

Unearthing the Secret Lives of Alaska's Mushrooms

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There is a reason people refer to “hunting” mushrooms instead of “picking” them. Mushrooms may not move fast, but they are elusive. Because the processes that drive fungi to fruit are still not fully understood, even choosing the right time and place to look for them takes a combination of guesswork and luck. Not to mention that many prime edibles, such as highly sought morels, can be easily lost among leaves or confused with spruce cones.

Researchers at the University of Alaska Fairbanks (UAF) have added a challenging twist to the hunt: They're collecting mushrooms and other fungi they *can't* see. Using the same techniques that helped unravel the human genome, a National Science Foundation-funded team is seeking to understand the diversity and role of the fungal life hidden in the soil beneath Alaska's boreal forest. Already, their work has begun to reveal the secret lives of some Alaska fungi, as well as their potentially large role in ecosystem responses to climate change.

The hunt

An invisible mushroom hunt begins the same as any other, with foragers bearing baskets and buckets through fall-reddened underbrush and yellow birch leaves, eyes cast down. But shortly after they enter the woods, it's obvious prime mushroom season has already peaked. A frost the day before has reduced most aboveground mushrooms to mush. Normally, this would be disappointing, but it doesn't disturb microbial ecologist Donald “Lee” Taylor, graduate student

Ian Herriott, postdoctoral fellow József Geml, and undergraduate Hope Gray.

Mushrooms are the “fruit” of a much larger mycelium, the vegetative portion of the fungi that gathers and processes nutrients, explains Taylor, principal investigator of the fungal genomics project. “The aboveground mushroom part you see is like a fruit on a huge dogwood tree,” he says. “It's the sexually reproductive part of a much larger organism. When you see a mushroom, there could be ten, a hundred, a thousand times the

quantity of that biomass material that's invisible to you underground.”

It is this belowground biomass his team is after. With each plunge of a hand-held soil-coring device into the ground, researchers gather scores of microscopic, hairlike filaments, known as hyphae, that make up fungal mycelia. The samplers stand an even distance apart, take their samples, walk 10 paces, and repeat the process. The most complicated element of the sampling procedure is avoiding fallen, recently burned trees covered with various stages of overgrowth. Soils collected from similar depths at each site will be pooled to produce homogeneous samples, each of which could contain hundreds to thousands of fungal species.

Unraveling the genetic information each sample contains is far more complex. The samples are sent to the Broad Institute, whose sequencing labs (formerly known as the Whitehead Institute Center for Genome Research) were a major player in the Human Genome Project. The process involves using segments of known fungal DNA sequences that are likely to be shared among all fungi—but not so common, or conserved, that they might also be found in other organisms—to locate and amplify any fungal DNA sequences within a given sample.

Using the same high-throughput methods that were developed to sequence the human genome, says Taylor, “we can process many, many more samples and generate many more sequences than can be done in individual-investigator labs.... Other sequencing projects that I'm aware of have sequenced a couple hundred sam-



Researcher Lee Taylor waits for other members of his sampling team to align themselves with him before taking a core sample. The forest types they are sampling are representative of varying successional stages. Photograph: Sonya Senkowsky.

ples to maybe a thousand samples," he says. "We're going to do 20,000 to 30,000."

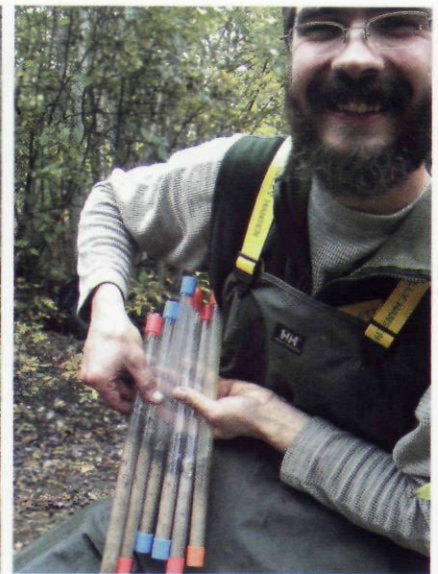
In addition to DNA extraction, the work involves sequencing selected genes of about 4000 collections of aboveground fungi (mushrooms) preserved in the University of Alaska Museum Herbarium. Among the goals of the work are to compare the diversity of belowground and aboveground fungal life, discover new species, and build a database of fungal genetic sequences and related information that is expected to be the largest of its kind. The effort, which started in 2003 with funding from the National Science Foundation, is expected to produce a storehouse of the most comprehensive information ever collected on one region's fungi.

