus progressively eliminates the primary mycobiont, taking over the photobiont to produce a new thallus of its own; the stability of such a union can be fragile, since in some cases it has been discovered that the original photobiont can be exchanged for another preferred photobiont after the takeover.

Within the realm of what we call lichens, the degree of lichenization varies tremendously from a few photobiont cells that seem to be almost haphazardly associated with a fungus (e.g. some Caliciales) to the more typical well-integrated thallus, in which a distinct photobiont layer is found beneath cortical fungal tissue (Chapters 4 and 5). In most of the latter cases, the lichen bears little morphological similarity to the bionts that form it. Because of the differences in degree of lichenization, no single definition may adequately cover the full range of relationships found within lichens.

The morphology of the lichenized thallus is strongly influenced by the photobiont and its direct contact with the mycobiont (Chapters 4 and 5). In nature there are at least a few cases where the same mycobiont, as ascertained with molecular techniques, is able to form two very different, interconnected thalli with respectively a cyanobacterium and a green alga (Armaleo and Clerc 1990). These different morphotypes are called photosymbiodemes, and their occurrence implies ontogenetic control by the photobiont. In culture, unlichenized mycobionts remain relatively amorphous, but initiate thallus development when they first come in contact with their photobiont (Ahmadjian 1993; Chapter 5). Subsequently the mycobiont may completely envelop the photobionts and, particularly in the case of green algae, penetrate the surface of the photobiont with structures called haustoria. Because haustoria are sometimes associated with dead photobiont cells and because parasitic fungi frequently form haustoria, Ahmadjian (1993) interprets lichenization as an example of controlled parasitism. Although there is limited experimental evidence, these haustoria are assumed to facilitate carbohydrate transfer from the photobiont to the mycobiont. In the future it would be interesting to determine whether haustoria can also facilitate nutrient delivery from the mycobiont to the photobiont.

Certainly there is variation in the degree to which the symbiosis is an obligate one for the partners involved. The green algal *Trebouxia*, which occurs in approximately 20% of all lichens, has rarely been found free-living (Chapter 2). In contrast, other photobiont genera, such as *Gleocapsa*, *Nostoc*, *Scytonema*, and *Trentepohlia*, occur commonly both in lichenized and free-living states. In at least some cases, both free-living and lichenized populations occur in the same habitat, such as free-living *Nostoc* and *Scytonema* in desert soils and their lichenized counterparts respectively in the terricolous lichens *Collema* and *Peltula*. The degree to which the same photobiont species occurs in both free-living and lichenized states (Beck 2002) is not well established, because relatively few lichen algae have been definitively identified to species, and more generally, the systematics at the species level of many cyanobacteria and unicellular green algae are not well resolved (Chapter 2). Nevertheless, it appears that most lichens are highly specific in their choice of photobiont (Beck *et al.* 1998; Rambold *et al.* 1998). In contrast, the systematics of the mycobiont is well known. Because isolated mycobionts grow so slowly, they are unlikely to survive well in the free-living state due to competition with other fungi or consumption by other organisms. Thus, most mycobionts are assumed to have an obligate relationship to lichenization, although the specificity of the mycobiont for a particular photobiont may not be as great as one might assume. In addition to the photosymbiodeme example cited above, more than one species of *Trebouxia* have been isolated from the same thallus (Friedl 1989*b*; Ihda *et al.* 1993).

Overall, the lichen symbiosis is a very successful one as lichens are found in almost all terrestrial habitats from the tropics to polar regions (Chapter 14). Certainly as a result of the symbiosis, both photobiont and mycobiont have expanded into many habitats, where separately they would be rare or non-existent. For example, most free-living algae and cyanobacteria occur in aquatic or at least very moist terrestrial habitats, but as part of lichens they occur abundantly in habitats that are frequently dry as well. Not only may the fungus enhance water uptake due to its low water potential (see below), but also it substantially reduces the light intensity to which the photobiont is exposed (Ertl 1951). High light intensity adversely affects the photobiont (Demmig-Adams *et al.* 1990), and hence lichenization is one mechanism by which photobionts may expand into high light environments. Thus, there may well be benefits to lichenization from the perspective of the photobiont. Overall, it may be less important to evaluate lichenization from a strict cost/benefit perspective than to recognize it as a prominent example of a successful symbiosis. Additional studies will doubtlessly help to elucidate further our understanding of the symbiosis.

