


Is lichen symbiont mutualism a myth?

William B. Sanders 

William B. Sanders (wsanders@fgcu.edu) is affiliated with the Department of Biological Sciences at Florida Gulf Coast University, in Ft. Myers, Florida, in the United States.

Abstract

Lichens are classic examples of symbiosis, but some biologists have questioned whether the algal partner benefits from the relationship. Among the diverse lichen symbioses, the carbon transfer systems show remarkable convergences. When a compatible fungus is encountered, the alga proactively releases large amounts of carbohydrate, suggesting active participation rather than victimhood. Some lichen-related fungus–alga symbioses appear obligatory for the algal partner. Within true lichens, algal symbionts can persist at microsites where they might not otherwise be competitive, because of improved stress tolerance, reduced photoinhibition, protection from herbivores, and the more efficient moisture management and positioning for light interception that fungal structures provide. Algal clones continually disperse from the lichen thallus by diverse means, allowing the genotype to pioneer aposymbiotic colonies from a stable refuge. Because lichen-forming fungi conserve rather than consume their algal symbionts, the mutual self-interests of both partners substantially align in the stressful microhabitats where lichens are successful.

About 20,000 known species of fungi live and reproduce as lichens in intimate partnership with specific algae (Lücking et al. 2017). This often involves a radical transformation of growth form to produce a macroscopic, collaborative structure (the *thallus*) that is frequently plant-like, bearing no resemblance to its component microorganisms (figure 1). The integration of the symbionts is so striking that lichens were thought to be unitary organisms until well into the nineteenth century, when Schwendener (1868, 1869) elucidated their composite nature. Shortly thereafter, they became and have remained among the most widely recognized examples of symbiosis, a physiologically intimate, enduring partnership between organisms of different species.

As it was originally formulated, the concept encompasses a spectrum of relationships ranging from mutually beneficial to more one-sided and exploitative (de Bary 1879). In popular usage and among some biologists, *symbiosis* may also be applied more narrowly to mean specifically mutualistic or at least nonparasitic relationships (Smith 1992, Douglas 2010), although, in science, this usage has receded recently (Martin and Schwab 2013).

With their emergent, superorganismal properties (Sanders 2006), lichens offer spectacular examples of symbiosis in the broad sense. But are they equally good examples of mutualism? The heterotrophic fungus (mycobiont) clearly receives photosynthate from its partner; less obvious is what the alga (phycobiont or photobiont) might get in return. Although the relationship is generally treated as mutualistic (Hawksworth and Honegger 1994), some prominent biologists have disputed that interpretation. Douglas and Smith (1989) judged the available evidence insufficient to support the claim of any benefit to internal, microorganismal symbionts such as lichenized algae. Kappen (1994) referred to the lichen symbiosis as a mutualism but saw little evidence for benefit to the algae; he recognized a generally parasitic character inherent in lichen-forming fungi. According to Richardson (1999), much evidence supports the view that lichen algae are the slaves of their fungal masters. In his early work, Ahmadjian (1962) found phycobionts to unquestionably benefit

from lichenization but later came to view them as victims of fungal parasitism; he called lichen symbiont mutualism a myth (Ahmadjian 1993, 1995, 2001, 2002). Citing Ahmadjian, Nash (2008) described the issue as controversial in his introduction to *Lichen Biology*. A parasitic view of the lichen symbiosis was further affirmed by Chapman and Chapman (2010), for whom lichen algae are enslaved and abused; those authors characterized the mutualistic interpretation as simplistic, faulty, false, wrong, and incorrect. Where does the weight of available evidence currently fall?

Contours of the lichen concept

To consider this question, it's necessary to first clarify what counts as a lichen, because there are many kinds of fungus–alga relationships. The definition that seems to best coincide with how most lichenologists view their subject characterizes the lichen as a stable, self-supporting relationship in which a fungus is the exterior *exhabitant* and the alga an interior but extracellular *inhabitant* (Hawksworth 1988, Hawksworth and Honegger 1994). In the most familiar cases, the surrounding fungus transforms itself to build complex tissues that enclose the algal symbionts, which may themselves be modified structurally (figure 2a–2c).

However, many lichens are much simpler, particularly those that grow within substrata such as bark or rock, where the fungus may simply contact algal cell surfaces without differentiating any special tissue (figure 2d). The mycobiont–exhabitant definition recognizes that the lichen thallus uniquely everts the usual position of a fungus within its food source (Sanders 2001). It excludes those symbioses where the tissues of certain seaweeds (multicellular algae) are simply invaded by fungal hyphae. Most lichenologists have not been keen to include this more conventional arrangement (a “mycophycobiosis” according to Kohlmeyer and Kohlmeyer 1972) within the lichen concept. Nevertheless, there can be significant connections (Garbarý 2009), with relevance to the question of lichen mutualism.