"We have an ambitious goal," says Taylor: "to obtain genomic sequence data for all of the fungi that occur in interior Alaska. Now that's an impossible task. We won't get there. But through all the mushrooms that were collected, we'll get as close as we can....We'll know more about fungal diversity in this region than probably anywhere else in the world when this project is finished in a year or two."

Behind the scenes of climate change

Why use such sophisticated methods just to learn about a single region's mushrooms? This hunt is not just about the fungi, say researchers. It's also about their role in the boreal ecosystem, and possibly the planet's climatic health.

Fungi perform four primary roles, explains Gary Laursen, a research mycologist in the Institute of Arctic Biology and curator of the UAF Mycological Herbarium. First, they act as decomposers. "If not for them, there would be dead trees and limbs all over the place," he says. They also associate with algae to form lichens, act as parasites to cause damage to living plants and animals, and associate with the roots of plants in mutually beneficial mycorrhizal relationships. "There wouldn't be a single tree in Alaska if fungi weren't associated with the roots of trees." Additionally, they may play an as yet unquantified but crucial role in the carbon cycle. But to date, the belowground ecosystem is so little understood



Ian Herriott begins to take a core sample at a research site (left) and shows the results of one site's core sampling (right). Photographs: Sonya Senkowsky.

that the role of fungi has remained an unknown factor in climate change models.

The boreal forest that stretches across Alaska as well of parts of Canada and Russia is known as one of the world's major storehouses of organic carbon. Previous studies have shown that the mycorrhizal hyphae that create mutually beneficial relationships with trees to access and process nutrients make up the largest part of temperate and boreal forest soil microbial biomass—a finding that implies the fungal role in belowground biogeochemical processes is significant. But even the most basic information about such communities and their role in nutrient cycling is lacking.

Very recent investigations of the role of microbial communities in boreal soils are intriguing, says Roger Ruess, a terrestrial ecologist at UAF. "The notion that we have this massive pool of organic carbon really is only partially true," he says. "What we know now is that there are tremendously fast rates of nutrient and carbon turnover in these systems." Fungi, which drive carbon, nitrogen, and phosphorus cycling, are most likely responsible, adds Taylor, "but we don't know very much about which fungi occur up here, and which play roles in the nitty-gritty aspects of these biogeochemical processes."

For example, according to one recent climate change finding, Alaska's winters

are warming more than other seasons, with snow-covered soils releasing significant levels of greenhouse gases, says Herriott. His graduate research aims to identify whether fungi are responsible—and if so, which ones.

Aboveground observations also suggest something is changing. In recent years, mushroom experts in the state have received calls about fungi never, or rarely, seen here before. One species of earth star mushroom, a type of puffball with a star-shaped base, is usually found with sagebrush, but this summer it was found on two lawns in Fairbanks. Another unusual find: Matsutake mushrooms, prized in Asian markets, were found for the first time in southeastern Alaska.

"In the last three or four years, we have seen greater numbers of species of fungi actually fruiting," says Laursen. "Is that an effect of global change, or climate warming? It's hard to tell. Are the fungi here already and the conditions just opportune, with the [recent] warming trends, for them to fruit? We're not sure, but we think that's what may be going on. We think that the fungi have been here as mycelia, the imperfect, nonfruiting state.

