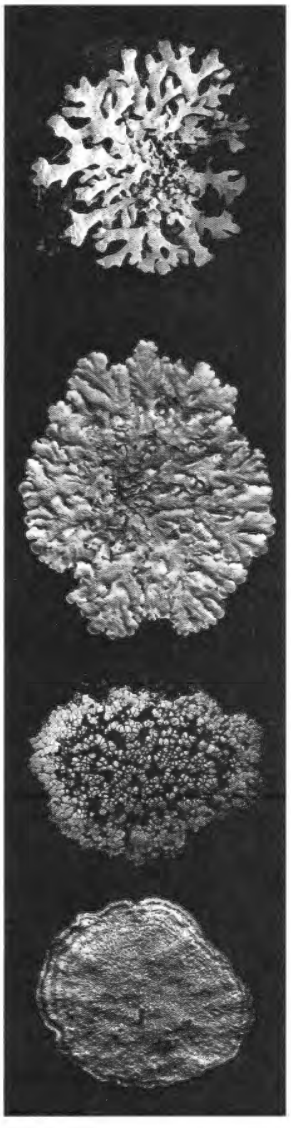
Chapter Five

Radical Lichenology

By Nastassja Noell

Lichens are expressions of pure joy. Their variety of color, morphology, chemical constituents, and inexhaustible capacity for adaptability is proof that the living world is not purely utilitarian. Lichens remind us that life is art and that deeply integrating into one's environments is the most refined expression of that art. Lichens paint the rocks of the desert with living murals, drape the temperate forests with lace ribbons, and thrive in the harshest of climatic conditions. From Antarctic to tropical systems, from rainforests to deserts, lichens are ever-present, showing us a way of fungal being that is always exposed, always present. Humbly, they slowly grow by crystallizing sunlight and vapor[1] into delicate but resilient symbiotic systems. Inside the ecosystem of a lichen are most of the primary components of life: fungi, bacteria, algae, and cyanobacteria, all living in a discrete synergistic system that can rarely be synthesized *in vitro* but can withstand the extreme conditions of outer space.



Lichens form a terrestrial version of kelp forests and coral reefs. Like their cousins the aquatic algae, lichens absorb all their nutrients from their surroundings: the sea of vapor permeating the terrestrial world. Without a fungal partner, such a lifestyle would be nearly impossible for the algae in lichens. The fungal symbiont creates a thick protective skin around the algae to protect it from desiccation. In exchange, the algae gives the fungus photosynthesized sugars. And together, they form shapes and pigments that help them survive and thrive in their other-worldly surroundings.

This symbiosis of fungus and algae is thought to date back to the first ancestors of terrestrial life. As landforms diversified and developed, as mountains rose defiantly and weathered into soft hills, lichens have been patiently watching from their perches. Some contemporary lichens are over 5,000 years old—relics from a distant age. Lichens are the beholders of stories on landscapes and climate, if one takes the time to witness them clearly.

Though their slow and ancient nature lends to lichens being lost in the shadows of the larger members in their ecosystems, these fascinating beings are not static. Rather, they perform numerous mutualistic roles with bacteria, insects, rodents, ungulates, and humans. And there are four main reasons that humans work with lichens: as medicine, as a natural dye source, to monitor environmental health, and to study ecosystem biodiversity and dynamics.

What is a Lichen?

A lichen is not a singular organism but a symbiotic relationship between a fungus and algae and/or photosynthesizing cyanobacteria. What we see when we look at a lichen body—technically known as the *thallus* (pl. *thalli*)—is a complex structure comprised largely of these partnered organisms.

The Mycobiont

Over 95% of the lichen thallus is the fungal partner, or *mycobiont*. Compared to the underground mycelial networks of wild mushrooms, the fungal mass of a lichen lives almost entirely exposed to harsh conditions that would kill most non-lichenized fungi. The fungus protects the lichen from the harsh above-ground environment by building a *cortex*, a dense layer of fungal tissue that prevents water loss, and a *medulla*, a fluffy, hydrophobic network of hyphae in the interior of the lichen. Inside the thallus is where most of the lichen magic happens, from the production of powerful medicinal compounds to the mind-boggling interplay between bionts.

Curiously, research on the 14,000 known species of lichenized fungi (around 3,000 species are thought to be currently undiscovered) demonstrates that lichen mycobionts do not form a common clade on an evolutionary tree (i.e. they don't have a common ancestor). Rather, the lichen relationship seems to have independently arisen at least five times over the eons, demonstrating the success of this relationship and serving as a clear example of convergent evolution. These relationships can also shift over time, as shown in genetic analyses that suggest some major lineages of currently free-living fungi were previously lichenized.[2] About one-fifth of all known fungal species are lichenized and nearly all of these fungi are obligate symbionts. Over 98% of lichenized fungi belong to various branches of the Ascomycota. There are some lichenized Basidiomycetes; most of these are found in tropical regions, but some live in boreal and temperate regions.

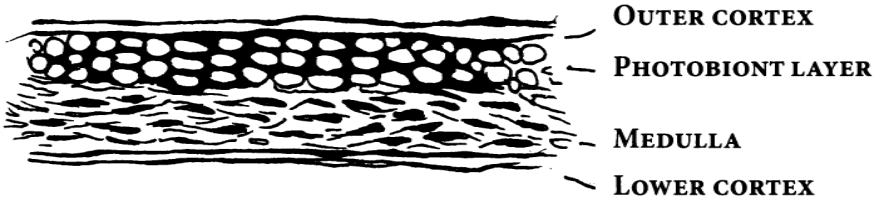


Diagram of a typical stratified lichen.

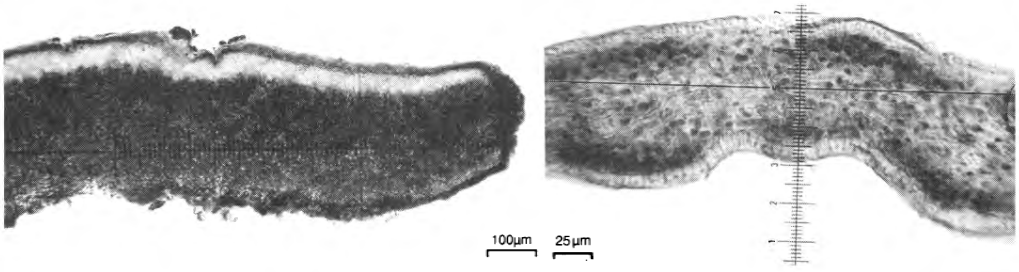
The Photobiont

The photosynthesizing partner (*photobiont*) is what makes the lichen an autotrophic organism. About 30 species across two kingdoms are known to act as the primary photobiont within lichens. Most photobionts are eukaryotic green algae species, though about 10% of lichens have a prokaryotic cyanobacterium as a photobiont. Approximately 4% of lichens contain both algae and cyanobacteria as photobionts.[3]

Lichens that contain a green alga as their photobiont are called *chlorolichens*. Lichens with cyanobacteria as their primary photobionts are called *cyanolichens*. *Tripartite lichens* have both types, with the cyanobacteria generally being contained within a specialized structure called a *cephalodium*. Cephalodia are not well studied, but they seem to help maximize nitrogen fixation in the thallus in a manner similar to the anaerobic gas chambers of root nodules in legumes.

In the lichenized state the photobiont's natural life cycle is usually suppressed. Filamentous algae and cyanobacteria are often deformed into shorter filaments or unicellular states, and cyanobacteria cells are typically larger than in the free-living or cultured state. Sexual reproduction is also suppressed within the lichen, and it is assumed that most algal growth in the lichen occurs primarily by asexual means. But asexual reproduction is not always as simple as cell division and may include sporulation by flagellated motile zoospores, which are occasionally found within the lichen thallus.[4]

Structurally, the primary photobiont forms a green or blue layer underneath a protective *outer cortex* of dense hyphae. This outer cortex acts as the glass roof of a greenhouse of sorts and regulates gas and moisture exchange, providing a homeostatic environment for the photobiont to thrive. This photobiont layer is nested within the upper part of a loose, cottony, hydrophobic layer of hyphae called the *medulla*.



(Left) Cross-section of a chlorolichen. Note the distinct stratification of the green algal layer and the gray fungal medulla, as well as the thickness of the outer cortex (the white tissue above the green algal layer that is melanized brown at the very edge).

(Right) Cross-section of a cyanolichen, the Jelly Lichen Leptogium lichenoides. *Note that the cyanobacteria and the medulla are intermixed in the interior and the outer cortex is very thin.*

The Relationship Between Bionts

While the fungus comprises the bulk of the thallus, it is by no means the dominant partner. For example, the photobiont is just as responsible as the mycobiont for determining the shape of the thallus that will maximize photosynthesis. These shapes take a variety of forms, often reflecting the lichen's habitat and substrate. They may be leaf-like structures clinging to hillsides, or flat crusts on rocks that capture light coming from one direction in a way that is akin to solar panels. Or they may be hair-like curtains draping from tree branches that can capture diffused light in misty habitats, or a blend of all of these shapes. As with mushrooms, it is the unique blend of these and other features that define a given lichen species.

Lichens have been found in recent years to be comprised of much more than just two or three partners. More like a miniature ecosystem, a lichen thallus can also contain hundreds of other microbes and fungi. These organisms include *epibiont bacteria* that live on the surface of lichens and may play key roles for cell wall function and nitrogen fixation.[5] There are also *endolichenic fungi* that seem to be cohabitating inside the lichen thallus performing unknown beneficial functions. Endolichenic fungi cannot be seen with normal light microscopes and do not create symptoms in the lichen. Other fungi grow on the surface of lichens. Some of these *lichenicolous fungi* have been found to be parasymbiotic, often with unknown relations to the lichen, while most others, such as *Carbonea* species, are purely parasitic. This group includes some odd parasitic Basidiomycete fungi that grow on lichens, such as *Biatoropsis usnearum* and *Cystobasidium usneicola* which form galls on *Usnea* species. Most of the 3,000 known species of lichenicolous fungi are obligate to a particular genus or family of lichens. However, as our understanding is currently limited about their range, distribution, and biodiversity, more research is needed to form a more thorough analysis of their roles and niches.

Some lichenized fungi are transiently parasitic. In the early phases of their life cycle they live on or within an established lichen thallus in order to take algae from the lichen host. In some instances, this new fungus-photobiont pair may even take over the original lichenizing fungus and form a completely new type of free-living lichen.[6] Phylogenetic evidence suggests that a tremendous amount of algal switching occurs between lichens.[7] Further, as there are so few photobiont species compared to mycobiont species, it has been suggested that the lichenized fungi may be suppressing the algae's ability to reproduce in an effort to stabilize their genetics and reduce speciation events. This might explain why *Trebouxia* species are not found free-living, despite being the most common photobiont in lichens. *Trebouxia* may have evolved to be dependent on the lichen biome and is only able to survive and procreate through the algal swapping between lichen thalli. As lichenology is still in its infancy—much more so than mycology—more research needs to be done to determine where the line is drawn between what does and does not constitute the lichen microbiome.

Lichen Needs

Lichens crystalize airborne water and nutrients into complex molecular arrangements that are used to build their bodies, construct secondary chemicals, and create microhabitats that favor their niche. While lichens have adapted to nearly all terrestrial habitats, there are a small number of ecological constraints that lichens must creatively respond to in order to thrive:

- **Water:** Unlike most plants, lichens do not have a vascular system that conducts water throughout their thallus. Rather, lichens absorb water from mist, dew, rainwater, waterfall spray, ocean air, and the humid microclimates created by moss. Whatever is in this water (e.g. salts, heavy metals), the lichen will absorb.
- **Nutrients:** Lichens lack true mycelia in the sense that lichen hyphae do not penetrate the substrate that they grow on in order to absorb nutrients. Most of these nutrients are obtained from rainwater and airborne particulates. A smaller amount are provided by water that has leached nutrients from deciduous tree bark, limestone boulders, or other substrates and subsequently dripped onto the lichen. Some species prefer certain nutrient sources. *Nitrophiles* are lichens that thrive in areas impacted by nitrogen pollution, such as agricultural areas or near popular bird roosts. *Calciphiles* thrive on calcium-rich substrates, such as limestone or calcium-rich soil. The pH of the substrate or water source is critical as most species have adapted to a particular pH range. Limestone and deciduous tree bark generally have a high pH; coniferous trees and granitic rocks tend to be nutrient poor and have a low pH.