1.2 Systematics

Lichens are classified as fungi (Chapter 17), and estimates of the number of species vary from 13 500 (Hawksworth and Hill 1984) to approximately 17 000 (Hale 1974). Because many regions of the world have been poorly collected, the higher number may well be more reasonable. By far the largest number of lichens are Ascomycetes and in fact almost half of the described Ascomycetes are lichenized (Chapter 17). In addition, there are a few lichenized Basidiomycetes and Deuteromycetes (= Fungi Imperfecti). The latter group is an artificial class, in which sterile species are placed. If fruiting structures are eventually found, then these lichens may in due course be classified as either Ascomycetes or Basidiomycetes. In addition, in the Actinomycetes, Mastigomycetes and Myxomycetes, there are a few symbiotic associations with some properties similar to lichens, but in general these are excluded from lichen classifications.

Although one might hypothesize that cyanobacteria, green algae, and fungi evolved from lichens, it is generally assumed that lichenization occurred subsequent to development of these organisms. In the fossil record there is limited evidence for the occurrence of lichens, but this may be more due to lack of preservation than their absence from earlier eons. In fact, several quite old fossils have recently been interpreted as being lichens (Chapters 5 and 16). The diversity of lichenized fungi and the fact that some groups contain both lichenized and free-living fungi has led to the inference that lichenization and delichenization have occurred more than once and in fact may have occurred several times (Gargas *et al.* 1995; Lutzoni *et al.* 2001). The initial inference is supported by the occurrence of lichens in different classes of fungi, and, within the Ascomycetes, by the fact that lichenization occurs exclusively in only five of the 16 orders, in which lichenization has thus far been found (Hawksworth 1988a). If lichenization has occurred multiple times, then in an evolutionary sense lichens cannot be regarded as one group or, as a phylogeneticist would say, lichens are polyphyletic (Chapter 17).

1.3 Diversity and ecological domain of lichens

Among the terrestrial autotrophs of the world, lichens exhibit intriguing morphological variation in miniature (Chapter 4). In color they exhibit a fantastic array of orange, yellow, red, green, gray, brown, and black (Wirth 1995; Brodo *et al.* 2001). Lichens vary in size from less than a mm² to long, pendulous forms that hang over 2 m from tree branches (Chapter 4). Almost all lichens are perennials, although a few ephemerals (e.g. *Veizdaea*) are known. At the other extreme some lichens are estimated to survive well over 1000 years and may be

useful in dating rock surfaces (Beschel 1961; Section 10.7). Linear growth varies from imperceptible to many millimeters in a year.

Lichens occur commonly as epiphytes on trees and other plants, and in some ecosystems epiphytic lichen biomass may exceed several hundred kg ha⁻¹ (Coxson 1995). In addition, they frequently colonize bare soil, where they are an important component of cryptogamic soil crusts in arid and semi-arid landscapes (Evans and Johansen 1999; Belnap and Lange 2003). Furthermore, lichens occur almost ubiquitously on rocks with the most obvious ones occurring as epiliths, either growing over the surface or embedded within the upper few millimeters. A few lichens even occur endolithically within the upper few millimeters of the rock, such as occurs in Antarctica (Friedmann 1982). In the tropics and subtropics, some rapidly growing lichens even colonize the surface of leaves as epiphylls (Lücking and Bernecker-Lücking 2002). Although most lichens are terrestrial, a few occur in freshwater streams (e.g. *Peltigera hydrothyria*) and others occur in the marine intertidal zone (e.g. *Lichina* spp. and the *Verrucaria maura* group).

