



Erratum: Introduction to the lichen ultrastructure series

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A lichen is a slow-growing organism that forms a body – a thallus – via scripted symbiotic/mutualist relationships between fungi, algae, and bacteria – in some cases cyanobacteria. Some 20,000 different ascomycete fungal species are lichenogenic, and every fifth species of fungus on the planet, and every second species of ascomycete, resides within a lichen. An estimated 12% of the planet's terrestrial ecosystems are lichen-dominated, including arid deserts and vast regions of arctic tundra. Lichens have been found that are 9000 years old. Humans are generally unaware of their existence since they are not common sources of food or fuel.

Each thallus is constructed using the same basic body plan. A mycelium of fungal hyphae occupies the central region of the thallus, called the medulla. The medullary hyphae send out branches that differentiate to form the outer layers, including a green-algal layer, that encase the medulla. The outer layers, which also carry bacterial biofilms, mediate the attachment of the thallus to its substratum, its interactions with the environment, and its reproductive strategies. The layered tissues in turn generate the large-scale architecture of the thallus: some lichens (foliose) produce flattened lobes, some (fruticose) cylindrical stems, some (squamulose) tubular structures called podetia. The unique shapes, sizes and colors of these tissues allow the skilled lichenologist to recognize thousands of lichen species, much as a skilled ornithologist recognizes thousands of bird species.

1. Lichen architecture at the ultrastructural level

Our studies [1–5] focus inward, probing the small-scale architecture of the lichen thallus. What do the fungi and algae and bacteria look like when they construct and inhabit these layers? How do they interact with one another? What kinds of matrices and coats and secondary products do they produce, and how do these contribute to lichen assembly and the properties of particular tissues?

We used three data sources: 1) electron micrographs of lichens using the quick-freeze deep-etch technique; 2) light micrographs of lichens using the polyethylene glycol-embedding technique; and 3) published images using a variety of microscopic techniques. By integrating these sources, and coupling them with published studies on lichen

biochemistry and physiology, we have been able to deepen our knowledge of lichens, in some cases by confirming and expanding previous understandings and in other cases by making novel discoveries.

Here we highlight the key concepts that have emerged from our studies, where the supporting data and discussion are presented in the five papers that follow. We conclude by offering some general perspectives on the nature of lichens.

1.1. Medullary hyphae have stem-cell properties

All lichens possess a central medulla whose resident hyphae engage in homotypic branching in their peripheral growth zones called pseudomeristems, bifurcating to form new hyphae that are replicates of the mother hypha and increasing, albeit very slowly, the lichen's overall size (lichens typically expand 1–2 mm per year). They also engage in heterotypic branching: these branches grow at right angles to the mother hyphae and into specific regions of the thallus periphery, where they differentiate into specific cell types and assemble into tissues that collectively surround the medulla. Some heterotypic branches attach to algae to form the green modules of the algal layer; others grow into the outer cortex of foliose lobes where they form sub-branches and secrete polysaccharides; others align in the outer layers of fruticose stems; others mediate substratum attachment in the inner layers; others form asexual propagules called soredia with crystalline coatings; others form asexual propagules called fibrils; others differentiate into sexual tissues in the apothecia; and others participate in forming the many additional structures that are produced by specific lichen lineages.

The medullary hyphae therefore resemble stem-cell systems in other organisms: they maintain themselves as a steady-state inter-communicative multi-potent population, and are poised to send out branches to perform differentiated tasks. In some cases the branches may initially be programmed to simply engage in surface-directed growth, with specific differentiation programs triggered by external signals encountered during their migration to or arrival at their destination. In other cases the “fate” of a branch may be determined at the time of branching. There are as yet no molecular-level understandings of these processes.

During the course of their differentiation, the medullary branches

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commonly interface with bacterial biofilms that coat the lichen surface, and complex bacterial matrices attach to the walls and extracellular materials (ECM) produced by the fungi. Presumably co-evolution has occurred and is occurring at this juncture, with specific bacteria adept at colonizing specific ECM displays by the fungus. Not yet known is the extent to which the fungal ECM is genetically optimized to participate in the recruitment of favorable bacteria and to what extent specific bacteria are genetically scripted to associate with favorable ECMs. In either case, these interactions likely stimulate novel patterns of gene expression in both the fungus and the bacterium, and they doubtless play vital roles in lichen physiology.