Seeing Lichens

Lichens embody the principle of symbiosis at many different scales, yet their misrepresentation throughout history has led to their ecological importance being overshadowed by shortsighted descriptions of their internal and external dynamics. Depending on how one chooses to perceive Nature, the lichen symbiosis is often described in one of three ways:

Reductionist Perspective

A lichen is a symbiotic organism, composed of an alga or cyanobacterium and a fungus. This relatively bland description—if indeed it says anything at all about the other organisms living in the lichen thallus—treats each biont as an isolated entity that reacts predictably and mechanically to the other bionts one at a time. The reductionist extracts a fungal spore, tries to grow it in isolation in a plate of agar, and is stumped when the resulting undifferentiated slime refuses to magically turn into an elaborate and colorful lichen when she drops an algal cell into the dish.

Mycocentric Perspective

A lichen is a dietary choice of a fungus—a fungus that discovered agriculture. In this perspective, the mycobiont is said to create a structure that is similar to a greenhouse, solely to provide a beneficial growing environment for the algae that keeps it alive. Like a good farmer, the mycobiont produces sunscreen-like compounds that protect the algae from harmful UV radiation during dry periods, anti-herbivory and anti-microbial compounds that reduce predation, and a three dimensional structure that regulates moisture and gas levels within the lichen in response to the growth needs of the algae. The algae, in this perspective, is scarcely distinguishable from the substrate or air surrounding the lichen; it supplies photosynthetic sugars to the fungus and that's about it.

Systems Perspective

A lichen is an ecosystem, it is an emergent property. In the systems perspective, a lichen is understood to be entirely different than the sum of its constituent organisms. Such cumulative associations are found in the experience of consciousness arising from the random firing of individual neurons and in the collaborative power of a worker's cooperative or social movement. The systems perspective of lichens requires a conceptual leap that challenges traditional biological concepts of species and the linear phylogenetic arrangement of the Tree of Life. Within the systems perspective, the ecology of the lichen symbiosis is emphasized over the individual roles of each biont, and the boundaries between the bionts blur. Lichens are understood as ecosystems, where both autotrophs and heterotrophs are present and in balance, and gas and nutrient exchange between the two bionts creates a miniature biosphere that regulates the temperature, moisture, and light and gas levels of the system in relation to its surrounding environment. The shape, features, colors, and morphological particularities of lichens are a dynamic and complex interplay of the bionts, the lichen, and the surrounding ecosystem.

- **Substrate:** Given enough time, lichens can grow on nearly any surface, from rotting couches, to rusting metal, to evergreen leaves and desert soil. But the finely tuned nutrient, water, and light requirements of most lichens tend to limit a species' growth to one type of substrate. *Epiphytes* grow on trees or shrubs, *saxicoles* grow on rocks, and *terricoles* grow on soil.
- **Light:** Light is critical to the generation of photosynthates and, indirectly, the secondary metabolites that protect the lichen from parasites and herbivores. Lichens usually thrive in areas that have intense to diffuse light, however many species, such as the pin lichens, are adapted to grow in darker habitats like the underside of logs or in rock crevices.
- **Water and light regimes:** Unlike most fungi and plants, lichens are *poikilohydric*, meaning they can readily withstand desiccation. During dry periods, lichens go into a dormant state that can last more than 100 years. When these dormant thalli are rehydrated they can return to life within minutes and begin photosynthezing. During periods of darkness the lichen cannot produce additional photosynthates. If the lichen does not enter dormancy via dessication it will eventually run out of the materials to produce secondary metabolites, making it more susceptible to parasitic fungi and bacteria, particularly if temperatures are warm and the climate is moist (accelerating the fungal metabolic processes).

Lichen Chemistry

The range and complexity of the chemicals and secondary metabolites produced by lichens is almost unparalleled by other similarly sized groups of organisms. Most lichen species have a distinctive chemistry that tells a story about the habitats they call home, the challenges they've encountered, and their adaptive resilience. Many of the pigments that give lichens their range of colors have been found to act like a sunscreen, reflecting or absorbing light (e.g. atranorin), while other lichen chemicals stored in the interior medulla of the lichen have been found to have anti-herbivory properties against snails (e.g. gyrophoric acid). Most lichens also have antibiotic properties that are effective against fungal and bacterial parasites (e.g. usnic acid). These compounds can be used as medicines for humans. While chemistry can be used to distinguish lichens at the species level, most chemicals are not limited to a particular genus or family. The vast multitude of secondary chemicals can be found within most families or orders of lichens. The phylogenetic implications of this may be suggestive of genetic bottlenecks, such as during the K-T extinction, or of the horizontal transfer of genes between different groups of lichens.

The Reproductive Structures of Lichens

Though the algae and fungi in a lichen cohabitate, they do not share DNA. Both organisms reproduce independent of the other, and a lichen as a whole may have multiple ways of replicating itself. The mycobiont tends to reproduce much like other fungi, often through the production of sexual spores and/or asexual conidia. The Ascomycete lichenized fungi have sexual reproduction patterns that tend to reflect those of their mushroom-forming kin, especially the Cup Fungi in the order Pezizales. In Ascomycete dominant lichens, the spore producing ascomata are generally apothecia or perithecia. Some Basidiomycete lichens do exist, however these are rare and often mistaken for mushrooms. The fruiting body structure in a Basidiomycete lichen is often similar to an agaric mushroom, however the mycelium and associated green algae form a distinct, superficial vegetative thallus (e.g. the basal scales of *Lichenomphalia hudsoniana*). The exception is the basidiolichen *Dictyonema s.l.*, which looks and feels like a polypore mushroom, but the photobiont lives in the interior of the thallus.

Apothecia.

Perithecia with asci filled with sexual ascospores. Pycnidia look similar but contain asexual conidia.

After sporulation by the ascomata or basidiomata, the spores grow independently for a short while until an appropriate photobiont is found. These spores take on a variety of shapes, sizes, colors, and forms. As with mushrooms, the general spore types are usually consistent across genus or family. Most lichens also have asexual fruiting bodies (*conidiomata*) that produce conidia. In some species, conidia have been found to act as spermatia, fertilizing another lichen through a structure known as a *trichogyne*.

Interestingly, most lichens also feature one or several methods for asexual cloning of the lichen itself. Instead of, for example, producing new spores with unique DNA sequences, these lichen clones are little bundles of fungal hyphae containing several photobiont cells. These bundles, called *diaspores*, bud off and fall away or are carried by the wind to new habitats where they serve as "seeds"—cottony clones of the mother lichen—that will eventually grow to be a new thallus. Diaspores can be dispersed hundreds of miles on the feet of traveling birds and in the air currents of the upper atmosphere, or more locally on the backs of insects and animals. Often the diaspores simply fall from the mother lichen to establish on a lower branch or below a host boulder.

When a diaspore lands on a suitable substrate and the right moisture and nutrients are present, it will first grow rhizomorph-like structures over the surface of the substrate. From this structure the thallus' tissues will begin to grow from the center outwards on top of the substrate, forming the cortices, the medulla, and a layer of photobiont cells. Diaspores come in two main forms:

- **Isidia:** These diaspores grow from within the medulla and push up through the cortex, bending the cortex around the diaspores, forming a protective cortex that then breaks off in finger-like pieces.
- **Soredia:** These diaspores lack any protective cortex. They are granular outgrowths of the medulla that grow up through openings in the cortex called *soralia*.

Isidia Soredia

Diaspore-producing lichen species have distinct morphologies that aid in their identification. Lichens that produce isidia will not produce soredia, and vice versa. The diaspore type and location of origin are significant characteristics that likely reflect speciation events in the evolution of a particular group of lichens. Most sorediate and isidiate species will also occasionally still produce viable apothecia (in addition to their usual asexual diaspores), however there are a few species (e.g. *Lepraria spp.*) that have never been observed in the sexual state. Very rarely one will encounter sorediate or isidiate forms of species that normally do not produce diaspores. This terminology might be confusing at first, but just remember that diaspores contain both algal and fungal symbionts and thus reproduce the whole lichen as a clone, while spores reproduce only the mycobiont.

Lichen Phylogenetics

The Mycobiont

About one-fifth of all fungi are lichenized and nearly all of these fungi are obligate symbionts to the lichen: they cannot carry out their entire life cycle without their symbiotic partner. Although all lichens share a similar nutrient acquisition strategy of deriving photosynthates from a phototrophic organism, lichenized fungi do not form a common evolutionary group, or *clade*. Depending

on the researcher, around five clades of Ascomycete fungi are considered lichenized, and some of these clades include both lichenized and non-lichenized fungi.[8] Additionally, phylogenetic research suggests that some lineages of non-lichenized fungi were previously lichenized (e.g. the chemically powerful genera *Aspergillus* and *Penicillium*[9]), suggesting that lichenization is not necessarily genetically predetermined, but rather a system built by the bionts that can be abandoned in certain situations, such as has been found in some *in vitro* culturing situations.

Most lichenized fungi (about 98%) belong to various branches of the Ascomycota, but little is known or speculated as to what characteristics make ascomycetous fungi more favorable to the lichen symbiosis than other fungal groups. To turn the question on its head, we might ask, why have lichenized Basidiomycetes not evolved and diverged with the same exuberance as their ascomycetous brethren? Basidiolichens make up only 2% of all lichens.[10] The answers to these questions remain highly speculative at best.

The Photobiont

About 100 taxa of algae and cyanobacteria are known to act as photobionts within lichens.[11] Although there are relatively few species of photobionts in comparison with mycobionts (ca. 17,000 taxa), the potential photobionts range across different kingdoms. The favorite photobiont for lichens is by far the eukaryotic green algae, which belong to the Plant Kingdom, while the less prevalent prokaryotic cyanobacteria are part of the Eubacteria Kingdom. The Verrucariaceae lichens—some of which are renowned for growing on tidal rocks where nothing else can grow—include species with a red algal photobiont from an unknown and unresolved kingdom, and one species with a brown algal photobiont from the Chromalveolata Kingdom.

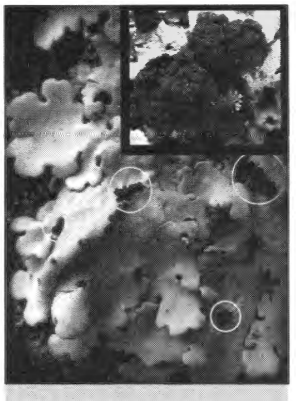
Proper identification of a photobiont requires culturing in order to see the distinguishing morphological features associated with different parts of its life cycle. Due to the unstable taxonomy of algae and bacteria, most photobionts are known to genus at best. Due to these limitations, there may be many unnamed species or even genera of photobionts and a far greater diversity of photobionts than is currently understood.

Coevolution of photobionts and mycobionts has not yet been demonstrated by phylogenetic research, rather it appears that specialization is unidirectional. Molecular research suggests that lichenized fungi are extremely faithful to a particular set of photobiont species and evolved to adapt to that species or species group. The reverse does not hold up in molecular analysis: while photobiont species are found to associate with a wide range of lichens, most are also found free-living. Existing phylogenetic research does not yet demonstrate that lichens harbored their evolution and diversification. But there are exceptions, including the green algae *Trebouxia* (a huge exception considering it is the most common lichen photobiont) and *Myrmecia*. These algae are rarely found free-living and the lichen symbiosis appears to be their primary mode for growth and dispersal.

The Classification of Lichens

For various reasons, lichens are taxonomically classified by the fungal partner, not the photobiont, nor the symbiosis of the two entities. This taxonomic system is challenged by some lichenologists who emphasize that a lichen is the combination of two genomes, thus the sum is distinctly different than the single fungal genome. Taxonomic classification based upon symbioses between two or more organisms challenges the linear-hierarchical system of Linnaean taxonomy. An emergent classification system would have to be born from the old standard system.

Recent research is showing that there is also a range of associated bacteria whose relationships to the lichen symbiosis are currently unknown, but they are very specific to the lichen thallus.[12] The bacterial flora on the surface of a lichen is often distinctly different from the flora of the surrounding soil. Research into the roles of these organisms in the lichen symbiosis is an emerging area of research.



The white foliose lichen is built by the same fungal species as the brown corraloid Dendriscocaulon *lichen (insert). Why do they look so different? They have two different photobionts. Until traditional taxonomic concepts can incorporate co-dominance of bionts, these lichens are considered the same species:* Lobaria amplissima.

