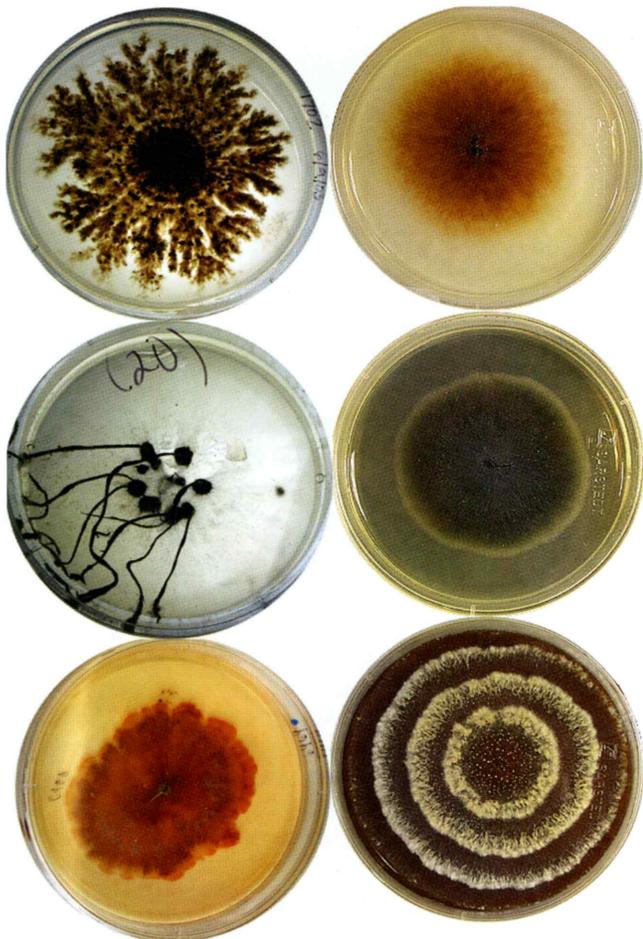


The Diversity Inside: Cryptic Fungi on the Navajo Nation

Native American students contribute to a new area of biodiversity discovery

By Susan McGinley

Betsy Arnold



Array of endophytic fungi growing on Petri dishes

They hide inside leaves, living silently and invisibly, only revealing themselves when forced to grow on a laboratory agar plate. Then they burst forth, their silvery, threadlike growth expanding into a nimbus of unexpected color. Endophytic fungi are among the world's least understood plant-associated fungi, living within leaves and stems without causing disease. These tiny fungi are found in trees, grasses, crop plants and shrubs all the way from arctic tundra to tropical rainforests, but only recently have their diversity, potential benefits for plants and applications in biotechnology been discovered and explored. That potential is vast and untapped.

"What's really remarkable about these fungi is that they live inside plants without harming their hosts, and in many cases protect those plants against invasive pathogens," says Betsy Arnold, an assistant professor of Plant Pathology and Microbiology in the University of Arizona College of Agriculture and Life Sciences, and curator of the Robert L. Gilbertson Mycological Herbarium.

"Think about that when you see green leaves—or woody trunks—or even cactus pads around you. Endophytes are ubiquitous among mosses, liverworts, ferns, conifers, and flowering plants. And plants that look perfectly healthy on the outside in fact can harbor dozens or hundreds of these fungi, the vast majority of which are expected to be new to science." Arnold collects and

studies the ecology, evolution, and taxonomy of these mysterious endophytic fungi by the thousands.

"I got hooked on these when I was a graduate student and haven't looked back," she says. "We've got 12,000 isolates living in the lab, representing several thousand species of fungi harvested from all over—from tiny plants in the tundra to chocolate trees in tropical agroforestry systems."

Over the past decade she has gathered these organisms from healthy plants in taiga (boreal coniferous forests); southeastern deciduous forests; montane ecosystems in the Appalachians, Canadian Rockies, and western US; the hyperdiverse rainforests of Costa Rica and Panama—and even from cultivated plants on the UA campus.

"I'm really a field biologist at heart," Arnold says. "The questions are satisfying and the applications in agriculture and pharmaceutical research are promising. But for me, the best part is collecting, and then getting to see these unknown organisms for the first time."

One special aspect of studying endophytes is that while divining their ecological roles or applications is a complex process, many of the techniques needed to gather them are straightforward and are well-suited for student researchers. Arnold capitalized on this during the summer of 2006, when she initiated a special collaboration with Diné College on the Navajo Nation.