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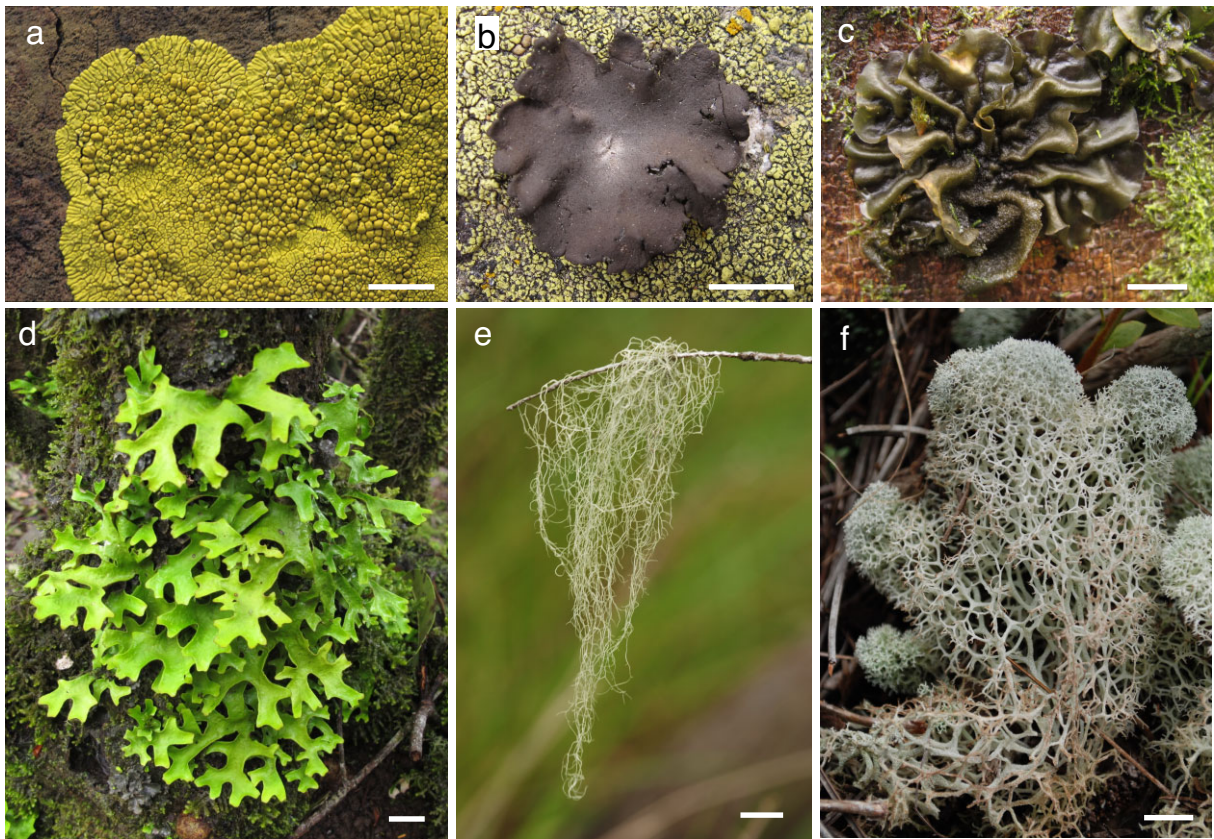


Figure 1. Some lichen thallus forms. (a) Crustose *Acarospora* sp. (b) Umbilicate *Umbilicaria polyphylla*. (c) Foliose gelatinous *Leptogium austroamericanum*. (d) Foliose *Sticta canariensis*. (e) Fruticose *Ramalina usnea*. (f) Fruticose *Cladonia evansii*. The scale bars represents approximately 1 centimeter.

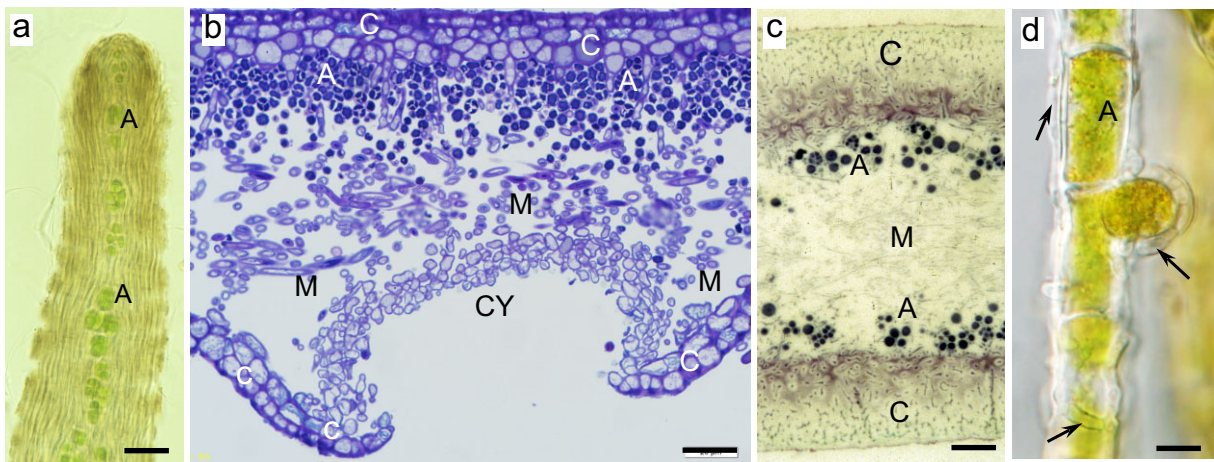


Figure 2. Enclosure of algal cells within complex fungal tissues (a–c) or simple contacting mycelia (d). (a) *Bryoria* sp. with *Trebouxia phycobiont*. (b) *Sticta canariensis* with *Symbiochloris phycobiont*. (c) *Niebla homalea* with *Trebouxia phycobiont*. (d) *Coenogonium* sp. (arrows) with *Trentepohlia phycobiont*. Abbreviations: A, algal symbiont; C, cortex; CY, recessed gas-exchange pore (cyphella) in lower cortex; M, medulla. The scale bars represent (a, b) 20 micrometers (μm), (c) 25 μm , and (d) 5 μm .

One such fungus, *Mycophycias ascophyllae*, grows within the tissue of the common rockweed, *Ascophyllum nodosum*, a highly visible colonist of northern Atlantic shores. The intertidal habitat where the seaweed and its symbiotic fungus are found is subject to diurnal extremes of hydric stress, exactly the sort of conditions under which the lichen symbiosis has been so successful on land. Studies have repeatedly concluded that the relationship is mutualistic, with the infected alga showing faster growth and better

protection from desiccation compared to uninfected individuals, which have never been reported in situ (Garbary and London 1995, Garbary and MacDonald 1995, Xu et al. 2008).

Another such fungus, *Mastodia tessellata*, is a member of the Verrucariaceae, a family that consists mainly of rather typical lichen formers found in terrestrial and intertidal marine habitats. In maritime polar regions, *Mastodia* invades the tissues of the multicellular green alga *Prasiola*, whose cells it surrounds and

separates. This makes it somewhat harder to distinguish exhabitant from inhabitant (Lud et al. 2001), leading some to consider the symbiosis a borderline lichen (Pérez-Ortega et al. 2010). The alga's tolerance of freezing temperatures is increased in symbiosis (Fernández-Marín et al. 2019).