Lichens occur in most terrestrial ecosystems of the world, but their biomass contribution varies from insignificant to being a major component of the whole ecosystem (Kershaw 1985; Chapter 14). In many polar and subpolar ecosystems, lichens are the dominant autotrophs (Longton 1988). In addition, lichens are conspicuous components of many alpine, coastal and forest ecosystems, such as the temperate rain forests of the southern hemisphere (Galloway 2007) and taiga of the northern hemisphere (Kershaw 1985). Because most lichens grow relatively slowly, their primary productivity contribution is fairly small in most ecosystems (Chapter 10). On the other hand, the more rapidly growing species may increase their biomass by 20–40% in a year and these species may play an important role in the mineral cycling patterns of their ecosystems (Section 12.10), particularly if cyanolichens are the dominant component (Chapter 11).

1.4 Lichens as poikilohydric organisms

Most flowering plants and conifers have developed the capacity to maintain the water status of their leaves or needles at fairly constant levels and hence are referred to as homiohydric organisms. In contrast, lichens are prominent members of poikilohydric organisms, whose water status varies passively with surrounding environmental conditions (Chapter 9). Other poikilohydric organisms include the bryophytes, some ferns and other primitive vascular plants. All of these organisms become desiccated relatively rapidly and, as a consequence, water availability is of prime importance for their

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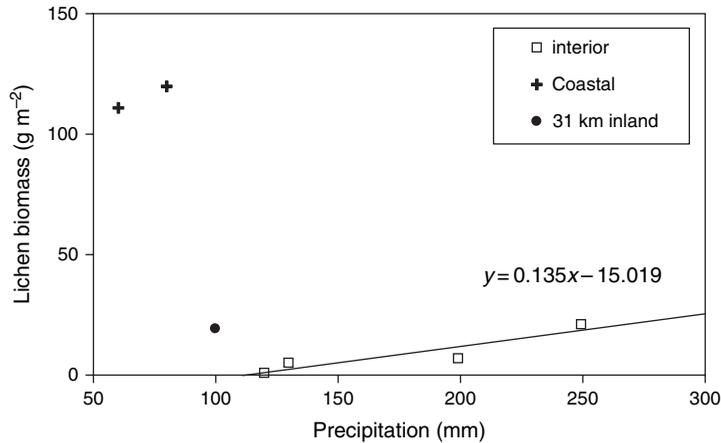


Fig. 1.1 Relationship of biomass of lichen communities within the Sonoran Desert region to mean annual precipitation (Nash and Moser 1982).

survival and in explaining their patterns of occurrence (Chapter 9). One might assume that poikilohydric organisms are highly dependent on precipitation, primarily in the form of rain. Certainly this is true for many lichens, as can be seen for the lichen biomass relationship among interior desert sites (Fig. 1.1, the straight line). On the other hand, lichen biomass near the Pacific Ocean in the western part of the Sonoran Desert vastly exceeds values that would be predicted based on precipitation alone (Fig. 1.1, crosses). This illustrates the ability of lichens to utilize other water sources, such as fog and dew. In addition, lichens have the remarkable ability to extract some moisture from non saturated air under conditions of low temperatures and high humidities. This is essentially the reverse of transpirational water flow occurring through vascular plants and is due to the low osmotic values of lichen thalli. However, under intermediate to high temperatures and intermediate to low humidities, the water potential gradient from the lichen to the atmosphere is reversed and evaporation occurs.

1.5 Practical applications

Many of the secondary products formed by lichens are unpalatable and may serve as defensive compounds against herbivores as well as decomposers (Rundel 1978; Chapter 14). As a consequence, it is not surprising that these secondary products are frequently used by the pharmaceutical industry as antibacterial and antiviral compounds. In addition, lichens have long been used as a source of natural dyes and in the making of perfumes. In both cases the secondary products provide the chemical basis for these applications (Chapter 7).

The differential sensitivity of lichens to air pollution has been recognized for over a century and a half, and the application of lichenological studies to biomonitoring of air pollution is now well developed (Chapter 15). For example, patterns in lichen communities may be correlated with sulfur dioxide levels in the atmosphere (Hawksworth and Rose 1970). In recent years sulfur dioxide levels have been reduced, either by improved controls on emissions or by more efficient dispersion strategies, and, as a consequence, lichens are now reinvading areas from which they had previously disappeared (Rose and Hawksworth 1981; Bates *et al.* 1990). However, the recolonization is incomplete because other factors, such as high nitrate deposition, modify lichen community composition as well. Finally, lichens are efficient accumulators of metals and persistent organic pollutants and are frequently used as surrogate receptors for documenting deposition of these pollutants (Chapter 12).

