

Lichen Subscribe

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[Music begins]

Izzie: Welcome to Morel Dilemma, an exploration of fungi in the natural world and human society. Today I have a huge piece of news! It's so huge that I actually bumped this episode up like five weeks in my podcast schedule, just to talk about it while it's still "breaking". The paper was published online in Science last Thursday, July 21, and it's going to be on the front page of the next issue, July 29. This paper is ripping textbooks apart. And it's all about... lichens.

[Music ends]

Izzie: As with all science courses I've ever taken, I'll start this episode by telling you the long-established rule, and then after the break, I'll tell you how it's been broken.

You've probably seen lichens if you've ever wandered the woods, visited a rocky beach, or walked through a cemetery. They grow on trees, leaves, mosses, stones, walls, soil, other lichens, almost everywhere. They can be vibrant yellow, dusky brown, olive green, bright red, or have a bluish tinge, and some change colors depending on whether they are wet or dry. Lichens may branch forever like tiny leafless trees; they may look like a spot of spilled latex paint; or they might appear like tiny bushes full of leaves.

Don't let the branch or leaf shapes fool you, however. Lichens are absolutely not plants. In fact, lichens don't snugly fit into any category at all. As you may have guessed by my excitement about lichens, they do contain some fungi - but where fungi don't photosynthesize, lichens do. That's because every lichen is a symbiotic relationship, which means it's an example of more than one species working side by side. The fungus in a lichen acts as a frame that protects an inner layer of algae or cyanobacteria, tiny green microbes that use the sun's energy, air and water to make sugar. That's the photosynthetic part of the lichen.

Lichens were first described in the 1860s, and in 1868 the Swiss botanist Simon Schwendener proposed that they were two organisms working in concert. This controversial idea was rejected at first, which is understandable - the concept of symbiosis had not even been conceived yet, and the word was actually first coined a few years later in order to describe what was seen in lichens. *Symbiosis* is Greek for "living together", and it is a very common way of life. You've probably heard about your gut microbiome, and know not to ingest antibiotics unless you really have to. That's because maintaining your own health and nutrition involves keeping a ton of species of bacteria alive and happy in your gut. Thanks to you, they get access to food,

and thanks to them, you get more nutrition out of the food you eat. You're living together in a *symbiosis*.

There are a few recognized types of symbiosis. The one I've just described in your gut is known as *mutualistic symbiosis*, where both organisms in the relationship gain something and neither is harmed. There is also *commensalistic* symbiosis, where one organism benefits from the other, which is not affected by the relationship at all. Orchids engage in commensalistic symbiosis with their host trees in tropical forests. The orchids send out just enough roots to anchor themselves to the trees, then unfurl their leaves and do their own thing. By using the trees, the orchids gain a place in the sun, but the tree isn't really affected by the orchid's presence one way or another.

Finally, there are *parasitic* symbiotic relationships, and I'm sure you know plenty of examples. These are relationships in which one organism benefits to the detriment of the other. Think about hookworm, ringworm, tapeworm, and other pests that feed on the host, weakening them and sometimes putting their lives in danger. It doesn't have to be that dramatic; as long as one organism is benefitting from the other's decline, it's parasitism.

Lichens are generally considered to be in the mutualistic category. The fungus of the lichen provides structure and creates a safe little home for the photosynthesizer, while bringing nutrients in and keeping the interior moist. In return for this cozy house, the algae or cyanobacteria makes sugars that both it and the fungus can enjoy. The way a lichen is structured, the algae or bacteria is one layer surrounded on top and bottom by fungus.

Lichens' names are usually the same as the fungus's name, and the photosynthesizer is implied based on its known relationship with the fungus, because any species of fungi that can form a lichen only does so with a specific species of algae or cyanobacteria. Quick note: to make the rest of the episode less cumbersome, I'm going to just say "algae" to refer to the photosynthesizer. But remember that it could just as easily be a cyanobacterium.

The inner layer of the fungus anchors the lichen to the substrate - the rock, tree, plant, etc. that the lichen lives on. The outer layer is called the cortex, and it's the skin of the whole operation, tightly packed fungus solidified with a biological glue. All together, the two cortex layers and the algal layer are called the *thallus*. The alga is sandwiched between two layers of fungus, which means it's basically stuck.