Because *Mycophycias* and *Mastodia* inhabit rather than enclose their macroalgal symbionts, however, they don't fit the prevailing lichen concept, despite ecological and phylogenetic connections to those that do (box 1). One could likewise argue that the mycobiont *Collema*, which grows within the gelatinous sheath material of its phycobiont *Nostoc*, is not really an exhabitant. But its close relatives (*Leptogium* s. lat.) form very similar-looking thalli with the sole addition of an external parenchymatous layer (Sanders and de los Ríos 2019), and it would hardly seem reasonable to place these often macroscopically indistinguishable sister taxa on opposing sides of the lichen concept. In summary, the mycobiont-exhabitant definition, although quite useful, does impose somewhat artificial boundaries on the variety of intergrading fungus-alga symbioses that qualify as lichens. But even at the periphery of those boundaries, mutualistic relationships have been recognized.

The phylogenetic diversity of lichens raises another important caveat to any critical assessment of symbiont relationships: to what extent can one generalize about them? Recent analyses suggest that the lichen-forming lifestyle evolved at least 20–30 times independently in more than five different classes of Ascomycota and several different orders of Basidiomycota (Lücking et al. 2017) that associate with some 55 genera of green, blue-green and stramenopile algae (Jung et al. 2021, Sanders and Masumoto 2021). It would seem unlikely that these diverse algal symbionts all receive similar treatment from their likewise diverse fungal partners. But there are remarkable similarities in the photosynthate transfer systems that have evolved independently in the numerous lichens investigated physiologically. Responding to some still unidentified signal, the lichenized alga releases carbohydrate en masse, a process that quickly ceases when they are isolated into culture. The eukaryotic algal symbionts transfer carbohydrate as sugar alcohols—ribitol, erythritol, or sorbitol, depending on the genus—whereas cyanobionts transfer glucose; the mycobionts convert the sugars received into mannitol and arabitol (Richardson et al. 1967, 1968, Smith 1980). An important structural adaptation related to the mass release of carbohydrate are mycobiont-derived hydrophobic materials that coat symbiont surfaces at their contact zones, thereby sealing an apoplasmic conduit to the fungal cells while maintaining a water-free external surface for efficient carbon dioxide diffusion to the alga (Honegger 1986b, 1991, Trembley et al. 2002). These common features of the substance transfer system represent convergences that may be fundamental to the lichen concept. They could well indicate common underlying mechanisms (Hill 1976) and suggest that at least some generalizations concerning the relationship between lichen symbionts may be justified.

Evidence that phycobionts receive protective benefits

Just what the lichen symbiosis offers to—or imposes on—the algal partner has been the subject of much speculation and commentary but has not been extensively explored experimentally. Although numerous studies have shown mass transfer of carbohydrate from alga to fungus, along with fixed nitrogen where the alga is prokaryotic, active transfer of substances directly from fun-

gus to alga has not been definitively demonstrated (Smith 1980, Smith and Douglas 1987). However, both partners secrete a variety of potentially relevant metabolites (Pichler et al. 2023). In a lichen with both green and blue-green algal symbionts, radiolabeled nitrogen fixed within thallus nodules (cephalodia) by the cyanobacterium *Nostoc* was subsequently detected in the mycobiont *Peltigera aphthosa* and, afterward, in its green algal symbiont *Coccomyxa*; presumably, the fungus was the conduit for this transfer (Rai et al. 1981). More generally, the interior position of lichen algae may mean that their only access to water and nutrients is through their contacts with the surrounding fungal symbiont (Honegger 2009). The question might therefore be reframed as whether the mycobiont serves merely as a passive apoplasmic channel or whether it actively apportions substances to the algal symbiont. As Spribille and colleagues (2022) point out, much of the research on substance transfer in lichens was done 40–60 years ago, with little follow-up since then. Some recent studies of gene expression during lichen resynthesis in the laboratory have reported upregulation of fungal transporter proteins that export ammonium and phosphate, likely destined for algal use (Armaleo et al. 2019, Kono et al. 2020). New transcriptomic data may stimulate further physiological investigation of intersymbiont transfer, particularly in the fungus-to-alga direction. With growing recognition that additional microorganisms often inhabit the surfaces and interior of lichen thalli, a more complex “exchange of goods and services” has also been postulated (Spribille et al. 2022). But at present, most cited evidence for algal benefit has been based not on detection of substances received, but rather on the various protections that may be obtained in symbiosis. Such protections might contribute significantly to the algal lineage's fitness—its relative success in passing on its genes to future generations. This parameter can be notoriously difficult to assess (Douglas and Smith 1989). But fitness has nothing to do with fairness. The anthropomorphic comparisons to slavery and abuse, while thought provoking, will not illuminate how lichen algae have fared under the amoral workings of natural selection.

To understand how lichens might offer protection to the algae they enclose, one must consider their special talents as extremophiles. Lichens are found in the most inhospitable environments, such as hot deserts, arctic tundra, and Antarctic dry valleys, where only the most highly adapted organisms can eke out a living (Kappen 1974, de los Ríos et al. 2005, 2014, Jung et al. 2019). Even in more moderate habitats, lichens are well adapted to colonize the most stressful microsites, such as the surfaces of bare rock, soil, tree trunks, or branches, that experience severe conditions of water, light, and temperature flux. Their growth may be very slow, but they have few competitors there. Most lichens are highly desiccation tolerant, regularly entering into and returning from a state of metabolic suspended animation (Kranmer et al. 2009). In that state, lichens have been shown to endure punishing extremes of heat and cold (including storage in liquid nitrogen); exposure to vacuum, cosmic rays, and unfiltered electromagnetic energy in outer space; and immersion in organic solvents (Kappen 1974, Solhaug and Gauslaa 1996, Honegger 2003, Sancho et al. 2009). The desiccation tolerance mechanisms include sizeable pools of sugar alcohols that lower water potential and likely stabilize membranes with their numerous hydroxyl groups as water is lost. Also essential are enzymes and antioxidants that neutralize the dangerous free radicals and reactive oxygen species generated by a cell's energy-harvesting machinery under the stresses of dehydration and rehydration (Beckett et al. 2008). Significantly, antioxidants used by both fungal and

Box 1. What counts as a lichen, and who is the host?