"But the conditions now—temperature, moisture, and seasonal length—are conducive to their fruiting, so we're beginning to see things here we have never seen before. And that's really exciting for

us." He adds, "As climate is changing, I think we're going to see more of those things fruiting."

Deciphering their genetic code may also solve other mysteries. "For Alaska, the mushrooms are poorly studied," says Laursen, "so the genetics are going to allow us to jump up a notch," perhaps helping to identify species that could be harmful if eaten, and explaining why.

Already, some intriguing finds have come from the genetic results, including previously unknown fungi. "The most impressive result to me so far is just the magnitude of contribution of mycorrhizae to the richness of the soil fungal pool," says Ruess. The first couple of hundred sequences the team compiled suggested that about 60 percent of the fungal biomass was associated with trees. This implies a "staggering" role for mycorrhizal fungi in nutrient and carbon cycling, says Ruess.

In the category of species-specific research, Geml has found that one mushroom, *Amanita muscaria*, includes three clades, or groups, not previously seen together in one geographic region anywhere other than Alaska. The finding suggests the mushroom originated during the Tertiary Period in Beringia (a region including today's Alaska and northeastern Siberia) and probably migrated south on both sides of the Pacific over millions of years as the climate cooled. One of Geml's goals is to test whether Beringia represents a hotspot of genetic diversity for high-latitude fungi.

Another finding, says Taylor, may have uncovered the oldest fungal lineage ever sequenced. "We don't know yet if it's legit or artifactual—but it appears to branch off earlier than any other known fungi at this point in the tree of life, and yet it is clearly a fungus."

A legacy of data

Making the genetic data usable and accessible to a wider community was the task of a team of nonbiologists, led by bioinformatics expert Tom Marr, a research professor at the Institute of Arctic Biology, who has previously worked on the Human Genome Project. Although assembling mushroom data might sound like an easier task than working with

human genetics, Marr says, in some ways it was far more complicated. "From my perspective, this turned out to be more of a challenge than I thought."

The first step in setting up the new database involved quality-checking and assembling a collection of more than 31,000 existing fungi genetic sequences from sources such as GenBank, an annotated collection of publicly available DNA sequences maintained by the National Library of Medicine.

The database was designed so any user could enter an unknown segment of a newly sequenced fungus and the program would call up related sequences. But the problem, as the computer team soon learned, was that mushrooms are genetically more complicated than humans, thanks to having had more time to evolve—hundreds of millions of years to humans' six million.

"The fungi, being very old, have remarkable diversity," says Marr. One fungal segment might turn out to be one that was broadly conserved, or also present in a wide range of other life-forms. In the database, that meant that a search using that segment might bring back not only related fungi but also bacterial—or even human—hits, making the results next to useless for research. Ultimately, the computer team pioneered



Hope Gray shows off some coral fungi, one of the more substantial above-ground finds of this late-season foray. Photograph: Sonya Senkowsky.



Mycologist Gary Laursen takes an up-close look at tiny cup fungi growing on a moose nugget (dung). He teaches students not to overlook excrement when seeking fungi for collection. "Poop is an excellent substrate for fungi," he says. Photograph: Sonya Senkowsky.

a way to screen out "noninformative" parts of a genetic sequence, a process that is still being refined.

The legacy of this project will be a massive and searchable database of fungal genetic information, the Fungal Metagenomics Project Web site (<http://iab-devel.arcs.edu/metagenomics/>). Although designed primarily to help researchers, the site will also be accessible to the general public, says Laursen, with images and descriptions accompanying the sequence information. "What we want to do is, if you click out on a database—boom—a picture comes up, so it becomes extremely hands-on and user friendly." The result: a virtual Alaska foray, within reach of anyone who can use a Web browser.

Whether this foray into genetic sequencing nets the project goal of more than 30,000 usable sequences or only a fraction of that number, one result seems assured: At its end, the elusive and mysterious world of fungi will no longer be so invisible.

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