Photosymbiodemes: Why Photobionts Matter

Photobionts are not merely passive photosynthesizing cells that are subordinate to their fungal superiors. Hardly. Subordinance is rare outside the animal kingdom. Although the extent to which different photobionts drive lichen morphology is not clear, there are some striking examples of photobionts that completely change the structure and function of a particular lichen symbiosis. A good example of this is found with the branching fruticose cyanolichens in the genus *Dendriscocaulon*. Until recently, *Dendriscocaulon* was considered taxonomically unrelated to the foliose (leaf-like) genera *Sticta* and *Lobaria* because the morphologies of the two groups are so strikingly different. But recent molecular research has shown that the mycobionts in Dendriscocaulon are identical to some of those also found in the *Sticta* and *Lobaria* genera; the only difference is the photobiont. These trans-photobiont pairs, called *photosymbiodemes*, calls the concept of a mycocentric lichen taxonomy into question. For this puzzle to be adequately resolved, the phylogenetic Tree of Life would have to anastomose, creating a Mycelium of Life in which species are the nodes.

Lichen Biodiversity and Bioindications

From coastal deserts to tropical rainforests, from temperate deciduous and coniferous forests to prairies and talus slopes, anywhere you look, lichens thrive in vast numbers. They are so globally abundant that 6% of the Earth's land surface is estimated to be covered in vegetation dominated by these miniature ecosystems.[13] Yet, despite their ubiquity, it is surprising to find that only a few lichen species are globally distributed.

With no roots, mycorrhizal structures, wings, or feet, lichens are specially adapted to the quality of the air and type of climate that surrounds them, as well as the structure and nutrient cycles of their habitat. Worldwide, the highest biodiversity of lichens tends to be found in areas with a mosaic of diverse habitat types, different levels of continuity (e.g. ancient forests mixed with various seral stages of forest), and, of course, clean air. In general, the more diverse the topography and potential substrates, and the more pristine the habitats, the greater the lichen biodiversity. Thus, some of the best lichen hotspots in the world include the Great Smoky Mountains of southeastern United States, the Yukon of Canada, the tropical mountain systems of the northern Andes, the Himalayas and the Central American highlands. Other optimal sites include temperate rainforests in northeastern China and the Pacific Northwest of the United States, the forest and bogs of northern Ireland and Iceland, and southern Chile and Argentina. Even Antarctica hosts at least 484 species of lichens; of these more than 60 are not found anywhere else in the world.

In many of these areas *oligotrophic* lichens tend to dominate. These species prefer low levels of nutrients in the air and water, making their presence a strong indication of pristine air. These habitats tend to be rather stable and homogenous, so lichen biodiversity tends to only be increased by small-scale disturbances, such as a small fire or the falling of a large tree. A mosaic of small, isolated disturbances help increase light into the forest or release ash-borne nutrients into the canopy of trees. Both of these events can help increase lichen establishment and growth.

Conversely, large-scale disturbances greatly threaten lichen health and diversity. This is seen most clearly in urban centers—our modern "lichen deserts"—where air pollution and the destruction of habitats is directly correlated with a lack in lichen diversity. Most lichen species are so sensitive to the effects of acid rain and heavy metals that they will slowly disintegrate and die when exposed to the air around industrial areas. Some pollution-tolerant macrolichens, such as *Physcia* species or the bright orange species in the genera *Xanthoria* and *Xanthomendoza,* are often the only species found in heavily polluted areas, and their dominance is usually a clear indication of low air quality.

The distribution ranges of different lichen species also provide interesting insights into not only lichen biogeography, but also into the history of a place. Some lichens are endemic to very small areas, suggesting they are relics of a Pleistocene climate or remnants of a rapidly disappearing hab-

itat. The presence of slow growing pin lichens often indicates that a forested habitat has undergone little disturbance over a period of decades to centuries, and thus most species of pin lichens are associated with older growth forests. In desert environments, soil crust lichens have successional stages that correspond with the increasing stability of the soil. This creates a positive feedback loop whereby primary succession soil crust lichens create the habitat required by secondary and tertiary succession soil crust lichens.

Lichen distribution patterns also give us a look back into geologic time. For example, the genus *Heterodermia* is most diverse in southeastern United States and eastern Asia, strongly suggesting that their range was continuous during the Arcto-Tertiary geoflora, when the Appalachian mountains of eastern North America formed a contiguous landscape with modern China.

These responses to ecological variables make lichens strong bioindicators of climate and climatic regimes, air quality, acid rain levels, and the continuity of a habitat both spatially and over time. As such, a variety of biomonitoring methods can be employed to measure anthropogenic impacts on lichens and their surrounding environment. These skills are discussed in the Citizen Science section of this chapter.

Lichens Being

Within terrestrial ecosystems around the world, lichens co-create ancient temperate forests and add to the stoic resilience of desert ecosystems. These small creatures are not passive members of our local habitats, but critical ecosystem drivers.

In Deserts

Desert lichens form some of the most striking displays of a symbiotic relationship between an ecosystem and organism. In undisturbed desert landscapes, lichen soil crusts form a living skin: a mosaic of living biological soil crusts (biocrusts) that hold soil particles together and paint the desert floor in a pinnacled topography of yellow, white, pink, brown, green, and black biocrusts. This living skin is composed of lichens, moss, cyanobacteria, bacteria, actinomycetes, and fungi, together forming their own miniature ecosystem.

Biotic soil crusts on the Colorado Plateau, Canyonlands, Utah, USA. Notice the texture and height of the crusts compared to the worn down trail that weaves through them.

Biocrusts are vital for the desert ecology as they bind together surface soil particles and precious organic matter into a biological crust that is usually at least several millimeters thick. This living crust stores carbon to create organic matter. Jelly lichens, such as *Collema tenax,* also fix nitrogen. But the biocrust network goes beyond the production of essential nutrients: biocrusts create a state of homeostasis that supports the restoration and structural capacity for an arid ecosystem to thrive. And they do this in a way that is similar to the skin of humans:

- **UV protection:** The sunscreen-like pigments of lichens scatter or absorb UV radiation, protecting sensitive microfauna and microflora from the DNA scrambling effects of the sun.
- **Erosion control:** The biocrust's sticky photosynthates and the hyphae of fungi,

Don't bust the crust.
—U.S. National Park Service

actinomycetes, and lichens bind soil particles together a couple of centimeters deep beneath the surface of the soil. When intense precipitation occurs, the biocrust rapidly absorbs the water, sending it deeper into the soil through the hyphal web, while at the same time allowing excess water to slide across the surface of the biocrust. During heavy rain events, bareground areas that are covered by biocrust are able to retain nearly all their soil, nutrients, and organic matter relative to areas lacking biocrusts.

- **Infiltration:** Precipitation events in arid lands are precious, and the pinnacle-and-valley topography of biocrusts form miniature rainwater catchments that enable water to infiltrate the ground and reach the deep roots of native plants and shrubs. The height of the pinnacles and the depth of the rainwater catchment valleys can vary from 1–10 centimeters or more, depending on the extent of frost in the region and the length of time that the area has been undisturbed by heavy grazing or trampling.
- **Reservoirs of biodiversity:** The topography of the soil crusts create humid microclimates that nurture the seeds of native plants, insects, and microfauna, which are also supported by the nutrients produced by the crusts.[14] Further research into the miniature webs of life that are supported by biocrusts is greatly needed.
- **Soil organic matter:** In arid lands, most organic matter is limited to the areas around shrubs and perennial grasses, leaving large, barren interspaces. Biocrusts fill these interspaces and create a thick (1 cm or more) layer of fixed carbon and organic matter that feeds and protects the soil microfauna, yielding a more fertile desert that can harbor greater biodiversity.
- **Soil nitrogen:** Nitrogen is a limiting nutrient in most ecosystems, but particularly in deserts where overall diversity of nitrogen-fixing plants can be quite low and atmospheric input from lightning is easily lost. Biocrusts at various stages of development have been found to contribute 2–365 kilograms of nitrogen per hectare each year.[15] One of the most productive biocrust species is the jelly lichen *Collema tenax*.

In Forests

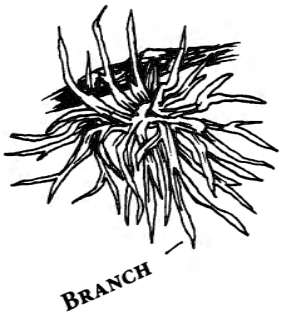
Lichens are probably best known for their majestic displays in temperate forests around the world and their strikingly bold presence in tropical forests. Where there is clean air and moisture, forest lichens thrive and are integral members of their ecological communities.

- **Protection:** Many lichens create anti-herbivory chemicals to protect themselves from insects. This protection is also imparted to the trees on which they grow. This is notably important for young hardwood trees, which are susceptible to insects sucking on their sweet cambium juice. In clean air forests, smooth barked hardwood trees are usually covered with a mottling of white, green and blue crustose lichens that grant the tree an anti-herbivory shield.
- **Structural:** Lichens form a variety of canopy and surface structures which moderate and enhance humidity and temperature, helping support other epiphytes, native plants, insects, arthropods, and microfauna. Canopy lichens and the biotic community of forest canopies help to create a furry skin over the surface of forest ecosystems that traps in humidity, stabilizes temperatures, facilitates the resilience of forest ecosystems, and even influences the precipitation patterns of downwind ecosystems.
- **Nutrient sponge:** Lichens function as biological sponges that absorb nutrients from the air. In coastal areas, tufts and dangling lacy nets of fruticose lichens trap and absorb the nutrient content of ocean air, slowly releasing these nutrients into the terrestrial environment by rainwater leeching or decomposition.
- **Carbon and nitrogen fixation:** All lichens fix carbon, and many also fix nitrogen. Lichens in temperate rainforests contribute up to 50% of the nitrogen budget

Along with the anti-herbivory layer provided by crustose species, the foliose lichens on older trees provide habitats for beneficial insects, thereby nurturing insect biodiversity, which in turn helps combat detrimental pests.

for the forest. Nitrogen is a primary limiting nutrient in most ecosystems.

- **Food web:** Lichens provide critical winter forage in temperate to boreal forests. Horsehair Lichens (*Bryoria spp.*) are the primary winter forage of keystone species, including woodland caribou. A variety of small mammals depend on them for food as well.
- **Insects:** Insect-lichen associations are relatively unknown. Some insects such as Lacewings use lichens as camouflage, but there are likely many more intersections to be discovered.



Fruticose lichen.

Identifying Lichens

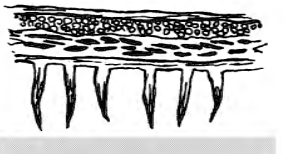
Before one can begin to work with lichens, it is essential to be able to first learn how to identify them. Identifying lichens is one of the most rewarding ways of engaging with lichens for it not only enhances personal and ecological resiliency but also increases one's connection to a habitat. As you learn how to identify lichens, more and more species begin to reveal themselves. A forest that previously looked like a wash of only one or two lichens soon turns into an ecosystem covered in hundreds of species.

Luckily, learning to identify most of the larger lichens is not too difficult and requires little equipment. If you're an herbalist, a good 10x or 20x loupe, field guide, and practice differentiating between look-alike species is all you will need. If you're an artist and want to collect dye lichens, you'll also need to do spot tests, as described later. If you're a citizen scientist doing environmental monitoring you'll probably also want a dissecting scope in order to identify lots of different species within a shorter period of time. And if you're a naturalist measuring total biodiversity, you'll eventually also want a compound microscope and the chemicals known as P and I.