If a mycobiont were judged a parasite, one would expect the phycobiont to be called its host. However, there are quite different views of this concept. In the terminology of Smith and Douglas (1987), the physically predominant partner is the host, and the diminutive, internalized partner its “symbiont.” In figures 3b and 4b, the lichen-forming fungus fits the parasitic model quite awkwardly, because that would make it both parasite and host! Kappen (1994) criticized this usage, pointing out that from the standpoint of nutritional sustenance, the alga must be considered the host. Applying the terminology of Smith and Douglas (1987) to the examples in figures 3 and 4, algae are hosts to fungal symbionts in figures 3a and 4a, whereas fungi are hosts to algal symbionts in the lichens in figures 3b and 4b. In the nutritional sense, by contrast, the photosynthetic algae host their fungal partners in all four examples.

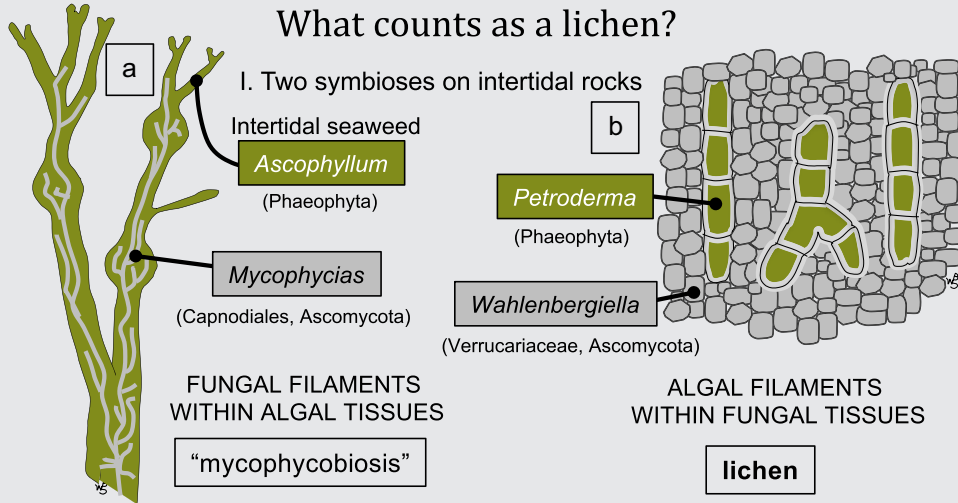


Figure 3. Two symbioses on intertidal rocks compared with respect to the lichen concept (a) *Mycophycias* versus (b) *Wahlenbergiella*, an undisputed lichen. Both of these intertidal fungal species associate with brown algal symbionts. *Mycophycias* is currently placed in the Capnodiales (Toxopeus et al. 2011), an order that encompasses fungi of diverse nutritional strategies, among which lichenization has arisen several times (Creus et al. 2009).

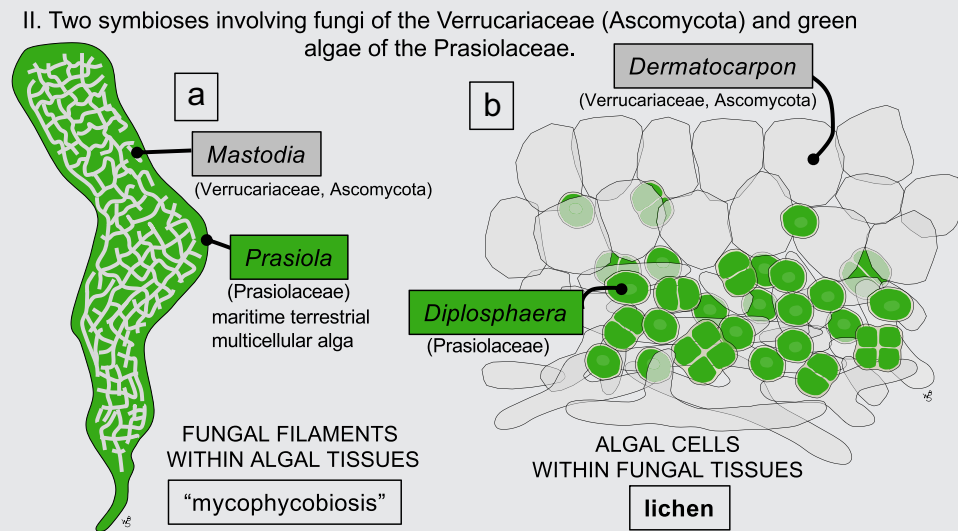


Figure 4. Two symbioses involving fungi of the Verrucariaceae (Ascomycota) and green algae of the Prasiolaceae compared with respect to the lichen concept (a) *Mastodia* versus (b) *Dermatocarpon*, an undisputed lichen. In these two associations, the fungi share a common family (Verrucariaceae), as do their algal symbionts (Prasiolaceae).

algal symbionts for this protection may be present in much greater quantity in the lichenized state compared to that of the isolated symbionts. Intersymbiotic signaling seems therefore to be necessary for the mutual upregulation of these crucial protections (Kranmer et al. 2005).

At the exposed microsites that many lichens colonize, the symbionts may be subjected to extremely high radiation loads from which strong photoprotection is required. Many aerophilic algae are already supplied with protective pigments, such as the abundant carotenoids produced by *Trentepohlia* and its relatives. But

protection from photoinhibition (a decline in photosynthetic rate resulting from excess light) is substantially augmented within a lichen thallus, because of screening by overlying fungal cortical tissues (Büdel and Lange 1994) and pigments such as parietin and melanins (Solhaug and Gauslaa 1996, Beckett et al. 2019). More intimate symbiont interactions may also be involved in strengthening algal protection against excessive light energy. Kosugi and colleagues (2009, 2013) showed that desiccated *Trebouxia* phycobionts were significantly better at dissipating energy in lichen symbiosis with *Ramalina yasudae* than in isolation; mycobiont-derived arabinol appeared to be somehow implicated. The additional sun-screening and physiological protections obtained in lichenization undoubtedly expand the range of exposed microsites that the alga can successfully colonize. Other products of mycobiont metabolism may also serve to deter herbivores that would otherwise consume an algal colony (Lawrey 1983, Asplund and Wardle 2013, Boch et al. 2015).