This isn't necessarily a bad thing; the cortex controls the amount of light the alga receives, providing it with enough sun to photosynthesize, but preventing it from roasting. Besides, algae need to be wet, and without the fungus, landlocked gravestones aren't going to be a great place for the algae to live.

However, there's evidence that the algae and the fungus have, shall we say, an uneasy relationship. Experiments in the lab have shown that while inside a lichen, algae

export, or give up, about 40 percent of their sugars to the fungus, and use only 2 percent of their sugars for their own growth and development. That's a lot of sugar to give up. You might guess that the algae just send a lot of their sugar out into their environment normally, and the fungi have found a way to use it, while helping the algae out too - real mutualism. Unfortunately, it doesn't look like that's the case. Outside their lichen, the algae completely switch, using 50 percent of their sugars for personal use and only exporting 2 percent. It seems like the fungus is holding the algae captive, denying them the sugar they'd rather keep for themselves, in more of a parasitic relationship. Kind of like a typical 9 to 5 job. "Al, I need you to work this weekend so you can finish that Sucrose order!"

Either way, whether or not the algae and I are happy about it, lichens represent a total departure from the forms either fungus or algae take on their own, in terms of appearance as well as behavior. Lichens also occupy different habitats than fungi or algae do on their own. Alone, the partnered fungi and algae could never survive in the places lichens are found - from the desert to sheer sea cliffs to the arctic, lichens are everywhere. They cover an estimated 6% of the earth's surface, and there are even so-called "vagrant" lichen that are happiest when they aren't attached to anything at all.

For the most part, vagrant lichen aren't very useful for people, but anchored lichens are handy in a lot of ways. For example, lichenometry is the practice of dating exposed rock by the lichens growing on it. Radiocarbon dating, which is used to figure out the age of artifacts like mummies or the Dead Sea Scrolls, isn't super reliable for things suspected to be less than about 500 years old. That's where lichens come in. Because of their tough, adaptable morphology, an undisturbed patch of lichen can live for close to a thousand years. By measuring how fast a particular lichen species grows each year, scientists can infer the age of an artifact anchoring the same species.

My favorite story of lichenometry involves Franklin's lost expedition to the Arctic. A 129-man British crew led by Captain Sir John Franklin disappeared during an 1845 journey to map the Northwest Passage. The ship from that voyage wasn't found until 2014, but the graves from three crewmen, who we can only presume were the first to go, were discovered in 1850. And there is a Jewel lichen, *Xanthoria elegans*, growing on the tombstones. It's thought that as soon as the gravestones were placed, they offered perches for arctic birds, whose excrement would have been excellent nutrition to a lichen - as such, it's assumed that the lichens began growing in 1846. In 1990, the lichen patches had diameters of 4.4 centimeters, indicating that *Xanthoria elegans* grows about one third of a millimeter every year (at least in the Arctic). Now, if scientists want to date another grave with *Xanthoria elegans* growing on it - or a chest, building or rock shelf - they should have an easy time!

Lichens can also be used to monitor air pollution. They only grow where the air is very clean, and heavy metals and air pollution that fill the air in industrial areas are

poison to them. If there are lichens near your home, congratulations! It means your neighborhood is probably a good place to breathe. Come to think of it, I haven't seen as many lichens in my neighborhood in New York City as I used to see in Colorado...

Lichens have a good place in traditional medicine and food around the world. *Peltigera leucophlebia* was used in Europe to fight the oral yeast infection called thrush. The misnamed Iceland Moss, really the lichen *Cetraria islandica*, has been used to treat cough, stomach ache, and even cancer. Also in Iceland, it's a useful food item, whether stewed with milk and brown sugar to make porridge or ground up and used as flour. In the United States, some native peoples relied on lichens to get through the winter.