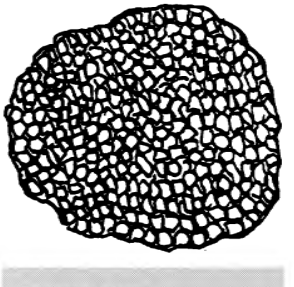
Identifying lichens first begins with determining the overall structure of the lichen, generally classified by the following three forms:

- **Fruticose:** These lichens have a tree- or beard-like form and are found in the greatest abundance growing in temperate rainforests. They tend to hang from trees where their large surface area is able to absorb as many nutrients and as much water from the air as possible. In more arid forests or areas with air pollution issues, fruticose lichens are often low in abundance and diversity. Unique features of fruticose lichens include *branches* and a uniform *outer cortex* (no distinction between upper and lower cortex is possible).
- **Foliose:** Foliose lichens are flatter and more leaf-like. They come in a wide range of shapes and sizes and are often found in the greatest abundance in moist temperate forests on the bark and branches of trees or on top of moss at the bases of trees and rocks. Most are attached to the substrate by *rhizines* (short root-like structures) and the thallus usually forms a *rosette* (rose shapes), where each section is called a *lobe*. Lobes can be elongated like fingers or squat like rose petals. Lobes that are smaller than 2 millimeters in length are called *squamules*; lichens with many squamules are called *squamulose*. Unique features of foliose lichens include lobes, differentiated upper and lower cortices (usually both are present), and rhizines.
- **Crustose:** These lichens are the most diverse group of lichens. They are found growing in all habitats, from the bark in tropical rainforests, to the soil of arid deserts, to frigid rocks in Antarctica. These lichens grow along or within the surface of their substrate, forming a living skin that facilitates water absorption and erosion prevention in desert habitats, while also providing an anti-herbivory shield for thin barked trees in temperate and tropical forests. Unique features of crustose lichens include an upper cortex (no lower cortex) and *areoles* (the tile-like subunits making up the thallus of many crustose lichens).

Foliose lichen.



Rhizines.



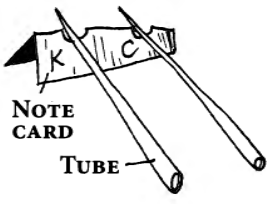
Crustose lichen.

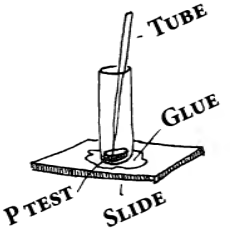
Basic lichen identification flow chart.

The Lichen Rainbow

Are lichenologists colorblind? Sometimes it sure seems like it! Describing the colors of lichens for identification purposes is a highly subjective and rather contentious topic among both amateurs and professionals. Is a lichen containing usnic acid called yellow, or yellow-green, or pale green? Ask three lichenologists and you might get three different answers. Similarly, a lichen containing the compound atranorin may be called blue by one person or white by another. It's all a bit ridiculous, but the matter is more confounded by the fact that lichen colors tend to vary when they are wet, dry, shaded, or exposed to the sun. Thus, some tips are offered to aid in determining a lichen's color:

- Try to ID lichens only when they are dry. This is when their pigments are most visible and consistent.
- Learn to recognize lichen pigments instead of colors. Begin associating the color you see with the chemical produced by the lichen, that way you can learn the range of color variation of "usnic green," "atranorin gray," etc.
- When collecting lichens remember to note if the lichen was in a shaded location. Lichens exposed to less sunlight produce less pigment and are thus more pale or almost green colored.





Spot test gear.

Spot Tests

As with identifying mushrooms, proper identification of a lichen may require the use of color change-inducing chemical reagents. This process is slightly different from that of working with mushrooms, but with some practice it can often be done quickly in the field. The materials for spot tests include:

- **2–4 small glass containers:** These are for holding the chemicals. I prefer glass tincture bottles with eyedroppers that seal at the top.
- **Chemical application device:** I prefer glass capillary tubes, others use a dissecting probe. Eyedroppers apply too much chemical, producing inaccurate reactions.
- **Razor blade**
- **Dissecting microscope or large magnifying glass**
- **Chemicals:** The most commonly used chemicals for lichen identification are K (10% potassium hydroxide [KOH]) and C (normal household bleach). As you get more comfortable with lichen identification you will want to add E (ethanol or methanol at 70% or higher), P (*p*-Phenylenediamine), and I (Lugols iodine) to your repertoire.
- **UV lamp:** Centered on 350 nm (see below).

Spot tests often need to be applied to both the cortex and the medulla of the lichen, and often in a specific order, so make sure the capillary tubes are specific to only one chemical. I accomplish this by making my K tube longer than my C tube since KOH is more commonly called for in most ID keys. To limit having the toxic P test rolling around, I make the P tube so long that it rests in the P mixing container.

A UV lamp is also important for identifying lichens in tropical or subtropical areas, less so in temperate areas. Tropical lichens often contain xanthones, subtle yellowish pigments that fluoresce under UV light. UV lamps are also useful for other groups of lichens, including *Cladonia* and *Parmotrema*. The lamp must emit UV with a wavelength of around 350 nm in order for most UV+ substances to fluoresce. Cheap UV LED flashlights do not work! Some experimentation may be required to find a suitable lamp.[16] To conduct a UV spot test, simply go into a dark closet or cut holes out of a cardboard box for your eyes and hands, and turn on the UV lamp, being careful not to damage your eyes. If the lichen cortex has xanthones, it will fluoresce as a dull to bright orange or yellow color. If the lichen contains alectoronic acid or other subtler medullary chemicals, you will need to first flake off some of the cortex to expose the medulla before conducting the UV test. Alectoronic acid and other medullary chemicals turn a subtle to bright white or "ice blue" under UV, depending on the concentration of the chemical. This can be a confusing spot test if the results are not obvious; just know that a dull or vibrant purple color indicates a negative UV reaction.

My preferred capillary tubes are made by Fisher Scientific (70 µL, product number 22-260-943). You can get 100 for ten dollars. Before you use one, first create a narrow application point by holding the middle of the glass capillary tube over a small flame until the glass is soft. Then pull from opposite ends to break the tube at the center. Using sand paper or a rough surface, gently rub the narrow tip until there is a small hole. The capillary tube will pull chemicals up inside using capillary force and will pour them onto the lichen when the tip touches the thallus.

Lichen Chemistry		Color	K	C	KC	UV	P	Group	Examples	Notes
Yellow, Orange, and Red Pigments	Parietin	Orange/red	Wine-red			Orange-ish		A	*Caloplaca, Teloschistes, Xanthoria*	Highly variable according to sun exposure.
	Calycin	Yellow	Faint reddish		Faint reddish	Pale orange		PA	*Candelaria*	Pulvinic acid derivatives are typically a purer, brighter yellow than any type of anthraquinone. Some *Candelariella* are orangeish. Beware pale *Xanthoria* and *Caloplaca* species in deep caves.
	Rhizocarpic Acid	Yellow				Bright orange		PA	*Rhizocarpon geographicum* group	
	Pinastric Acid	Yellow						PA	*Vulpicida*	
	Vulpinic Acid	Yellow						PA	*Vulpicida, Letharia*	
	Pulvinic Acid	Yellow						PA	*Pseudocyphellaria aurata*	
	Secalonic Acid	±Yellowishh	Yellowish	±Yellow-ish	Yellow-orange	±Yellow		A	Some *Myelochroa, Physconia*	
	Thiophaninic Acid	±Yellowish		±Yellow-orange	Yellow-orange	Bright orange		X	Some *Pertusaria*	Xanthones are most common in tropical species.
	Coronaton	±Yellowish				±Pink-orange		X	Some *Pertusaria*	
Other Pigments	Usnic Acid	Yellow-green			Gold			Df	*Usnea, Flavoparmelia*	Highly variable from yellowish to milky greenish; absorbs UV, resulting paradoxically in an extremely useful UV+ dull yellow for some cheap, broad-spectrum UV lamps.
	Atranorin	Pale gray	Pale yellow				Pale yellow	βOD	*Physcia, Parmelia*	With bluish tinge if in high concentrations.
	Lichexanthone	Pale gray?				Bright yellow		X	Some *Pertusaria, Pyxine, Ochrolechia*	Commonly found as a replacement for atranorin in cortex of tropical species.
	Melanins	Dark brown							*Melanelia, Cetraria*	Mostly northern and alpine.
	Fumarprotocetraric Acid	Brownish	Dingy brownish		±Pinkish		Orange-red	βODo	Cortex of many *Cladonia*	Turns brownish in sun.
C and/or KC Positive	Strepsilin			Blue-green	Blue-green			Df	*Cladonia strepsilis*	Related to usnic acid.
	Alectorialic Acid		±Yellow-ish	±Reddish	Red	White	Yellow-orange	βODE	*Gowardia nigricans*	
	Barbatic Acid Complex			±Orange-ish	Yellow-orange	White		βOD	Some *Cladonia*	
	Olivetoric Acid			Dark red	Dark red	White		OD	*Cetrelia olivetorum, Tuckermannopsis ciliaris*	
	Erythrin			Dark red	Dark red	White?		OD	*Roccella*	
	Lecanoric Acid			Dark red	Dark red			OD	*Flavopunctelia, Melanelixia, Pseudevernia*	
	Gyrophoric Acid			Fleeting pink	Rosy-red			OD	*Umbilicaria, Trapelia, Ochrolechia*	Typically weaker, more fleeting C+ than lecanoric acid, but both vary according to concentration; tiny needle-shaped crystals in water.
	Cryptochlorophaeic Acid		Slow reddish	±Purplish	Purplish	±White ish		OD	*Cladonia cryptochlorophaea*	
	Protocetraric Acid		±Yellow-ish		Rosy-pink		Orange-red	βODo	*Flavoparmelia*, some *Hypogymnia*	

Lichen Chemistry		Color	K	C	KC	UV	P	Group	Examples	Notes
C and/or KC positive	Physodic Acid				Rosy-pink	±Whit-ish		ODo	Many *Hypogymnia*	
	Alectoronic Acid				Pink-violet	Whitish		ODo	*Alectoria sarmentosa*	
	Lobaric Acid				Pink-violet	White		ODo	Many *Stereocaulon*	
	Divaricatic Acid				±Pink-violet	White		OD	*Some Canoparmelia, Dirinaria, Evernia*	
	Perlatolic Acid				±Pink-violet	White		OD	*Canoparmelia caroliniana, Icmadophila*	
K and/or P positive	Psoromic Acid					White?	Yellow-orange	βODo	Some *Cladonia, R. geographicum* group	
	Thamnolic Acid		Deep yellow				Yellow-orange	βOD	*Imshaugia, Thamnolia,* some *Cladonia*	K and P usually much stronger and quicker than atranorin.
	Norstictic Acid		Yellow-> red				Yellow-orange	βODo	Many *Usnea, Xanthoparmelia*	Produces distinctive red needle-shaped crystals in K.
	Salazinic Acid		Yellow-> red				Yellow-orange	βODo	Many *Parmelia, Xanthoparmelia*	Typically K turns darker and less bright than norstictic.
	Stictic Acid Complex		Yellow-> orange				Orange	βODo	Some *Lobaria, Pseudocyphellaria*	K varies from yellow to almost red, but P is distinctly orange with less of the yellow intermediate stage of norstictic and salazinic acids.
	Galbinic Acid		Yellow-> orange				Orange	βODo	Some *Myelochroa, Usnea*	Usually mixed with other acids making it hard to detect.
	Pannarin						Orange	βODo	*Pannaria*	Produces distinctive red needle-shaped crystals in P.
	Physodalic Acid		±Brown-ish				Orange-red	βODo	Many *Hypogymnia*	
	Lividic Acid Complex		Pinkish-brown					ODo	*Hypotrachyna livida* and *H. pustulifera*	
Just UV positive	Evernic Acid					White		OD	*Evernia prunastri*	
	Sphaerophorin					White		OD	*Sphaerophorus, Haematomma*	
	Squamatic Acid					White		βOD	Some *Cladonia*	
	Diffractaic Acid					Whitish		βOD	*Usnea ceratina* and *U. trichodea*	CK+ bright yellow-orange, but rule out K+ first!
	Sekikaic Acid					Whitish		OD	*Dirinaria confusa, Ramalina montagnei*	
	Homosekikaic Acid					Whitish		OD	*Cladonia rei*	
	Stenosporic Acid					Whitish		OD	*Ramalina stenospora*	
All negative	Confluentic Acid							OD	*Lecidea tessellata*	Thin sections produce bubbles in K (use microscope!).
	Fatty Acids								*Cetraria, Kaernefeltia*	e.g. Aliphatic, caperatic and lichesterinic acids.
	Triterpenoids								*Nephroma, Peltigera*	e.g. Zeorin and eucotylin.

Secondary Chemicals Chart

OD – *Orcinol depsides*
ODo – *Orcinol depsidones*
βOD – *β-orcinol depsides*
βODo – *β-orcinol depsidones*
βODE – *β-orcinol dibenzyl esters*
A – *Anthraquinones*
X – *Xanthones*
PA – *Pulvinic acid derivatives*
Df – *Dibenzofurans*

Compiled by Jason Hollinger.