Lichens also have the remarkable capacity to hydrate sufficiently from humid air to carry out net photosynthesis, even at temperatures far below freezing (Kappen 1993). The high osmolarity of their cells apparently lowers water potential sufficiently to achieve this. Within several types of lichens first examined in this regard, the phycobiont *Trebouxia* was reported capable of net photosynthesis at significantly lower water potential than when isolated from the lichen, suggesting another important algal benefit in symbiosis (Brock 1975). But these results were not corroborated by later investigators, who found the isolated phycobionts as capable of hydrating and activating photosynthesis as when lichenized within the thallus (Lange et al. 1990). It should not be surprising that many lichen phycobionts, like other aeroterrestrial algae, are themselves adapted to making a living under conditions of stressful exposure (McCourt et al. 2023). They produce their own intracellular pools of protective, osmophilic polyols in the aposymbiotic state (Gustavs et al. 2010, 2011), although seemingly at lower levels than in symbiosis (Hill and Ahmadjian 1972). This, of course, raises the standard for recognizing advantages derived from lichenization; any putative protection has to clearly exceed what the alga enjoys on its own. The ability to hydrate from humid air might therefore not qualify, but in hydrating from liquid water sources (rainfall, dew, fog), the lichenized alga will often have a distinct advantage over a simple layer of free algal cells on the substratum. The elaborate surfaces, tissues, and hydrophilic wall materials of the lichen thallus are functionally adapted to specific strategies of moisture condensation, absorption, and retention (Larson and Kershaw 1976, Larson 1979, 1981, Jahns 1984, Valladares 1994, Pintado et al. 1997, Esseen et al. 2015). Thallus structure also elevates and more efficiently displays its phycobiont population to incident light, even in crustose forms (e.g., Vondrák and Kubásek 2013). Notably, no contemporary aeroterrestrial alga has itself evolved the size and structural complexity that would allow it to directly compete with macrolichens. Most are unicellular; very few are macroscopic. Were they as capable of tolerating full exposure and stress to the same degree on their own as in lichen symbiosis, at least some lineages might be expected to have body forms rivaling those of foliose and fruticose lichens. After all, green algae are morphologically diverse in marine and aquatic environments, where they build a wide variety of macroscopic forms (Oltmanns 1922, Fritsch 1935). But on land, only one descendant lineage (embryophytes) ever managed this. All other terrestrial chlorophytes that enjoy the functional advantages of plant-like structural complexity do so exclusively through lichen symbiosis.

Mycoaggressions?

If the frequently elaborate protective housing provided by the mycobiont suggests algal benefit, the cellular details of symbiont interaction have often led to parasitic interpretations of mycobiont behavior. Several observers have noted the occasional disintegration and fungal invasion of algal cells in certain lichen thalli (Tschermak 1941, Peat 1968, Peveling 1968). Others have concluded that the cycle of algal cell senescence and death observed within a lichen thallus is unrelated to any penetration by the mycobiont (Galun et al. 1970). Indeed, mycobionts often invade their own moribund cells by hyphal outgrowth from adjacent compartments—the so-called intrahyphal hyphae routinely observed in transmission electron microscopy studies (e.g., Sanders and de los Ríos 2017's figure 7e). In laboratory synthesis experiments, the destruction of phycobiont cells was often extensive when paired with incompatible mycobionts (Ahmadjian et al. 1980, Ahmadjian and Jacobs 1981). In compatible resyntheses, deep fungal penetration of algal cells was noted in partnerships where such intrusions were absent in the naturally occurring thalli (Ahmadjian et al. 1980). Significantly, the numerous attempts to cultivate or resynthesize lichens in a controlled environment have repeatedly shown that the symbiosis arises from a dynamic balance of fluctuating conditions that are not strictly favorable to either partner separately. Without this equilibrium, the symbiosis dissolves or cannot be established, and the fungus and the alga grow independently of one another (Thomas 1939, Scott 1960, Ahmadjian 1962, Stocker-Wörgötter 2001). For this reason, it may not be particularly informative to overemphasize symbiont behavior under conditions that are artificial or suboptimal for symbiotic development. It may tell us more about how the symbiosis first arose and the dynamic boundaries of its maintenance than about its current significance to algal symbiont fitness. Ahmadjian's interpretation of sporadic algal consumption is expressed in his provocative analogy: "To call a lichen association mutualistic is similar to believing that domestic cattle and humans have a comparable relationship because we provide them with food and shelter and increase their populations before we slaughter them" (Ahmadjian 1993, quoted in Chapman and Chapman 2010). The comparison is problematic, and not merely because the empathy it evokes has no application to the world of microorganisms. In most natural lichens examined, the destruction of algal cells, when evident, is actually quite minimal (Tschermak 1941). Although some have argued for its central importance (Webber and Webber 1970), consumption of algal cells cannot be the principal means by which typical lichen-forming fungi obtain carbon from their algal symbionts, as mass transfer of leaked photosynthate clearly indicates (Smith et al. 1969, Hill 1976, Smith 1980). And quite unlike cattle, the algal cells within a lichen thallus are not individuals but genetic clones resulting from mitotic divisions. If some of these cells are indeed sacrificed under certain conditions in some lichens, it is of no necessary consequence to the fitness of the genotype, which is conserved and perpetuated within the thallus.