1.6 Lichens as self-contained miniature ecosystems

The lichen thallus is a relatively stable and well-balanced symbiotic system with both heterotrophic and autotrophic components. From this perspective, the lichen can be regarded as a self-contained miniature ecosystem (Farrar 1976c; Seaward 1988), particularly if one considers the parasitic lichenicolous fungi colonizing lichens as this ecosystem's decomposers. The lichen fungus undoubtedly benefits enormously by obtaining its nutrition from the photobiont, but the photobiont's gain from the association is less obvious. Fundamentally, the photobiont gains protection from high light, temperature extremes and to some extent drought, but the premise that the alliance between free-living algae or cyanobacteria and the fungal partner enables them to live together in inhospitable areas where they could not do so independently cannot be fully justified. Pushed to its ultimate limit, this train of thought leads to the fallacy that lichens are the only form of life possible on other planets – a false assumption, because, even supposing that the environment there was capable of supporting life as we know it, then representatives of both symbiotic partners would have to be present in the first instance. However, lichens have recently been put to the test in terms of their ability to cope with extreme conditions of outer space, even Martian conditions, the symbiotic system and germination capacity proving remarkably resistant to UV radiation and vacuum exposure (de Vera *et al.* 2003, 2004).

The lichen symbiosis typically involves a close physiological integration. The usually dominant mycobiont is, of course, a heterotrophic organism that derives its carbon nutrition from the photobiont (Chapter 3). The flux of carbohydrates, as polyols in the case of green algal lichens and glucose in the case of

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cyanolichens, from the photobiont to the mycobiont is well established (Smith and Douglas 1987). This is a necessary benefit for the mycobiont and is the result of the photobiont's cell walls being more permeable to carbohydrate loss in the lichenized than nonlichenized state (Hill 1976). In addition, the mycobiont gains a nitrogen source in the case of cyanolichens, in which nitrogen fixation occurs in the photobiont (Chapter 11). No comparable flux of nutrients from the mycobiont to the photobiont has been demonstrated. However, the recent demonstration of recycling of nitrogen and phosphorus in a mat lichen is an exciting first step to providing such documentation (Hyvärinen and Crittenden 2000; Ellis *et al.* 2005). Does the fungus in general serve as a reservoir of inorganic nutrients for the photobiont through the haustoria? Certainly other fungi facilitate nutrient uptake in other symbiotic relationships, such as occurs in mycorrhizae and rhizospheric fungi. Another result of close physiological integration is the occurrence of a wide range of secondary products, many of which occur as crystals extracellularly within the lichens (Chapter 7). Most of these are unknown in free-living fungi (or other organisms) and hence their occurrence adds to the uniqueness of the lichen symbiosis.

From an ecological perspective, lichens may be even more complex, as free-living bacteria and non symbiotic fungi may be found associated with an "individual" (Section 13.4) and, as a consequence, some authors regard a lichen as a miniature ecosystem. Further support for accepting the lichen as an ecosystem is provided when one considers the range of other benign or harmful microorganisms associated with one or more of the above bionts; these include fungi and bacteria found both on the surface and within thalli, or in the microenvironment generated beneath thalli or within lichen-weathered substrata (Bjelland and Ekman 2005), and also invertebrates which graze upon them, or seek protection from predators through crypsis or by sheltering beneath thalli; the intimate relationship between the lichen and its substratum in the case of epiphytic, lignicolous and foliicolous species adds to the complexity of the microhabitat generated.