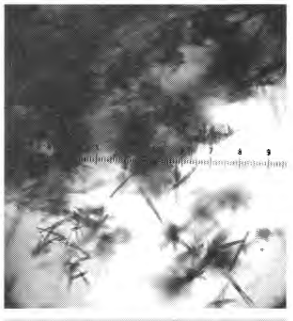
It can be hard to get only small quantities of these substances. If you're looking to get set up with just enough to get going, contact the webmasters at MushroomObserver.org or WaysofEnlichenment.net and they'll kindly set you up with everything a budding lichenologist might need.

DIY Chemical Spot Tests

- **C:** Fill a glass eye dropper bottle with regular chlorine bleach. C breaks down pretty quickly so change the C in your bottle every couple of months, and change your jug of C every six months or so. If it doesn't smell strongly like bleach, it's probably time to replace it.
- **I:** Easier to buy than make. Look for Lugols solution online.
- **K:** A bit trickier to make. Buy some reagent grade pellets of potassium hydroxide online from a science store. I use Fisher Scientific S71978. These pellets are 85% KOH, so you want to get it down to 10% KOH by adding 9 parts water to 1 part KOH pellet (e.g. 1.5 tablespoons water to 0.5 teaspoons KOH). Mix the ingredients in a glass container, put on the cap, wrap it in a cloth towel, and gently shake the container. Be careful, the reaction of water and KOH creates a lot of heat. Alternately, sodium hydroxide and ammonium hydroxide will work in a pinch. The former is sometimes available at supermarkets or hardware stores as Red Devil brand Drano™.
- **P:** Trickier still. P contains a chemical that is hard to get. P comes as little crystals, to which you add a couple drops of ***E*** (70% ethanol or methanol). The crystal(s) dissolve after a second, after which point they are taken up by a capillary tube. Take a chunk of the thallus, place it on a glass slide or piece of index paper to do the spot test, then throw out the thallus and carrier. Do not apply P directly to your specimen: over time it will turn your entire specimen black and bleed into the rest of your herbarium. Powerful stuff; use caution.

Lichen Microscopy

Macrolichens



Norstictic acid crystals from a Bryoria spp., *Southern Chile.*

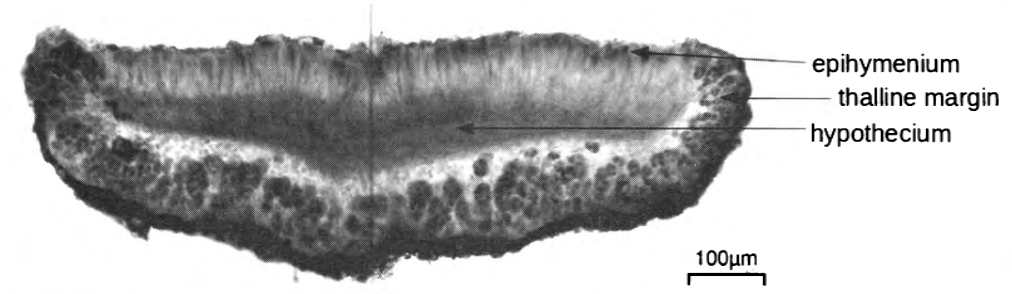
Compound microscopes are not necessary for identifying most macrolichens (non-crustose species). For these larger species, usually the only time you will need to use a microscope is to check for norstictic acid crystals. Norstictic acid is a relatively common lichen chemical; species with norstictic acid are scattered throughout most families and genera, so it's important to get comfortable with this test.

To test for norstictic acid, take a relatively thin section of the thallus, place it in a drop of water on a slide, put a cover slip on top, and press down with an eraser to squish the section. Then add a drop or two of K to one side of the cover slip, using a piece of tissue paper on the other side to wick up the extra water and pull the K across the slide. Look under the scope at 100x for a red hue forming around the thallus. At 400x you should slowly start to see linear-shaped red needles that form star shaped crystals. This is strikingly beautiful. If you see those crystals, you've got norstictic acid. If you don't get crystals then you probably have stictic or salazinic acid, both of which also react positively to K with a yellow to orange or red color.

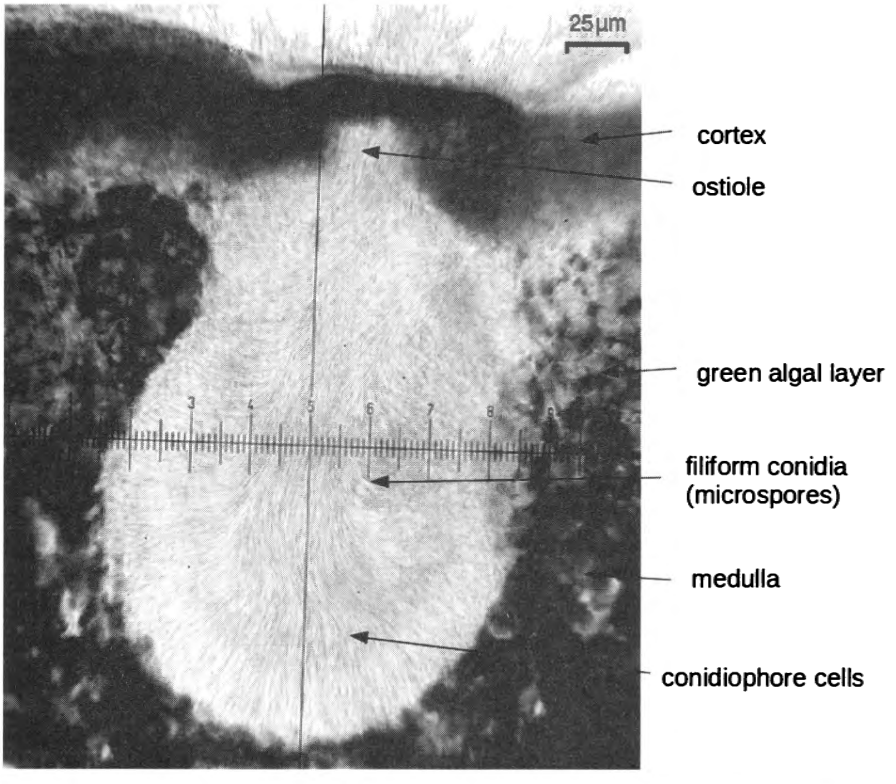
Crustose Lichens

To identify most crustose species you will need to use a compound microscope. Microscopy with lichens is nearly the same as microscopy with ascomycetous fungi. You generally do a vertical section through the apothecium, perithecium, or pycnidium using a razor blade and a dissecting scope at 10x or 20x. To prevent pieces from flying and to keep asci from shattering, wet the apothecium first. To get a really thin slice, try to cut little pie-shaped wedges, one side really thin, the other a little thicker. Getting thin sections that include all the important parts (i.e. the asci with spores still inside, the margin and exciple, the ostiole if it's a perithecia) can be challenging at first, but patience and practice helps—remember that even the best lichenologists had to learn this skill as well.

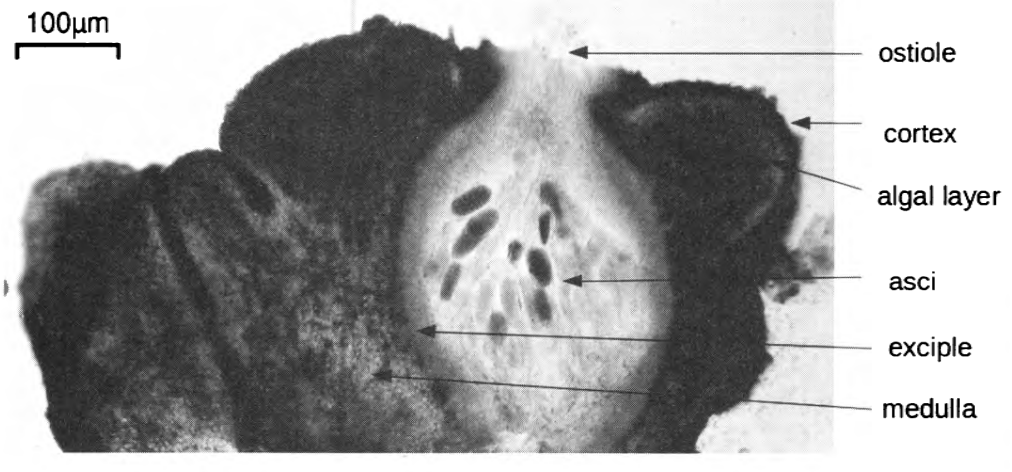
After slicing your sections (a few slices is enough), place a drop of water on the center of a glass slide. Then use a wetted tip of your razor blade to pull the section onto the blade (the power of hydrogen bonds!), carry the section to a glass slide, and then dip the tip of the blade into the drop of water so that the section is pulled onto the slide. Place a cover slip over the slide, and view it under



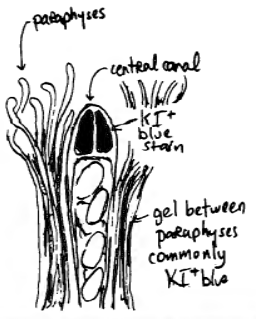
Apothecial section of cyanolichen Peccania subnigra. *Note the presence of the photobiont in the rim of the disc. The presence or absence of a thalline margin is a major classification in crustose lichens. Lecanorine apothecia have a thalline margin that is concolorous with the thallus. In contrast, lecideine apothecia lack photobionts in the apothecial rim and appear concolorous with the disc.*



Pycnidia of Rhizoplaca melanopthalma. *Note how tiny the condia (asexual microspores) are compared with the large muriform spores of the perithecia in the figure below.*



Perithecia of Staurothele aureolata. *Note the large muriform spores and the unusual presence of green algae amongst the asci, signatures for this species.*



Typical ascus stain for a Lecanora *species, notice the helmet shape of the KI+ stained ascus tip and the central canal that goes through the entire tip. This canal facilitates spore discharge.*

a compound scope at 100x and 400x. Larger power objectives are usually not necessary. If you are looking at an apothecium you'll want to note the presence or absence of algae in the margin. If you are observing a perithecium, look for the shape of the *ostiole* and *exciple*. Also note the number of spores per ascus (4, 8, or too many to count), the color of the spores (brown or colorless), as well as if the spores are broadly or narrowly ellipsoid, if they have any septae (cross walls), or if they have numerous jig-jag septae (known as *muriform spores*). In order to get an accurate spore size, you want to measure only mature spores. For this you will need to gently push some spores out of their asci. This can be done by pushing a soft pencil eraser on the cover slip, squishing the asci so that spores squeeze out. Lastly, note the color of the *epihymenium* (the thin and often colorful layer above the asci) and *hypothecium* (the tissue below the asci).

Chemical tests of ascomata sections are often required for crustose lichens. Luckily, they are not very difficult to do. The trick is to use a tiny piece of toilet paper to pull liquids from one side of the coverslip to the other—effectively washing the section in different chemicals—and then observing color changes to the ascomatal section. The most common chemical test is a KI test: potassium hydroxide followed by iodine. This test will reveal amyloid structures in your section. For lichen ascomata, first prepare your section as described above, then place a couple drops of KOH on one side of the cover slip, and place the piece of tissue paper on the other side. Under 100x you'll see cellular material move rapidly towards the tissue paper side as the tissue paper wicks up the water and pulls the KOH under the cover slip. Often, the tissue will only turn hyaline or pale brown in response to the KOH. A color change in the epihymenium to green, purple, or red is notable. Repeat this process using a couple drops of water to wash the KOH out of the asci, then repeat this process using a couple drops of I (Lugol's iodine). The iodine will change the water under the slide to a yellow-orange hue. If this does not happen, it may be because you didn't wash out the KOH sufficiently (KOH seems to repel iodine), so add a couple more drops of water to the side of the cover slip, and replace the tissue paper on the other side. If it's completely wet and not wicking anymore, try adding the iodine again. Usually part of the section will turn bright blue, and you'll be zooming in to look at the tips of the asci under 400x to see what parts turn dark blue.

In practice, this test can be exceptionally difficult to interpret at first. The hymenial gel of many species turns dark blue, completely obscuring the delicate reaction in the ascus tip. It is essential to gently separate the asci in order to see them clearly without mangling them beyond recognition. Even when done perfectly, it takes practice and experience to learn which asci are at the appropriate stage of development to display the desired internal structures. But when you finally get the hang of it, this ascus stain is an invaluable and essential character for identifying most groups of crustose lichens. One of its primary benefits is its ability to indicate the type of spore dispersal apparatus used, such as whether the asci are unitunicate or bitunicate.