For many observers, however, lichen "haustoria," fungal penetrations so named in reference to the absorptive structures produced by plant pathogenic fungi, irresistibly evoke parasitic interpretations. Intracellular invasion occurs principally in the simpler crustose lichens that lack organized layering of thallus tissues (Tschermak 1941, Plessl 1963, Galun et al. 1971, Honegger 1986a). The penetrated algal cells often appear healthy when examined under a microscope. The alga's frequent deposition of

additional wall materials as reinforcements surrounding the fungal intrusion (Tschermak 1941, Matthews et al. 1989) seems to highlight the balancing act through which symbiotic stability is achieved. In structurally complex lichens, unicellular phycobionts such as *Trebouxia* often respond to penetration by dividing into packets of spores with newly synthesized walls that shut out the haustoria, suggesting a mechanism by which the proliferation and distribution of algal cells may be coordinated by mycobiont growth and cell cycle control (Greenhalgh and Anglesea 1979, Honegger 1987, Hill 1989). But lichen haustoria do not show the extensively branched contact interfaces that multiply absorptive surface area in plant parasitic or endomycorrhizal fungi. Indeed, in most foliose and fruticose lichens, the so-called haustoria do not even fully traverse the algal cell wall or substantially invaginate it, and may be absent altogether (Tschermak 1941, Plesl 1963, Honegger 1986a, Tucker et al. 1991). Studies of carbohydrate flow from alga to fungus have not supported the hypothesis that haustoria play any prominent role in substance transfer (Jacobs and Ahmadjian 1971, Collins and Farrar 1978, Hessler and Peveling 1978). They might be homologues of absorptive structures deployed by parasitic ancestors, but from a functional point of view, the term *haustoria* as applied to lichens appears to be largely a misnomer. Unfortunately, naming them so has led to circular reasoning regarding their function and their implications for the lichen symbiosis. All evidence so far suggests that carbohydrate is not removed from phycobiont cells by fungal haustoria but rather released in quantity by the alga itself over its entire cell surface area (Richardson et al. 1968, Jacobs and Ahmadjian 1971, Hessler and Peveling 1978, Smith 1980). This is not the usual behavior of a cell being parasitized. Whereas a host/victim may expect to have its resources seized by the parasitizing fungus, the lichen phycobiont appears to hand them over proactively. Clearly, it is responding to symbiotic signaling, because shortly after isolation into culture, the alga downregulates carbohydrate release (Richardson et al. 1968). All this strongly suggests traits specifically evolved for symbiosis that are not easily reconciled with the view that the alga is a mere victim. And despite surrendering substantial amounts of carbohydrate, the algal cells in the lichen thallus usually appear to be growing and dividing with remarkable health and vigor. For all his colorful analogies to masters and slaves and spiders and their prey, Schwendener (1869) was also effusive in expressing his surprise that the presumably victimized algae were proliferating so luxuriously within the lichen thallus. This point is often passed over by those citing him in support of a parasitic interpretation of the lichen relationship. Schwendener (1869, 1872) certainly did treat lichen-forming fungi as algal parasites. But at the time of his groundbreaking recognition of lichens as dual organisms, fungal parasitism of photoautotrophs (e.g., de Bary 1866) was the only relevant interaction model in scientific circulation. Symbiosis and mutualism were novel concepts that had yet to be formulated in print (Frank 1877, de Bary 1879).

Perhaps the strongest challenge to a mutualistic interpretation could be made for the leaf-dwelling *Strigula* and its multicellular, subcuticular phycobiont *Cephaleuros* (figure 5). It is to this example that Chapman and Chapman (2010) give the most attention in their arguments favoring a parasitic view of the lichen symbiosis. In an ultrastructural study of *Strigula smaragdula*, Chapman (1976) observed most fungus–alga contacts to involve simple wall-to-wall apposition, whereas about 10%–20% showed mycobiont penetration into algal cells, both senescent and healthy looking; Chapman concluded that the poor condition of some cells was the eventual result of this penetration. Relatively large hyphal penetrations are evident in the micrographs provided, although most of the algal cells invaded actually look quite healthy.

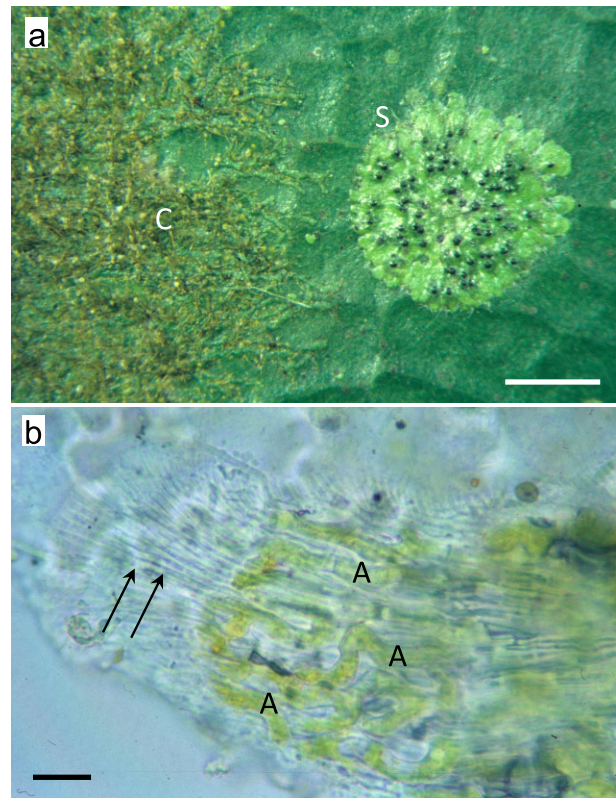


Figure 5. The subcuticular leaf-colonizing lichen *Strigula smaragdula* (S) at right, with an apparently unlichenized alga *Cephaleuros* (C) alongside it at left. (b) Lobe of *Strigula nemathora*, whole mounted. Healthy-looking algal filaments (A) are contained beneath an organized mycobiont layer composed of contiguous hyphae (the arrows), with no visible indication of the antagonistic interactions described by Ward (1884) for *S. smaragdula* and its algal symbiont. The scale bars represent (a) 1 millimeter and (b) 10 micrometers.