While lichens have been able to help geologists, environmental health workers, and traditional chefs, they've long posed an unsolvable puzzle to biologists. While they've represented the quintessential symbiosis since 1868, the fungi and algae in lichens have been very hard to study. When scientists attempt to culture lichens in the lab, nothing happens. When they try inducing the symbiosis between two species known to work in wild lichens, either the cortex forms incorrectly or nothing happens. Most frustratingly, sometimes two lichens that have identical species of fungus, and identical species of algae, are totally different colors; one might even be edible while the other is deadly.

But without being able to cultivate the lichens in the lab, scientists haven't been able to even start figuring out *why*.

Until last Thursday.

[Intermission music]

Izzie: For 150 years, lichens have represented a strange duality, being simultaneously the quintessence of a biological concept and largely impossible for biologists to study. Everyone wants to tease apart the relationships in lichens to find out how symbiosis began. Unfortunately, without being able to put the puzzle pieces together in the lab, biologists have had no way to look at the origins of the lichen relationship.

Two lichen scientists determined to crack the code and joined forces in 2011. Toby Spribille and John McCutcheon of the University of Montana began a study on two varieties of lichen that were apparently genetically identical. The fungal species is the same in each, as is the algal species. And now that we're in part two, I do mean specifically algae.

These lichens had different coloration and levels of toxicity. Where *Bryoria fremontii* is dark brown and nontoxic, *Bryoria tortuosa* - great name, right? - is bright yellow and produces high levels of a toxin called vulpinic acid. Despite being genetically identical, the baffling differences mean these lichens have had different species names for 90 years.

When Spribille and McCutcheon took on this puzzle, they decided not to look at the species in the lichens, which had proved fruitless for decades; instead, they would look at the proteins the different lichens produced. Every cell has a nucleus full of DNA, which knows exactly what the cell needs to do to stay alive. The parts of the DNA that tell the cell how to make a protein are called genes. When a gene is “expressed”, that means the protein is getting made. It was possible that these lichens were producing different amounts of certain proteins - expressing the same genes differently - and that could explain the differences between them, even though their DNA is the same.

When a gene is going to be expressed, small molecules like recipe cards, called mRNAs, copy the directions from the DNA and move them to the place where proteins are built. If more mRNAs are coming from one gene, that means the cell wants to make more copies of the gene’s protein: that gene is being heavily expressed. Scientists can use this relationship between mRNA and protein to find out how much of a protein is being made by a cell, as long as they know the language in the recipe card (which we do).

In this kind of experiment, biologists generate glowing markers, molecules that specifically stick to certain mRNAs. When they wash away the extra markers, scientists can see how many markers stayed bound to the cell, and since each one is bound to an mRNA for the intended protein, scientists know how much of the protein the cell is trying to make. So if there were a lot more messages in *B. tortuosa* that said “make vulpinic acid!” the scientists might have had a lead.

This was a cool idea, but sadly proved fruitless - there was no difference in the number of mRNAs the cells in *B. fremontii* and *B. tortuosa* were making.

Almost on a whim, Spribille widened the search. The team had been focusing on the mRNAs known to exist in the fungus in the lichen, an ascomycete, as well as in the algae.

Now they broadened their search to include all fungi, and suddenly they had something. There was a second fungus in there, and strangely, it was not an ascomycete like the first fungus, but a basidiomycete. Ascomycetes and basidiomycetes mainly differ in how their sexual spores are produced; ascomycetes develop their spores *inside* little pouches, where basidiomycetes develop their spores on the *outside* of little club-shaped structures. These are the main phyla of the so-called “higher” fungi and some molds, and they diverged four hundred million years ago. So finding basidiomycete signals in a lichen supposed to only contain ascomycetes and algae was a dramatic surprise. These basidiomycete messages were present in both lichens, but much more abundant in *B. tortuosa*. Could the abundance of this startling new member of the lichens be just the thing that set the two species apart? *Bryoria*

Spribille's team was wary. Signals like this have appeared before in lichen studies, and they usually seemed to indicate that the samples were contaminated somehow - either there was an error in preparing the samples, or the lichens they had collected had been infected. So they left the mRNAs behind and tried directly marking the DNA of these newcomers.