Tips

As with other fungi, Mushroom Observer is a great website for getting help with identifying lichens. The Consortium of North American Lichen Herbaria (CNALH) is an online database that has range maps for species found around the world, although there is a bias towards North American species. The International Association of Lichenology's website also provides a few links to additional resources.

Currently, there are no lichen books that reflect global diversity. Existing books are regional at best. If you are in the Southern Hemisphere, there is an online copy of David Galloway's *Lichens of New Zealand* that should get you to genus at the very least. Similarly, in the Northern Hemisphere, the keys in the book *Lichens of North America* may also get you to genus. For regionally specific lists of species and photographs, visit the webpages of a national or university herbarium. Armenia and Ireland have impressive lichenology websites devoted to exploring lichens in their respective countries.[17] Many university herbaria throughout the world have databases that will give you range maps of particular species so that you can find what is common in your area. And looking for and collecting what is common is the best place to start!

Harvesting Ethics and Tips

Considering that the average lichen grows only one millimeter per year, harvesting lichens must be done with great care and awareness of the lichen life cycle. Unlike mushrooms, lichens do not have an underground body. What you see is the entire lichen, and what you collect is an entire lifetime. Most lichens require a dozen years or more to grow just a few centimeters. Slow growing, determined little fellas that they are—collect with respect!

If you are harvesting lichens for dyestuff or medicine, it is best to harvest specimens from a disturbed area such as a recent logging operation, or from wind fallen trees. Generally, the more recent the disturbance, the more potent the medicinal compounds will be in the lichen.

If you decide to harvest in an intact forest, do so very sparingly. The forest uses all the nutrients that are gathered from the air by the lichen and subsequently deposited into the soil when the lichen decays. If you are collecting lichens for biodiversity sampling, be sure to collect off trail and with care to the substrate that you are taking from. Taking huge chunks of lichens off of trees to obtain samples is strongly discouraged.

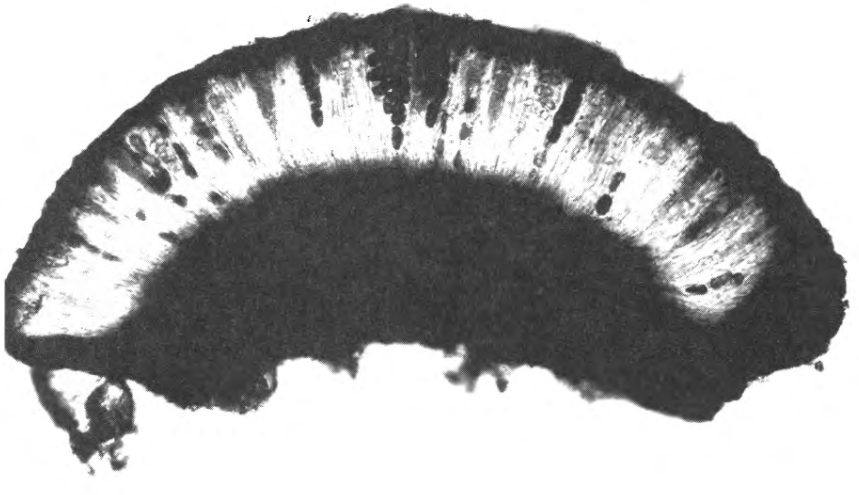
On your way out, give back to the forest by taking some of the beard lichens from a disturbed area and draping them over branches of the forests edge, thereby helping these elder lichens extend their reach. But most importantly, give thanks to the forest, to the tree you are collecting from, and to the dear lichen, whose life you hold in your hands.

Some lichen thalli have lived for over 1,000 years. Determining their age by their growth rate can also help determine the age of rock surfaces that they grow on. This discipline is known as lichenometry. Lichenometry has helped date the stones on Easter Island as well as approximate the time of ancient avalanches and earthquakes.

Lichen Herbaria

Whether you are an herbalist, lichen dyer, naturalist, or citizen scientist, creating a collection of the lichens you have obtained from the wild will help you stay organized while also helping preserve a local cultural memory of the species in your area. Herbaria are organized using similar methods to those described for mushrooms in Chapter 4. Simply air dry each lichen thoroughly and then place it into its own small envelope labeled with its species name, collection data, and identifying characteristics. To prevent mold growing on your specimens, herbaria should be kept below 50% relative humidity. It is also recommended to place specimens in a deep freezer for a few days to kill any insect eggs that are present on the thallus.

To easily track which collection corresponds to a given application (e.g. medicinal, ecological, or dyeing), it is best to number your collections starting with one and counting upwards for the rest of your life. If you'd like to bring your specimens back to the wild someday, just take them from their envelopes to a habitat that is similar to their original home and place them on their preferred substrate. Even if they've been sleeping in a paper packet for 100 years, in a few minutes after being exposed to humidity and light, they will come back to life.



The home lichen herbarium and lab of a much loved Canadian lichenologist.

Lichens and Humans

While lichens have played a helpful role in the development of many cultures around the world, their cultural significance amongst botanists and mycologists has largely been outshined by the intersection of humans with plants and mushrooms. Still, the small field of *ethnolichenology* holds a few fascinating insights into how lichens can assist in personal and societal resilience.

As Food

Lichens have been a part of diets from Asia to the Americas for thousands of years. Korean, Japanese, and Chinese cuisine highlights the flavor of *Umbilicaria esculenta* by using a cooking method that removes gyrophoric acid. The method involves boiling the lichen in a series of water baths, each at least an hour long, and avoiding breathing in the steam which contains the gyrophoric acid. There is even some evidence to suggest that the "manna," or "bread from heaven," spoken about in the Bible was actually the vagrant desert lichen *Aspicilia esculenta,* which today sustains herds of livestock in arid climates. Icelandic Moss (*Cetraria islandica*) has been used as a food source by aboriginal and Nordic people in the arctic areas of the Northern Hemisphere since the 9th century. Here, a porridge of the lichen is made by mixing its thalli with water or milk and boiled. It is said to be good if boiled for 10 minutes, bitter if boiled longer than 30 minutes, but sweet if boiled for 2–3 hours as the polysacchrides are released.[19] *Evernia prunastri* is similarly used to make bread or porridge in Turkey and Egypt.

As with mushrooms, it is important to be positive of your identification before eating any lichen. Lichens contain a vast range of chemicals, most of which have unknown biological effects. Many lichens can also absorb pollutants, such as heavy metals and radionucleotides.

Lichens are also used as a spice. *Parmotrema tinctorum* is used to flavor dishes in Saudi Arabia, Kuwait, and Oman, while *Parmelia abessinica* is used as a curry powder in India.[20] The beard lichen known as Wila to the first peoples of northwestern North America (*Bryoria fremontii*) has been used widely by different tribes as not only a food source, but also as a fiber for making fabric, and as a reliable source of tinder. Wila is traditionally baked at a medium heat with roots, meats, and berries for 12–24 hours in a covered pot placed in the oven or in the soil beneath a fire. For more information on lichens as food, and Wila in particular, check out the work and website of Stuart Crawford at the University of Victoria in Canada.[21]

Iwatake (Umbilicaria esculenta) *a delicacy in Japanese cuisine, on display at Japan's National Museum of Nature and Science, Tokyo.*

As Medicine

On the whole, lichens and their cultured mycobionts produce over 1,050 secondary metabolites (chemicals that are not necessary for the cellular functions of the individual bionts, but facilitate the emergent lichen symbiosis. Most of these chemicals are not found in plants or other fungi, and are exclusive to the lichen symbiosis). Medicinal uses of lichen chemicals range from menstrual teas to powerful antibiotics. Lichen-derived antifungal, anti-HIV, anti-microbial, and anticancer elixers have been a part of the pharmycopoeia of healers and health practitioners around the world for thousands of years.

The green beard lichens in the genus *Usnea* include some of the most widely used medicinal species, with recorded use reported from Traditional Chinese Medicine (ca. 500 CE), ancient Greek (Hippocrates, ca. 400 BCE), and traditional and modern Ayurvedic traditions. Bioscience research has demonstrated the powerful inhibitory effect of usnic acid against a variety of human pathogens including *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae,* and *Mycobacterium tuberculosis*. Usnic acid has also been found to have powerful anticancer properties against leukemia, endometrial carcinoma, breast cancer, and pancreatic cancer. Tinctures and other herbal remedies utilizing usnic acid, as well as other chemicals, may require preprocessing of the *Usnea* lichen prior to use if a more potent formula is desired (e.g. heating). For more information on preparing lichens such as *Usnea* for medicinal purposes, see the booklet by herbalist Christopher Hobbs, *Usnea: The Herbal Antibiotic and Other Medicinal Lichens,*[22] as well as the review article by Moreno Cocchletto.[23] For more information on the use of lichens in folk medicine, check out Stuart Crawford's chapter "Lichens Used in Traditional Medicine" in the recent book *Lichen Secondary Metabolites.*[24]

Other powerful lichen chemicals include gyrophoric acid, divaricatic acid, barbatic acid, norstictic acid, and diffractaic acid. These chemicals, along with others including atranorin and parietin, demonstrate inhibitory and/or cytotoxic effects against many types of cancer including breast cancer, prostate cancer, and lung cancer. For further information about antibiotic, anticancer, and immune stimulating effects of lichen compounds, check out the research of lichenologist Gajendra Shrestha, particularly the article "Lichens: A Promising Source of Antibiotic and Anticancer Drugs" in *Phytochemistry Reviews.*[25]

Most lichen chemicals are very potent and biologically active and should be handled with care when used medicinally. Some studies have shown usnic acid to be damaging to the liver in high concentrations, and that the cytotoxic activities of some anti-cancer lichenic compounds may be damaging to benign cells. Be sure to do your research, and above all, correctly identify your lichen species. Similar species in the same genera often have vast differences in associated chemistry. For instance, Usnea *is a genus of over 300 species and most species contain various arrays of associated chemicals, not just usnic acid. While these additional chemicals may be beneficial medicines, it is important to know the chemical content to account for contra-indications and dosage.*

As a Dye Source

Lichens have been used as a dye source from the Incan peoples in Peru to the First Peoples in North America, Australia, New Zealand, India, Japan, and throughout Europe. Written accounts date back to the 3rd century CE. Thin layer chromatography has been used on ancient Norse fabrics to demonstrate that lichens were used as dyes in Scandanavia during the Bronze and Iron ages.[26] The dyes found in lichens require no mordants, and the pigments can be used both to dye fiber as well as to paint objects, hair, and skin. Many henna formulas contain lichens such as *Anaptychia ciliaris, Lobaria pulmonaria, Parmelia karatschadalis, Parmotrema chinense, P. perforatum,* and *Roccella fuciformis*.[27] For more information on working with lichens as dyes, see Appendix D.

Culturing Lichens in the Lab

The two primary strategies for culturing lichens are *in vitro* and *in situ*. Each practice has its own applications, strengths, and shortcomings. Folks doing restoration projects will be drawn to *in situ* culturing as the results are often much more successful when culturing in the lichen's original type of habitat.

In vitro cultivation is often used for taxonomic purposes in order to identify the photobiont or endo- or epilichenic bionts. On plates of agar, the lichen symbiosis usually disintegrates as the fungi and algae begin to grow separately. The separate growth of each biont is distinctly different than the original lichen, more akin to fuzzy mold and green algae growing next to each other than the intricate, charismatic form of a lichen. A primary benefit of separating bionts is that the photobiont is able to carry on its full life cycle and thus can be identified to species. Similarly, the epibiont bacteria and endolichenic fungi will also disassociate from the lichen, allowing for identification of these otherwise invisible bionts. This makes an intriguing venture for people interested in photobiont taxonomy, endolichenic fungi and bacteria, and the construction and dissolution of the lichen symbiosis.

In vitro cultivation uses agar plates that are specialized to a particular biont. Methods for aseptic fungal cultivation are covered in depth in Chapter 8. The following information covers techniques specific to lichens and their bionts.