The *in situ* observations of Ward (1884) provide a sharper picture of antagonistic interactions between the symbionts, which need to encounter each other at compatible stages to establish an equilibrium. If the mycelium of *Strigula* encounters a germinating spore or few-celled germling of *Cephaleuros*, the alga is overpowered and destroyed; the fungus may then produce asexual spores but will not be able to form sexual fruiting bodies and complete its life cycle. Only when a well-established *Cephaleuros* is contacted can a stable balance be achieved. One portion of the multicellular algal body may be engulfed; the remainder continues independent development, although the alga may end up substantially consumed (Ward 1884). Both organisms reproduce sexually and independently under these conditions, with no codispersal of symbiotic propagules as occurs in so many other lichens. The alga manages the situation, but it is not easy to see how lichenization could be advantageous to it.

On the other hand, because the abscission of the leaf substratum will soon doom all colonists anyway, the eventual destruction of the alga might not be of much consequence if it completes its reproductive cycle beforehand. It would be interesting to determine whether the aggressive dynamics reported by Ward (1884) are typical of other species of *Strigula* under normal microhabitat conditions (figure 5b). In any event, the *Strigula*–*Cephaleuros* relationship cannot be considered typical of lichen symbioses. The unique position of the alga beneath the cuticle of the living leaf already provides it with important protections from hydric stresses and high radiation load. A lichen-forming fungus may have

little to offer in the way of additional benefit to an alga established in such a place. If that is the case, it would not be in the alga's interest to surrender its carbon resources to the mycobiont. If indeed *Strigula* normally "consumes" *Cephaleuros*, one may hypothesize that the relationship lacks the symbiotic carbohydrate transfer system present in more conventional lichens. Exploring this issue in *Strigula* and any other seemingly aggressive mycobionts could clarify whether there are distinct functional categories of lichen symbiosis, with potentially different implications for the algal partner.

Trebouxia and the question of symbiont dependence

The most persistent modern advocate for a parasitic interpretation of the lichen relationship simultaneously promoted another opinion, seemingly irreconcilable with parasitism: that *Trebouxia*—the most prevalent genus of lichen algae—is fully dependent on its mycobiont and incapable of independent, free-living existence (Ahmadjian 1988, 1993, 2001). Ahmadjian (2001) insisted that *Trebouxia*, which shows heterotrophic tendencies when isolated in culture, actually receives its carbohydrate from the fungal partner in what he described as a paradoxical reversal of roles. At present, there is little evidence supporting this intriguing hypothesis (but see Kosugi et al. 2013). Remaining unaddressed is how *Trebouxia* can be a victim of fungal parasitism—and therefore better off without the fungus—while at the same time so utterly dependent on it that the alga is incapable of surviving on its own. The issues raised by symbiont dependence were discussed by Smith and Douglas (1987) and Douglas and Smith (1989), who were skeptical that internal symbionts such as lichen algae receive benefit from symbiosis. They withheld judgement, however, arguing that any putative benefits cannot be evaluated once symbionts become fully dependent on a host and no longer exist in the free-living state for comparison. They stressed that becoming dependent does not imply improved fitness, because it involves the complete loss of alternatives.

Ahmadjian's belief that *Trebouxia* suffers from such dependence was influenced by his extensive experience culturing this phycobiont, which grows very slowly unless media are supplemented with glucose. But *Trebouxia*'s facultative heterotrophy in culture does not necessarily tell us anything about its aptitude for independent, autotrophic existence in nature. Similar heterotrophic behavior in culture has also been reported for *Apatococcus* (Gustavs et al. 2016), one of the most abundant and widely encountered aeroterrestrial green algae. In any event, the numerous reports of free-living *Trebouxia* that have accumulated in the last few decades (Tscheramak-Woess 1978, Bubrick et al. 1984, Cambra and Hernández-Maríné 1989, Mukhtar et al. 1994, Gärtner and Stoyneva 2003, Sanders 2005, Handa et al. 2007, Hedenås et al. 2007, Uher 2008, Wong et al. 2010, Neustupa and Štifterová 2013, Yung et al. 2014) should be sufficient to counter any further claims that this algal genus occurs only in lichen symbiosis.

Although we still know next to nothing about the character and dynamics of the free-living populations, they almost certainly make use of zoospores and flagellate gametes. With no possible role to play within the lichen thallus, the zooids are nonetheless repeatedly observed in cultured isolates of *Trebouxia* and its sister genus *Asterochloris*, with sexual fusions occasionally noted (Ahmadjian 1960, Škaloud et al. 2015). Although Ahmadjian (1988) held that the flagellate cells represent a vestigial trait without utility in the contemporary lives of these algae, such a scenario is

highly unlikely. Were there no selection pressure for their maintenance, the genes underlying flagellar function would soon accumulate crippling mutations, in the same way that vision is inevitably lost by animal lineages that adapt to life in total darkness. Functional zooids are yet another indication that *Trebouxia* and *Asterochloris* have an existence apart from their mycobionts, an existence likewise subject to natural selection. The question of benefit can therefore be legitimately addressed without the complication of dependence.

Are lichens prisons or refuges for phycobionts?

When evaluating putative algal benefits from lichenization, one might concede the protective advantages discussed above but question whether they are worth the expense. Lichenization comes at a high metabolic cost to the alga; some 30–90% of the carbon it fixes may be transferred to the fungal symbiont (Hill 1976, Smith 1980). And sometimes, free-living and lichenized individuals of the same alga may be observed growing right next to each other (figure 5a), a situation cited as evidence that lichenization offers no real benefit to the phycobiont (Chapman and Waters 2001, Chapman and Chapman 2010).

Under such circumstances, it may seem legitimate to judge the lichenized alga at a disadvantage. It must forgo sexual reproduction and divert much of its resources to supporting its mycobiont, a seemingly unnecessary burden when free-living individuals thrive alongside it. By analogy, one could invest substantial resources in a protective insurance policy for several years without needing to make a claim and thereby prosper less during that period than a neighbor who made no such expenditure. But evolutionary fitness, like risk management, plays a longer game. The onset of adverse conditions or events may quickly change the calculus of whether the free-living or lichenized state was ultimately more advantageous. (Of course, algae do not calculate anything; the insurance analogy merely serves to show how some benefits of lichenization may not be evident over the short term.) Among the fittest strains of aeroterrestrial algae should be those that can hedge their bets by maintaining a presence in both free-living and lichenized populations. And indeed there are many indications that lichen phycobionts manage this.