1.2. Some hyphal branches form junctions with algae

The lichens in our study harbor eukaryotic green algae in the Trebouxiphyte class. The algae form small wall-to-wall junctions with a population of medullary branches in the pseudo-meristem; presumably both the alga and the fungus participate in defining the junction's properties. The junctions have been posited to serve as avenues for algal/fungal metabolic exchange, an important product being the sugar alcohols (polyols) produced by algal photosynthesis and utilized for fungal metabolism. Algal/fungal junctions are found in all lichens analyzed and represent a key feature of lichen organization. Nothing is yet known about how they are constructed such that they might mediate metabolic exchange.

1.3. Hydrophobic films envelop algal/fungal consortia to form green modules

In foliose lobes and fruticose stems, conjoined algae and fungi become coated with hydrophobic proteins, produced and secreted by the fungi, to form insulated units that we call green modules. The modules are surrounded by cords of non-coated hyphal branches that differentiate into outer layers when they reach the lobe surface. The structural relationship between the hydrophobic faces of the modules and the hydrophilic materials in the hyphal walls has suggested a model for air/water conductance in the lobes of foliose lichens.

1.4. Hyphal branches transform into acellular struts and honeycombs

After hyphal branches have performed their differentiated tasks in the lichen, many if not all transform into acellular forms called struts and honeycombs. Strut transformations also occur when a lichenogenic fungus is cultured solo in the laboratory. The transformed hyphae are acellular but retain intact walls, and are posited to serve as structural support for the lichen and to perhaps also engage in water and polyol transport.

1.5. Medullary hyphae reside in a liquid matrix called fog

The medulla is usually described as being filled with air, but our images document that the medullary hyphae are embedded in a viscous fluid we call fog. While the composition of fog has not been analyzed, we posit that polyols, produced by algae, not only serve as sources of fixed carbon for lichenized fungi but also serve as an important component of fog. A polyol-based extracellular matrix would provide the medullary hyphae and their branches with a water/osmolyte buffer, an anti-freeze, and a protective glass-like interior in the desiccated state.

1.6. Lichen secondary products form crystals and aggregates

All ascomycetes synthesize and secrete an array of hydrophobic secondary/metabolic products. In lichens, >1000 different kinds have been identified, and they can represent 10–30% of the thallus dry weight. Crystallized products, posited to serve as photo-protectants, localize to fog and to fungal wall surfaces, and putty-like aggregates

localize to several surface tissues, where they participate in forming outer boundaries. Monomers of secondary products presumably contribute to fog composition. Much remains to be understood about the role of these materials in lichen construction and maintenance.

1.7. Bacterial biofilms associate with the lichen surfaces

Numerous bacterial species produce structurally diverse matrices on the lichen surfaces. Some matrix films serve as the sole boundary between medullary fog and external water; others collaborate with hyphal branches and secondary products to form more complex surface boundaries. Hence bacteria not only provide metabolic products but also physically participate in lichen construction. They are also thought to participate in forming attachments to bark and stone substrata, a hallmark activity of all lichens.

Lichen biofilms are hetero-specific. Two different bacterial species can be neighbors, their distinctive matrices meshed together seamlessly at the boundary between them. Hence the biofilm of each lichen is a multi-species construct, much like the lichen writ large.

2. Perspectives on lichens

2.1. The lichen as an organism

If we define an organism as a self, then a lichen meets the criteria: it self-organizes; it self-maintains; it self-protects; and it self-reproduces to form more selves of the same kind. On our planet, a self is usually a single-celled organism, dividing into two daughter organisms or engaging in sexual reproduction. In some cases it is a multi-celled organism wherein the constituent cells share the same genotypes; most perform differentiated functions in the service of that organism, while some form lineages that engage in asexual or sexual self-reproduction. Using this metric, a virus would be considered a self – it offloads the self-maintenance criterion to an infected cell, which it may or may not kill in the process, but the job gets done, as is the case for all parasitic organisms with stripped-down genomes.

Then there's the case of the social insects. The self in this case is the colony, the hive, the mound. Its constituents, all sharing the same genotypes, are the queen, responsible for the self-reproduction of the colony, and the workers that perform differentiated functions in the service of the colony. The concept of the colony as a self is counter-intuitive because the workers are free-living ants or wasps and not tissues or organs; they have “lives of their own.” Moreover, each worker self-organizes, self-maintains, and self-protects. But the workers lack the fourth criterion: they are sterile and are themselves programmed to die. It is the colony – self-organizing, self-maintaining, and self-protecting thanks to its constituent workers – that reproduces itself thanks to its queen.