Culturing Mycobionts

Spore Method[28]

Culturing mycobionts can be difficult because of the concurrence of bacteria, fungi, and other microbes that associate with the lichen symbiosis. The spore method limits the agar's exposure to anything other than mycobiont spores by placing agar in the lid of the petri dish and the spore bearing surface on the bottom of the dish. This works best with a lichen with visible apothecia and a low nutrient agar recipe, such as a 4% distilled water agar medium (4 grams of agar and enough distilled water to make a 100 milliter solution).

1. Obtain a fresh lichen thallus or thaw a frozen specimen (lichens can be stored in the freezer indefinitely).
2. Clean the thallus with running water then use a knife to remove the apothecia or perithecia. Soak these spore-bearing structures in distilled water for four hours.
3. Pour the agar into the top of the petri dish thick enough so that the spores only have to travel about 5–10 millimeters. Allow the agar to set.
4. Attach the apothecia/perithecia to the bottom of the petri dish using petroleum jelly. Make sure that the spore bearing surface is not covered in jelly.
5. Place the top of the dish over the bottom and wait about one day or more for spores to discharge onto the agar. Several different agar lids can be used with the same bottom dish to increase your chances of obtaining a sterile culture from that ascoma.
6. Place the inoculated agar medium on top of an empty sterile petri dish bottom and wrap the plate in Parafilm.

7. Place the plate in a humid, dark area at 59°F (15°C) to incubate and germinate the spores. This may only take one day.
8. Use an inverted microscope or a strong dissecting microscope with a lit up base to see if the spores have germinated. Once germinated, transfer the mycelium to a more nutrient-rich agar such as a Malt Yeast Extract Medium or Lilly and Barnett's Medium, though make sure your pH is between 5 and 6 for optimal growth.

Thallus Fragment Method[29]

Culturing thallus fragments can be more difficult than the spore method because of potential contamination by other unknown bionts. Culturing these unknown bionts is very fruitful and important research, just be sure you also figure out which fungus is the mycobiont so that it can be differentiated from the other fungal bionts. Isidia often harbor other fungi, so to isolate the mycobiont, culture the white cottony medulla.

1. Remove a thallus fragment from the interior of a clean lichen and place it on wet paper in humid, sterile conditions. Some researchers place the fragment in a test tube filled with a bit of distilled water. Store at 59°F (15°C) for a couple of weeks until a number of hyphae grow from the thallus.
2. Cut out a portion of the elongated hyphae and place it in a fresh culture medium of your choice. Nutrified media is fine.
3. Repeat step 2 with a number of elongated hyphae to ensure that you have the mycobiont and not some other fungal associate. The mycobiont is the most abundant fungal mass in the lichen, so it should appear in the majority of the plates while a smaller number of plates will contain other fungi.

Culturing Photobionts

To culture photobionts, they are first removed from the inside of a clean thallus and placed in an agar plate. Various media formulas are used to culture green algae culture, with Bold's Basal Medium being one of the most common recipes. To grow cyanobacterial photobionts, MDM media is recommended. For more information on culturing media, see the National Institute for Environmental Studies Microbial Culture Collection.[30]

1. Clean the lichen thallus with tap water and, using a razor blade, gently scrape off the outer cortex of the lichen and clean the tissue again.
2. Using a sterile razor blade, remove only the photobiont layer from the lichen's medulla as best you can and place it onto the media.
3. Incubate at 59–68°F (15–20°C) in low light conditions for a month. Direct sunlight is not recommended. Subculturing may be necessary to obtain a pure culture.

Culturing Epibionts and Endolichenic Fungi

Use the methods above for culturing photobionts or mycobionts. Contamination will undoubtedly occur, but this is an opportunity to examine the various other fungi and bacteria that associate in the lichen. Culturing endolichenic fungi can be incredibly fruitful. Over 640 endolichenic fungi have been found in the pelt lichen *Peltigera neopolydactyla*. This number of endolichenic fungi is not unusual, but the patience of the researcher is quite notable.

For more detailed information on culturing mycobionts and photobionts, see the research of Yoshikazu Yamamoto, particularly the article "Isolation and Culture of Lichen Photobionts and Mycobionts."[31]

In Situ Cultivation for Environmental Regeneration

Habitat restoration efforts are often so heavily focused on establishing plant and animal communities that the lichen component of an ecosystem tends to be entirely overlooked. Likewise, where habitats are threatened by human disturbance, the importance of lichens to those ecosystems is often missing from conservation dialogues. When lichens are left out of rehabilitation strategies, a systemic gap in nutrient acquisition and retention can develop, leading to a plateau or limitation in the success of a restoration project. Lichens play critical roles in healthy ecosystem functioning. As such, their integration into restoration efforts should be increased wherever possible.

Whether you want to help protect lichens or introduce them into habitat regeneration efforts, several simple methods can be utilized to intentionally cultivate lichen species in forest or desert ecosystems. Most of these methods are variations on techniques that have been used in research experiments but have not yet been applied outside of the academic arena. These techniques are presented below in hopes that they will be applied and elaborated upon by citizen scientists and Radical Lichenologists around the world.

Forest Lichens

One of the many outcomes of cutting down a forest is the loss of lichen communities. To help protect these lichens from such destruction, species in areas slated to be clearcut can be rescued and moved to a similar habitat. Just be sure that the host forest and recipient forests are located somewhat close to each other and share similar climate and flora. These considerations are important to maintain locally specific adaptations in the recipient forest.

To move tree-inhabiting lichens, remove a small piece of bark that contains a lichen fragment and place it in a piece of biodegradable gauze that you then adhere to the bark of a new tree using something that will allow the gauze to stay in place for at least a year (e.g. tree sap or pins). Alternately, take a couple of thalli and rub them on the bark of a recipient tree to spread fragments of the lichen across the bark surface. For lichens growing on the ground, such as *Peltigera* or *Cladonia* species, take a chunk of the substrate and place it in a similar location in the recipient area, noting whether the host surface was a rotting log, sandy soil, or humus.[32]

Rangeland Lichens

In overgrazed or post-fire rangelands, there is a tremendous need for immediate soil stabilization. Lichens and moss are essential for this process, and techniques are still being developed to best facilitate the recovery of these habitats via lichens.

Many methods create an inoculum by creating a liquid slurry of soil crusts collected from intact sites, distilled water, and various nutrients, then pouring the mix on soil at the recipient site.[33] The mixture should be poured on a soil that has a similar texture (fine soils will have the best results) and pH as the original site. To do a rough test of pH affinity, apply some diluted hydrochloric acid (HCl, sold in hardware stores as Muriatic acid). Put a drop of HCl on the host soil and observe the degree of effervescence. Is it non-effervescent (no bubbles), slightly effervescent (a couple of bubbles), or very effervescent (a ton of bubbles)? Be sure that your recipient site has a similar level of effervescence.

More research is needed on different types of slurry mediums. Some researchers have found composted sewage sludge slurry to be a very successful inoculant for biotic crusts.[34] However, there are major concerns about heavy metals and other chemical contaminants that are often associated with sewage slurries and the safety of spreading it over our landscapes.

Yogurt, used to culture moss, may provide nutrition and assist in the adhesion of biotic crust fragments to soil particles. A bit of compost tea (discussed in Chapter 9) mixed with distilled water and applied frequently to the inoculation site (once a day at dawn or dusk) may increase the growth of early succession species. The first species to establish will be green algae and cyanobacteria, which are nearly invisible. To tell if your culture is successful at the early stages, the soil particles

will clearly begin to aggregate together and if you pick up a piece of the soil (a *ped*) and break it apart you will see clear dangling filaments. As time passes and the conditions become right, the site should grow darker as the cyanobacteria increase in bulk mass. Several months to, usually, over a year later, lichens will begin to establish. If a watering system is employed, a drip watering system is recommended over a mist system.[35]

Biocrust restoration desperately needs further investigation. Climate change and disturbance are shifting arid ecosystems into depauperate versions of their former states. Bare ground is increasing in many ecosystems that were previously grass-dominated. The Dust Bowl catastrophe that tore topsoil from over 400,000 square kilometers in central North America may repeat itself in arid regions such as the Great Basin Desert.[36] Unfortunately, there is a deep lack of creative biocrust restoration methods that require minimal infrastructure and can be applied on a broad scale. There simply aren't enough people thinking about and experimenting with biocrust remediation, and there are an incredible number of unknowns—not just unknown answers, but unknown questions, which is exciting but also daunting. For more information, check out the work of Matthew Bowker at the University of Northern Arizona, especially his article, "Biological Soil Crust Rehabilitation in Theory and Practice: An Underexploited Opportunity."[37]

Biotic soil crusts create a pinnacles-and-valley topography that captures rainwater, creates microclimates for microfauna, and holds the soil together, preventing wind or water erosion.

Citizen Science

Lichens are incredibly sensitive to changes in their environment, a fact that provides humans with a low-cost means for measuring the health and vitality of an ecosystem. Lichen diversity and distribution can be directly correlated to air quality and habitat disturbance patterns. This simple practice was first conducted by British schoolchildren in the 1970s. Known as "The Mucky Air Map of Britain," this pioneering project created a lichen distribution map based on collections from schoolchildren around Britain that clearly demonstrated a low diversity of lichens in areas of highly polluted air, as well as a surprisingly low diversity in remote areas downwind of industrial sites.

Monitoring Air Pollution

Compared with the cost of deploying air quality canisters (>$10,000), working with lichens as air quality biomonitors is an inexpensive and effective means for citizen scientists to understand the long-term effects of industries on an environment. There are three primary ways to monitor lichens for air pollution: 1) monitoring reduction in diversity and abundance, 2) monitoring morphological changes of individual thalli, and 3) measuring the amount of pollutants in the thallus over a period of time. Methods for measuring a reduction in diversity and abundance vary from study to study. Below are a few methods that have been modified from various sources.[38]

Percent Cover Method

This accessible method requires limited knowledge of lichen species. Punch 50–100 holes across a stiff piece of paper, equally spaced about 1 centimeter or more apart. The exact measurements are not critical, just be sure they are repeatable and standardized across the sheet. This sheet is now your "quadrat." Make a photocopy of it and save it.

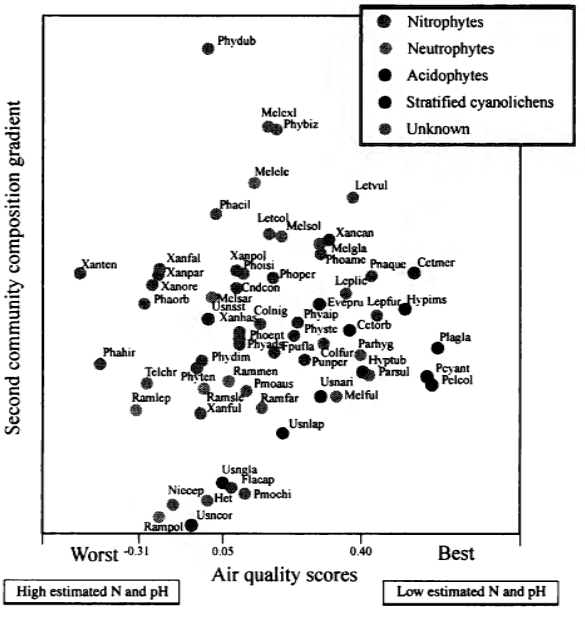
Pick a handful of trees that you'd like to sample. Put some sort of mark on each tree so that you can return to them later. You will need to be able to put the quadrat in the exact same location each time. It might be advisable to use a piece of degradable twine or stretchable cloth with a knot in the middle; the knot can be used to mark the location for the upper left corner of your quadrat. Other methods might be better. Do what works best for you. If you'd like to monitor lichens on rocks instead of trees, that's fine too, though epiphytes are usually more sensitive to pollution.

Take your paper quadrat and hold it over the surface of the substrate. Mark how many of the holes have lichens covering at least 50% of the surface beneath it. Divide that number by the total number of dots and multiply by 100. This gives you a "percent cover" estimate for that tree. You can also mark what percentage is covered by particular color groups (gray/blue, white, orange, green/yellow, brown/black). This may suggest the nitrophilic (tending toward orange) nature and general diversity of the assemblage. Ideally, the study would be repeated every three to six months or, at the least, after a few years.

In addition, you can also increase your sample size to 20+ trees in a polluted area and then survey the same number of trees in a non-polluted area. Increasing the number of plots you record at a site will increase the statistical significance and reliability of your estimates. Keep in mind that lichen cover may be different based on different types of trees, the height above the ground, and aspects (north facing versus south facing slope). Sunlight (i.e. percentage of canopy cover) may also have an impact on lichen cover. These factors can influence your results, so try to either keep these variables constant, or try to sample enough trees in different locations so that these variations can average out. Be sure to keep good notes!