An amusing cartoon included in *The Lichen Symbiosis* (Ahmadjian 1993's figure 3) shows algal cells with unhappy faces imprisoned behind hyphal bars by a mycobiont jailer, conveying the author's view that the alga suffers disadvantageous captivity within the lichen. But are phycobionts really captives? Assessment of their relative fitness would seem to depend on the answer, because genetic mixing is vital to the continued success of the algal lineage but is repressed within the thallus. Hill (2009) saw this as a major problem for the coevolution of the symbionts; he held that algal symbionts are not perpetuated when a thallus dies.

But regardless of what might become of the phycobionts when a lichen degenerates, algal symbiont clones take numerous opportunities to emigrate long before then. Lichens produce a wide variety of vegetative propagules that disperse fungal and algal components together. The wart- or lobe-like isidia that arise as corticated outgrowths of the upper surface, and the tiny granular soredia that erupt from below the cortex, are the two best-known examples of the diverse vegetative propagules produced in abundance by a sizeable percentage of lichens (Bailey 1976, Büdel and Scheidegger 2008). Aside from specialized propagules, thallus fragments readily detach and disperse both symbionts under the force of

wind, rainstorms, and animal trampling (Peirce 1898, Ullrich 1955, Bailey 1976, Armstrong 1990, Renhorn and Esseen 1995). The fragments contain viable symbiotic cells that can reproduce the lichen under the right conditions (Dibben 1971). But local conditions will likely favor independent growth of the alga in at least some of the microsites where dispersed fragments or symbiotic propagules end up.

In a number of lichens, particularly among those that colonize leaf surfaces, miniaturized phycobiont cells routinely attach to the surfaces of ejected ascospores, conidiospores or diahyphal propagules and travel with them, some detaching along the way (Stahl 1877, Sanders and Lücking 2002, Lücking 2008, Sanders 2014, Sanders and de los Ríos 2015, Sanders and Brisky 2022). Even in lichens without such specializations, algal cells have been reported to escape from the edges of thalli (Pyatt 1973) or grow emergent from the thallus (Reinke 1896's figure 205 II), and may sometimes also be fortuitously dispersed with ascospores (Pyatt 1973). There is substantial evidence that lichenivorous invertebrates such as snails and mites deposit viable phycobiont cells in their feces, suggesting that these animals may be significant vectors of algal dispersal (Frøberg et al. 2001, Meier et al. 2002, Boch et al. 2011). Recent environmental sampling studies have revealed the abundant presence of important phycobiont genera (*Asterochloris*, *Trebouxia*, *Coccomyxa*, *Chloroidium*, *Diplosphaera*, *Heveochlorella*, *Myrmecia*, *Symbiochloris*, etc.) on diverse natural and man-made substrata (Darienko et al. 2013, Hallmann et al. 2013, 2016, Yung et al. 2014, Zhu et al. 2018, Metz et al. 2019). The considerable genetic diversity present in major phycobiont lineages such as *Trebouxia* (Kroken and Taylor 2000, Muggia et al. 2020) further suggests that the algae are taking advantage of these periods of liberty to carry out sexual reproduction.

In the broad picture that emerges, the lichen thallus more closely resembles a base rather than a prison for algal symbionts. The rent may be high, and sexual activity prohibited, but from this sanctuary, a phycobiont genotype manages risk. Some of its emigrant clones will have the opportunity to generate new free-living populations and new lichens, with the lichenized populations functioning as more stable genotype banks that conserve algal clones in relative safety.

Conclusions: Alignment of symbiont interests

The elaborate fungal structures that protect the algal symbiont and optimize its access to light, moisture and carbon dioxide are, of course, really serving the mycobiont's own interests. This obvious fact is sometimes cited with the insinuation that symbiont selfishness is itself evidence against mutualism. But there can be no altruism among microorganisms. When symbiosis works mutually, it is only because the partners' interests substantially coincide. To the extent that the lichen-forming fungus is invested in sustaining its phycobiont population—as opposed to consuming it and moving on—the interests of the algal partner are also promoted. Repression of the algal sexual cycle is the usual situation for internal symbionts, because the production of genetic variants internally can be problematic for the maintenance of symbiont compatibility (Law and Lewis 1983).

Ultimately, however, the lichen mycobiont counts on the dispersal, independent proliferation and sexual reproduction of the algal symbiont for its own success. This is because most lichen-forming fungi disperse aposymbiotic ascospores or basidiospores in addition to—or instead of—symbiotic propagules. These mei-

otic spores are much smaller and potentially travel much greater distances than the symbiotic propagules, but their success depends on encountering compatible algal symbionts at their destination (Werner 1931, Clayden 1998, Sanders 2014). The algal genotype of the parent lichen might not be the optimal partner for the new fungal genotype that develops from the spore, and may not be best suited to the conditions of the new habitat, either (Peksa and Škaloud 2011). It is therefore in both partners' interests to be able to separate and recombine selectively in the course of their life cycles.

Because of the inherent asymmetry in the partners' needs (Hill 2009), the lichen symbiont relationship will never be one of parity. The alga can always be nutritionally autonomous; the heterotrophic fungus cannot. Natural selection has therefore shaped lichen-forming fungi to be highly specialized for symbiosis, and phycobionts to explore multiple options. Only under the contingencies of mutual deprivation are the partners induced to collaborate. There is much that remains to be clarified concerning the relationship between lichen symbionts, and many new tools have become available to approach such questions at different levels. But unless one holds biological mutualism itself to be illusory, there is little reason to dismiss lichen symbiont mutualism as a myth. Although the relationships may be varied and dynamic, most lichens thrive under conditions that align both symbionts' long-term interests. Central to this alignment is a fungal life strategy that revolves around maintaining and perpetuating its phycobiont. From the alga's perspective, whenever survival is at stake, maintenance and perpetuation will count as benefits well worth the costs of symbiosis.

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