Undergraduate volunteers Claire Heinitz, Kelly Farrell, Amanda Way, and Liang Shen in the Chuska Mountains, Navajo Nation

“We drove a sterile lab and all of the tools needed for molecular biology up to the college in the back of a pickup truck,” she says. And before long, six Navajo students were at work in a ten-week research program with Arnold, collecting leaves from native plants and isolating cryptic fungi from them.

“Their task was to get to know the biodiversity that hides out within the plants of the Navajo Nation. We looked at a completely understudied group of endophytic fungi,” Arnold says. “Soon the chemistry lab at the college was full of students extracting DNA and running PCR (the polymerase chain reaction), and coming in from the nearby mountains with more plant species to sample.”

Several studies suggest that Native American students from reservations succeed when they complete their education on-reservation and contribute directly to their communities. Because many students at Diné College could not travel to major universities for research training, two Diné College faculty—Barbara Klein and Mary Shimabukuro—worked with Arnold to bring the laboratory to the students instead. Funding came from the USDA’s competitive Tribal College Grants program. The grant proposal developed by these three scientists had its roots in Shimabukuro’s internship in the Arnold lab in 2005, which was supported by the Arizona Biology Network.

Recognizing the special opportunity encapsulated by living and working on the Navajo Nation, Arnold and her graduate students trained student peer-mentors who worked almost one-on-one with the Diné students to achieve the research goals of the project. These peer mentors, including three undergraduates from Duke University and one from the UA, volunteered for three weeks. During that time they lived in a hogan and assisted the Diné students in the field and lab. Together, the team completed ecological surveys, assessed plant diversity, gathered data on soil characteristics, and collected fungi from native plants.

“They collected over 600 fungi, and based on their DNA sequences, it looks like the vast majority are new species,” Arnold says. “We surveyed endophytic fungi from a variety of native plants in the Chuska Mountains near Tsaile—effectively the students’ backyards. It opened their eyes to this hidden biodiversity in the resources of their land.”

The research team brought leaves back to the mobile laboratory at Diné College and washed each with a series of chemicals to kill any fungal spores on the leaf surface. Each leaf was cut into small pieces that were placed on a sterile growth medium. Over time, the

fungi grew into the medium, extended into branches and blew out into starburst formations on the plates. Tiny pieces of each culture are now stored as a living library in the Gilbertson Herbarium and at Diné College, where they are available for further study.

In nature, endophytes produce spores from dead plant tissues. The spores then swirl around in the air, some landing on leaf surfaces, where they attach and grow into plants. Because the fungi only rarely sporulate in culture, the students had to sequence their colorful isolates to identify them.

“This provided a great opportunity to integrate both field ecology and molecular biology into the Diné College curriculum,” says Arnold. “And by using molecular tools to identify these fungi, the students provided a key piece of information needed for us to determine the evolutionary origins not only of endophytes, but of closely related pathogens.” These fungi are now a part of the living collection that Arnold and her graduate students study as part of a larger program examining the evolution of beneficial fungal symbioses.

“We are realizing that these fungi are linked in an evolutionary sense to virulent pathogens, including many that are important in agriculture,” she says. “My hope is that they will show us just what makes a pathogen a pathogen, and how we might ‘turn off’ that pathogenic activity in species or strains that cause disease.”

This type of research typifies what Arnold has done for the past decade. Most notably, in 2003 she and a team of scientists from the Smithsonian Tropical Research Institute in Panama discovered that endophytes from cacao (chocolate) trees can be helpful to their hosts.

“We found that natural assemblages of endophytes protect cacao trees against infections by *Phytophthora*, a virulent pathogen. An exciting aspect of that work was not that we found a ‘silver bullet’—but that defense was imparted by many endophyte species living together,” Arnold says.

In addition to the project on the Navajo Nation, which has already received funding for 2007, other projects in Arnold’s program include a study examining how beneficial fungi protect seeds from decay and death in tropical soils. She also heads a project examining the role of bacterial endosymbionts (bacteria living within the fungi themselves) in shaping fungal virulence. And she’s working with collaborators at the UA and beyond to explore potential applications of fungal endophytes in drug discovery and biological control. In the meantime, she manages ongoing efforts to catalogue the diversity, ecology, and evolutionary history of these little-known beneficial fungi in ecosystems from Arizona to western Africa.

“By studying these fungi we’re finding a new appreciation for the diversity of life on earth, a new source for potential medicines and other useful products, and a new framework for understanding plant ecology, physiology, and evolution,” Arnold says. “For me, that means a new understanding of what it means to be a plant.” ✨

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