Because scientists know the language of DNA just as well as the language of RNA, they can use similar markers to track the presence of specific DNA sequences in cells. Every species has a unique DNA sequence, so when Spribille's team made markers to label the DNA of one type of cell, every marker goes to that type of cell only. In the new experiment, Spribille used blue markers to label the ascomycetes and red markers to label the algae. For the mysterious newcomer they selected green. The team washed a sample of each *Bryoria* lichen with these markers, and when the results came back, there it was: an entire layer of basidiomycetes on the outer edge of the cortex. They're in the layer of sugars that gives the cortex structure and helps contain the algae. Now instead of an algae sandwich, the thallus looks like an ascomycete-algae layer cake... and the basidiomycete is the frosting.

Now the ball was rolling, and the team had to sort out whether what they'd found was a fluke, or an unknown rule of lichenology that had gone unnoticed for 150 years. Using more DNA markers, the team looked for basidiomycete fungi in the lichens that naturally grow near the *Bryoria* samples they'd taken. Then they sampled some more lichens from Montana. Then from a wider range of the United States. Then from Europe. As the search widened, they investigated more branches on the lichen family tree. They sampled 45,000 lichens from a variety of genera, "genera" being the smallest categories that sort similar species together. The team discovered 52 lichen genera, spanning six continents, that contain hitherto unnoticed basidiomycetes.

As with the algae and the ascomycete, each lichen incorporates a particular species of basidiomycete fungus. They're very picky: one species of lichen, *Letharia vulpina*, uses the same species of basidiomycete whether it's growing in Montana or Europe. Think about an organism that can't function properly without two other very specific organisms, no matter where it is in the world. That applies to more than 2,200 species* of lichen, or 6600 species of fungi and algae altogether. Based on molecular clock dating, a method by which scientists use the very DNA of a species to determine how old it is, Spribille and company determined that the algae and two fungi in each lichen are the same age - so that highly specific relationship might have been there from the very start, when lichens first helped colonize land hundreds of millions of years ago.

While the work is far from over and Pandora's Box of lichenology has just been flung open, it looks like Spribille and McCutcheon might finally have a working answer for the difference between *Bryoria fremontii* and *tortuosa*: there are more basidiomycetes in *tortuosa*, and they're located very close to the vulpinic acid.

Spribille has hypothesized that they are responsible for the toxin's production, or for causing the ascomycete to produce it. In other lichens, other basidiomycetes might be responsible for other defense mechanisms that have been attributed to ascomycetes. There might now be a key to understanding how these defenses are produced, where before it was just baffling that the ascomycetes weren't producing any defensive mRNAs.

Besides, now scientists have a better understanding of why lichens have been so hard to grow in the lab: one third of the party was missing!

With their new paper, Spribille and company ushered in a second revolution in lichen science and the study of symbiosis. They've literally rewritten the textbooks. Hopefully this will be the start of an era of greater lichen understanding, and scientists will start being able to learn more about how symbioses begin and organize themselves. As for me, I'll rest easier knowing that the algae isn't giving 40 percent of its sugar to just one officemate. It feels less like a 9 to 5 and more like the living expenses we all deal with. Maybe most of that exported sugar pays rent and utilities, and some extra goes to the super and the doorman. ... Why don't lichens grow here, again? That sounds like a typical New York apartment to me.

[Music begins]

Izzie: Morel dilemma is written and produced by me, Izzie Gall, and our theme song was written and performed by John Bradley. Special thanks this episode to Meg for doing our intermission.

If you'd like your voice on the podcast, call the hotline at 347-416-6735 and leave a mycophilic message.

Hey, exciting news - Morel Dilemma is now available on iTunes! You should definitely go check it out. And hey, if you like what I'm up to, please leave a review! It's really the best way for my hyphae to find other dormant mycophiles. Thanks to iTunes user KML926 for leaving the first one!

And for our iTunes listeners, don't forget to visit moreldilemma.org for extra content, like answers to user questions. Last week I wrote a post about the world's biggest fungi. The answer is more complicated than you might think!

I want to remind everyone that mushroom foraging is tricky business, and you should never eat a wild mushroom unless an expert has identified it in person and told you it's safe to eat. Even so, allergies to uncommon foods can be hard to predict, so please be careful. There are other ways to enjoy fungi. My favorite is photographs.

[Music ends]

Resources

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