Once the data is collected it should be processed using biodiversity statistics software. The best program is EstimateS, which is free and open-source.[39] There are also excellent and free biodiversity training lectures provided online by Biodiversity Informatics[40] that will give you an understanding of the different biodiversity indices used in EstimateS and how to evaluate the results.

Although numbers are powerful, they are essentially inert until they are brought to the attention of regional legislators and community action groups. Community organizing concerning industrial pollution and resource extraction often requires real life, measurable examples of impacts to be most effective. Thankfully, lichens give earlier responses, with more provable causal associations, than human impacts, such as health deterioration. But like most areas of lichenology, their interpretation as bioindicators has been underused and underexplored as a tool for community mobilization and action.



Air quality bioindicator species, as found along an air quality gradient

Species are listed as codes: the first three letters of the genera followed by the first three letters of the species, i.e. Bryfre is Bryoria fremontii. *Note that the occurrence of acidophyte genera in clean air locations is odd. The authors note that neutrophytes and acidophyte genera were classified based upon European indices and that species in these groups may need to be reclassified for North America. Table is from a publication of the U.S. Forest Service's lichen monitoring program.*[41]

Morphology Method

Lichens often show morphological distress in response to pollution, and photographs of these changes have the power to demonstrate the impacts of various pollution streams (e.g. coal powerplants, smelters, and vehicle exhaust) on the health of nearby communities and ecosystems.

To photographically measure morphological changes due to a new source of pollution, choose a couple sensitive and very showy species that are growing on trees or rocks near the new pollution source. Use a lichen pollution index[42] to find out which species in your area are sensitive to air pollution. Then take pictures of those lichens every month (if possible), starting before the pollution source is active, and then continuing to photo document the same lichen every month afterwards. Creating a cardboard frame that can be placed around the area being photographed may help with consistency and analysis of the photos later on.

Indicators of morphological distress due to pollution include curling up or bunching up very irregularly, turning white from the degradation of the chlorophyll, and crisping to a yellowish or brown color as the lichen dies.

If the pollution source is already in place, you can do a transplant experiment and then document morphological distress. Simply remove some pollution-sensitive lichens from the bark of a tree in a clean air area, attach it to the bark of the same type of tree in a dirty air area, and then document its morphological change over time. To increase the robustness of your study, set up a control so that you can be sure it wasn't the transplanting that caused deterioration of the lichen. For the control, transplant the same lichen species to a nearby tree in the clean air area, and then take photographs of it periodically as well. If the polluted air transplant shows greater morphological distress than the controlled transplant, then you've got a strong case for demonstrating the ecological impact of the pollution source in a tangible, visible way.

Dry-Weight Analysis

This method is used by state and federal agencies around the world as it gives quantifiable evidence for the amount of heavy metals, acid rain, and other pollutants in the air. If you are requesting air pollution canisters or resin accumulators to be placed in your community but the authorities are not providing you with funding, this method has the potential to give you hard, quantifiable evidence for authoritative action. The method involves using a mass spectrometer or similar device, which are rather common in most university chemistry departments. Depending on the pollutant being tested, mass spectrometry tests should be relatively inexpensive and easy to perform with a few days of training, if your local university will grant you access. If they won't, most graduate level chemistry students are trained in the basic procedure and analysis and might do it for a good cause. In either case, collect the lichens from upwind and downwind of the pollution source. The more sites you collect from along this transect, the more robust your dataset will be. After gently cleaning and drying the specimens, test for the suspected pollutants. Heavy metals such as lead and cadmium are among the easiest to test for. Gasses such as sulfur dioxide and radioactive isotopes are much more difficult to measure.

For comprehensive information on the particular pollutants that can be biomonitored by lichens, read the article by Marcelo Enrique Conti, "Biological Monitoring: Lichens as Bioindicators of Air Pollution Assessment—A Review."[43]

Monitoring Ecosystem Health

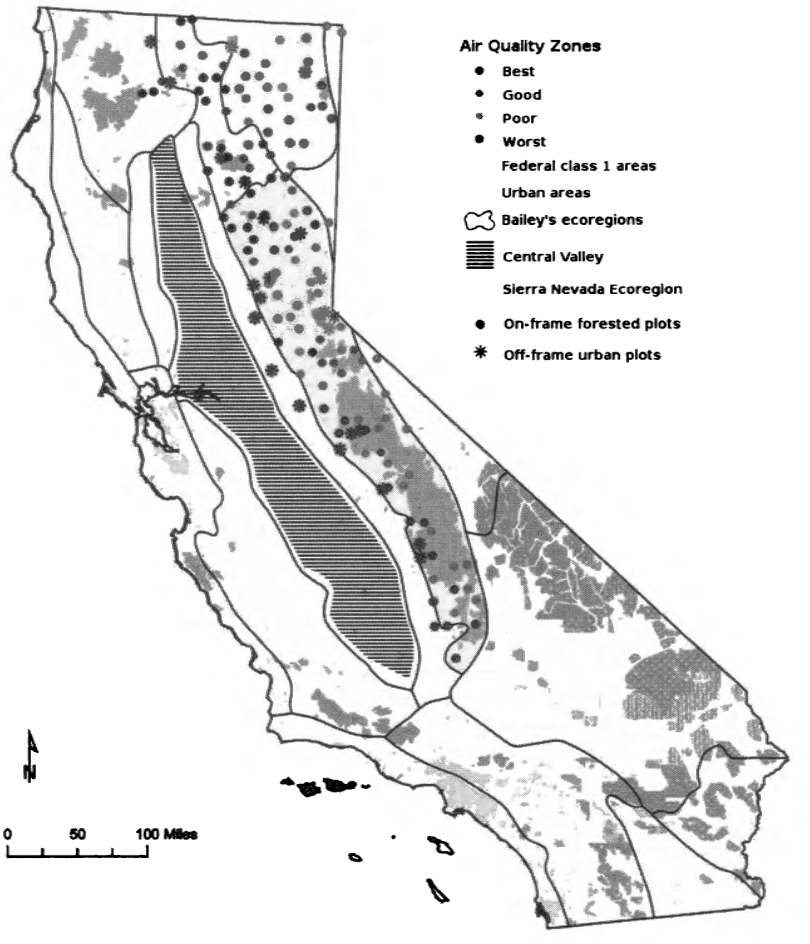
Conducting a biodiversity survey can tell you a lot about the quality and health of an ecosystem, from its air quality, to the continuity of habitat (old forest versus ancient forest), to its biodiversity. A total inventory is ideal in conjunction with plot-based methods for statistical rigor, but less intensive types of inventories and surveys can be conducted to assess an ecosystem.

A total inventory involves documenting all species present on all substrates within all habitat types in a given area. Often the habitat type is a vascular vegetation alliance or landform (i.e. mid-elevation canyon), which has many microhabitats within it (e.g. limestone outcrop, riparian granite boulders, riparian hardwoods, and upland shrub community). It is ideal to collect a voucher

specimen of every species found at each site, noting microhabitat and substrate for each voucher. Stratify these collection areas so that regions with different climatic variables (e.g. elevation, aspect, protection from wind), vegetation, and geology are visited.

Depending on time or experience limitations, various plot-based methods can be used to limit your survey areas to a particular size (lichen surveys conducted by the United States Forest Service are a 120-foot diameter circle plot), a particular group of lichens (e.g. epiphytic macrolichens), or target only bioindicator species. Excellent citizen science methods have also been developed for morphotype identification in place of species identification.[44] Many countries have indices for old growth forests or undisturbed arid land soil crusts. Although many of these are crustose lichens that take a bit of extra time to ID, targeting these species in your inventory can be very helpful in stressing the conservation value of particular areas.

For more information on rare lichens and their diminishing ecosystems, check out the Global Fungal Red List hosted by the International Union for the Conservation of Nature. For regional old growth lichen species indices for your region, do a search on www.scholar.google.com to find recent research in your area. Thus far, many ecosystems throughout the world have been assessed for valuable lichen bioindicator species, but much work remains to be done. For a general understanding of lichen bioindicators of forest health, see the work of Bruce McCune, particularly "Lichen Communities as Indicators of Forest Health."[46] For a general understanding of lichen bioindicators of rangeland health, check out the research of Jane Belnap and David Eldridge, especially their manuals on biotic crusts.[47]



Air Quality Bioindicators in California, USA

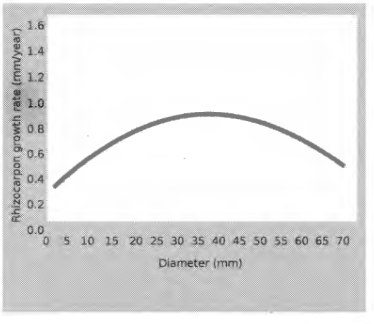
Lichen Communities are used by the U.S. Forest Service to monitor air quality along the Sierra Nevada mountains. Note how impoverished the lichen communities are in the Southern Sierra Mountains, presumably due to the nitrogen pollution coming from the Central Valley, one of the world's most productive agricultural regions. Figure adapted from a U.S. Forest Service research document.[45]

Lichenometry

Most species of rock crust lichens grow so slowly and regularly that they can be used to measure the age of rock surfaces. These might be unmarked tombstones, archaeological sites, or even moraines left behind after the retreat of a glacier. The most reliable time period is said to be between 50 and 1,000 years. Since radio carbon dating becomes inaccurate below 500 years and is limited to materials containing organic carbon, lichenometry can be a great tool in archeology and dating of rock surfaces.

The accuracy of lichenometry is based on the precision of calibration. To calibrate, find a rock surface that has had a surface exposed for a known amount of time. This might be a stone wall that has a commemorative plaque with the date, or a church that was built in a known year, or tombstone with a date on it. Ideally, the surface should be at least 20 years old. Then pick a crustose lichen that is common on that surface. The preferred species is the ubiquitous Map Lichen (species in the *Rhizocarpon geographicum* group), but other non-lobate crutose species should be fine as long as you calibrate. Note that lichens have a variable growth rate depending on climate and habitat, and little work has been done testing growth rates of other species, so although there are established growth rates of *R. geographicum*, it may or may not correspond with your region.

Once you've chosen your species, measure the diameter of the largest thallus in millimeters. Divide this by the number of years the stone has been in place to obtain an approximate annual growth rate. To refine the calibration, repeat it with several other rock surfaces that also have known ages. Be sure to note the rock type; granitic, basaltic/volcanic, and limestone/cement stone all have different nutritional profiles that will affect the growth rate and species composition. If possible, calibrate the annual growth rate using the same rock type as the rock surface being dated. These calibrations are location specific, and should be redefined in different bioregions, elevations, or habitat types. This consideration is not necessarily limiting, but rather opens up interesting possibilities of measuring how lichens respond to a changing climate or changing habitat.



Lichen growth rates are not necessarily linear. Rhizocarpon geographicum has been shown to have a curved growth rate. In Wales, when R. geographicum is about 17 years old it grows at a rate of 0.3 mm per year. When it is about 60 years old, it jumps to a rate of 0.9 mm per year and then decreases back down to growing at 0.5 mm per year when it is 100 years old. Graph adapted from Armstrong, 2004.[48]

Final Thoughts

"Lichens are *place*," says lichenologist Trevor Goward.[49] The meaning of this phrase twists around as it is considered. It is a koan that originates at the margins of lichenology, where philosophy blends with biological theory, and symbioses are recognized as keystones in the architecture of the biosphere. Place is philosophically conceived as the conceptual space where the subjective and objective overlap like colors in a kaleidoscope, where space and time intersect and reconnect, each changing the other. Similarly, lichens are where the concepts of the individual and the collective are seamlessly merged and dynamically changed by one another, and the distinctions between the two are nearly lost in complexity; where growth is no longer an aggregate building from DNA blueprints, but where growth builds from a dialogue of bionts, lichen, and ecosystem.

In lichens, we can find a biological analog for how to build healthy communities, gravitate towards a deeper understanding of the sacredness of relationships, and listen to the dangling murmur of emergent potentialities that can only become whole if we delve into relations the way that lichens do: fully dedicated to symbioses, fully present through all seasons of our experience, and always sharing the narratives of place.