2

Photobionts

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2.1 Major differences in cyanobacteria versus algae

Nearly 40 genera of algae and cyanobacteria have been reported as photobionts in lichens (Tschermak-Woess 1988; Büdel 1992). Three genera, *Trebouxia*, *Trentepohlia*, and *Nostoc*, are the most frequent photobionts. The genera *Trebouxia* and *Trentepohlia* are of eukaryotic nature and belong to the green algae; the genus *Nostoc* belongs to the oxygenic photosynthetic bacteria (cyanobacteria). Eukaryotic photobionts are also referred to as “phycobionts” while cyanobacterial photobionts are sometimes called “cyanobionts.” The vast majority of eukaryotic photobionts belongs to the green algae (phylum Chlorophyta) which share many cytological features and their pigmentation, e.g. the presence of chlorophylls *a* and *b*, with the land plants (Bold and Wynne 1985; van den Hoek *et al.* 1993). Only two genera of eukaryotic photobionts containing chlorophylls *a* and *c* (phylum Heterokontophyta *sensu* van den Hoek *et al.* 1993) have thus far been reported: *Heterococcus*, Xanthophyceae, and *Petroderma*, Phaeophyceae (Tschermak-Woess 1988; Gärtner 1992).

Cyanobacteria are of prokaryotic nature and lack chloroplasts, mitochondria, and a nucleus, all of which are found in eukaryotic algae. In cyanobacteria, thylakoids lie free in the cytoplasm, often more or less restricted to the periphery. The circular DNA is not associated with histones and is concentrated in areas of the cytoplasm free of thylakoids which sometimes are called “nucleoplasm.”

Metabolite transfer from the autotrophic photobiont to the heterotrophic mycobiont depends on the type of photobiont involved. In lichens with green algal photobionts, the carbohydrates are sugar alcohols; in lichens with cyanobacteria it is glucose (Feige and Jensen 1992; Section 10.2.1). The mode of

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activation of CO₂ uptake is another basic feature that varies depending on whether the photobiont is prokaryotic or eukaryotic. In many green-algal lichens, positive net photosynthesis is possible after water vapor uptake alone. In contrast, in cyanobacterial lichens no measurable gas exchange occurs because the water content level required to activate photosynthesis is higher and liquid water is needed to obtain such levels (Lange *et al.* 1986).

2.2 Identification, reproduction, and taxonomy of photobionts

2.2.1 *Cyanobacteria*

Identification of cyanobacterial photobionts in the intact lichen thallus is often impossible since the morphology of the photobiont is changed by the influence of the fungal partner. Filamentous forms may be deformed to such a degree that their originally filamentous organization cannot be recognized within the lichen thallus, e.g. in the genus *Dichothrix* (Fig. 2.1). Only the truly branched filamentous cyanobacterial genus *Stigonema* (Fig. 2.2) and the nonbranched genus *Nostoc* (Fig. 2.3) can often be identified within the lichen thallus. Furthermore, cyanobacteria do not show all characteristic stages of their life cycles in the lichenized state. Because it is essential to know these stages for positive identification of cyanobacteria, even at the genus level (Komárek and Anagnostidis 1998, 2005), isolation and cultivation of the cyanobacterial photobiont are necessary steps for positive identification. The mode of vegetative cell divisions is also important in the delimitation of many unicellular cyanobacteria at the genus level. However, using molecular techniques, at least determination on the genus level is possible directly from the lichen thallus, using specific primers for cyanobacterial 16S rDNA (e.g. Lohtander *et al.* 2003; O'Brien *et al.* 2005).

Cyanobionts with heterocysts like *Nostoc* (Fig. 2.3) increase heterocyst frequency up to five times when lichenized compared with the free-living state (Feige and Jensen 1992). Also, cell size of cyanobacterial photobionts may be increased compared with cultured or free-living material, as has been reported for the genera *Gloeocapsa* (Fig. 2.4) and *Chroococidiopsis* in the lichen genera *Lichinella*, *Peccania*, *Psorotichia*, *Synalissa*, and *Thyrea* (Geitler 1937; Büdel 1982). Increase of cell size can be a result of a very close mycobiont–photobiont contact, as in the deeply penetrating haustoria. This can be seen well in the vegetative trichome cells of *Scytonema* sp. within the lichen *Dictyonema sericeum* (Fig. 2.5). Unicellular cyanobacterial photobiont genera, e.g. *Chroococidiopsis* and *Myxosarcina*, very rarely show their specific mode of reproduction when lichenized, but frequently show these stages when cultured. For instance, *Chroococidiopsis* (Fig. 2.7) and *Myxosarcina* (Fig. 2.6) are characterized in culture