The lichen represents a fourth alternative: it is a multi-celled organism, capable of reproducing both sexually and asexually, wherein the constituent eukaryotic and bacterial cell types are genetically unrelated, and indeed from separate kingdoms. All are capable of free-living existence as independent self-reproducing organisms, but they opt to “lichenize,” collectively constructing a thallus. The algae and fungi each differentiate to perform various lichen-related functions, and the bacterial biofilms serve both architectural and metabolic roles. Hence the sole formal distinction between a multicellular organism and a lichen is that one differentially expresses the information encoded in a single kind of genome and the other differentially expresses the information encoded in multiple kinds of genomes. While this distinction is important, it does not disqualify a lichen from being regarded as an organism, just as the possession of a microbiome by a multi-cellular organism does not compromise its status as an organism.

2.2. The lichen symbiosis

Most facets of the project of building a lichen are encoded in, and organized by, the medullary hyphae and their branches. Medullary hyphae originate as hyphae that grow out of ascospores and bifurcate to generate mycelia, and they display no lichen-building traits until their heterotypic branches contact and form junctions with a suitable alga.

This junction-forming stage of the lichen-building program is elicited when compatible algae and fungi are grown separately in the lab and then mixed together. The branches in the vicinity of these *in vitro* partners may go on to form layers of various kinds, but the consortia fail to form lichens, fail to produce secondary products or to differentiate into specialized or sexual tissues. This failure has not yet been remediated by the addition of lichen-friendly bacteria or by other manipulations, and its basis is not yet understood.

While the fungus encodes most of the lichen-building information, and each species of lichenogenic fungi has a distinctive set of information, it is not correct to suggest that the alga is just a “prisoner” or “slave.” A lichen fails to form unless a suitable alga is present – hence the alga holds co-ownership of the lichen. Moreover, the benefits to the alga are numerous: it resides in a tissue with excellent provisions for light, water and air, and excellent protection from desiccation and harmful photoradiation. All it has to do is synthesize glucose/polyols, which it would do in any case as a free-living organism. It's a fair exchange, and hence a mutualistic symbiosis.

The numerous bacteria, and in some cases cyanobacteria, that inhabit lichens presumably also engage in symbiotic exchanges with the algae, the fungi, and one another, where the scope of these interactions is only just beginning to be probed at the molecular level. Many of the bacteria are capable of converting atmospheric nitrogen into reduced nitrates, providing the community with vital building blocks for nucleic acid and protein synthesis. Importantly, only a sub-spectrum of bacterial phylotypes, with a predominance of α -proteobacteria, take up lichen residency, and a sub-spectrum of these resides in each kind of lichen. Choices are being made.

Since the bacterial biofilms usually attach to fungal walls and extracellular materials, the fungi are presumably providing bacteria with favorable surfaces, but it is not yet known whether these matches are genetically scripted by the fungus (do fungi actively favor certain bacteria?) or are taken advantage of by the bacteria. Nor is it known whether the fungi actively “recruit” favored bacteria using, for example, soluble cues, nor whether unwanted bacteria can be rejected. What is clear is that lichen biofilms represent a highly complex consortia of bacteria, with different species forming matrix connections with one another and with the fungi, and that they participate symbiotically in both lichen construction and physiology.

2.3. The lichen as an ecosystem

Each lichen thallus is also a bounded, self-structured, self-maintained, and fully balanced ecosystem. Its fungi, algae, and bacteria carry out niche construction, photosynthesis, nitrogen fixation, external nutrient acquisition, air/water relationships, and biosynthetic/metabolic processes that collectively ensure the survival of each inhabitant and the preservation of their shared and co-constructed habitat. The ecosystem is structured to be resilient, bounding back after periods of desiccation, freezing, and UV bombardment. It does no harm to other organisms or ecosystems, and it is vulnerable to only a few exogenous agents, such as air pollutants, caribou, and certain snails. It is an ecosystem without evident internal predators or pathogens. All of its inhabitants grow very slowly, and they increase in number/length only as the niche itself expands in size to accommodate them. Lichens have been able to spread throughout the terrestrial world, including its most inhospitable terrains, because they bring their self-sustaining ecosystems with them and because they aren't in any hurry.

CRediT authorship contribution statement

Ursula Goodenough wrote this on her own.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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