Bridging the Gap BETWEEN Classrooms AND Research Laboratories

One teacher's RET experience working in a mycology lab

Brian Dempsey, David Hibbett, and Manfred Binder

In the ever-expanding realm of science, educators struggle to share new discoveries and techniques with their students. Keeping abreast of recent advances can be daunting, even for the most motivated teacher. Fortunately, the National Science Foundation's (NSF) Research Experiences for Teachers (RET) program helps educators keep up with the fast-moving research community. The RET program enables K–12 science teachers to perform research projects in NSF-supported laboratories and brings the excitement of cutting-edge science into the classroom. In this article, I describe my RET-supported experiences working in a laboratory that studies the ecology and evolutionary biology of fungi, and I provide advice on how teachers may find RET opportunities in their own communities.

RET awards are made as supplements to ongoing NSF research grants, which are conducted under the direction of Principal Investigators (PI), who are typically faculty members at universities. The PI of an active award may request an RET supplement, which can pay for the K–12 teacher's stipend, research supplies, and other expenses, such as travel to a scientific meeting or costs associated with curriculum development.

My RET project was conducted under the direction of David Hibbett, a faculty member at Clark University in Worcester, Massachusetts, and Manfred Binder, a research fellow in David's laboratory. David and Manfred are Co-PIs on a current NSF grant to study the evolutionary relationships of a group of mushrooms called the *Boletales*. With support from an RET supplement to David and Manfred's NSF grant, I performed research at Clark University for eight weeks during the summer of 2006 and attended the annual meeting of the Mycological Society of America in Québec City, Canada.

What led to my RET project

The path to my 2006 RET project actually began back in 2003, when I participated in a mycology workshop led by David at Clark University. The workshop was funded as part of another NSF grant, Assembling the Fungal Tree of Life, on which David was the PI. Thinking I would learn about traditional *taxonomy*—the branch of science that seeks to sort species into ordered groups based on morphology and anatomy—I was surprised to find that David's lab resembled a high-tech biotechnology facility. Researchers in his lab are using the latest tools in molecular biology and bioinformatics to probe the cryptic world of fungal classification and evolution. Ecology, molecular biology, and evolution—topics that can seem unconnected in the minds of most high school students—are being interwoven into a dynamic new science.

After taking the mycology workshop, I became active in the Massachusetts Association of Biology Teachers (MABT), a regional branch of the National Association of Biology Teachers (NABT). Through this network I reestablished contact with David and learned that he and Manfred were looking for a teacher to collaborate with, through their Boletales grant. The collaboration they proposed involved development of a learning module for high school students in fungal molecular ecology

(in which DNA sequences are used to identify fungi in the environment). I eagerly agreed, hoping I could learn more about mycology and ways to involve my students in a research-based curriculum. David came to my school in the fall of 2005 and together we led classes in basic fungal biology, molecular ecology, and evolution.

The experience was positive. Students learned about an ecologically important group of organisms and the connections between molecular biology and organismal biology, but I was not completely satisfied. One problem was that students did not actually generate the DNA sequences that they analyzed. Instead, these were provided by David's laboratory as text files. In addition, I was uncomfortable with my understanding of the details of the research. The solution, I realized, was for me to gain first-hand research experience, which would allow me to design more engaging classes for my high school students. I would also be empowered to pursue other grant support to develop a molecular biology laboratory at my school, with the ultimate aim of creating and leading a hands-on course (possibly a summer course) that would cover all aspects of a molecular ecology project, from data collection to analysis and interpretation. In February 2006, David and Manfred requested the RET supplement, which brought me back to Clark University in June 2006. Figure 1 provides a timetable summarizing my experiences and Figure 2 summarizes the basic steps I followed to isolate and analyze the genetic material of mushrooms.

The research experience

The Boletales (informally called boletes) include many symbiotic species that form underground partnerships with forest trees, such as oaks and pines. Boletes are important to the health of forest ecosystems and they include one highly prized edible species—*Boletus edulis* or the porcini. Boletes are highly variable in form (Figure 3, p. 36) and exhibit few distinctive anatomical characters that can be used to discriminate species. Roughly 1,000 described species of boletes exist, but the actual number of species is probably much higher. Some mycologists have estimated that only about 5% of living species of fungi have been named. All of this makes fungi very challenging to study.

In his research, Manfred addresses a classic and difficult question in taxonomy: How can we distinguish between variations within a species versus differences between species? The general problem can be illustrated with a familiar group of vegetables—broccoli, brussels sprouts, and cauliflower. Although we might think of these distinct "kinds" of plants as different species, they are in fact varieties that have been bred from a single species—*Brassica oleracea* or wild cabbage. Scientists sometimes make this same classification mistake and designate mushrooms as different species when in fact they are closely related "races" or varieties. Of course the opposite could also be true as one species could be a collection of similarly looking species. This classification confusion is notoriously problematic with organisms that lack much anatomical form or morphology, such as fungi.

Manfred and David use DNA sequences—which contain a vast number of independent characters—to identify species and estimate relationships. In my project last summer, I analyzed genes that code for ribosomes, the tiny construction sites for building proteins that are present in the cells of all living organisms. The organisms I studied include members of several easily confused species of boletes (Figure 3). The goal of my work was to reassess species boundaries and evolutionary relationships based on molecular characters. The research combined fieldwork, laboratory procedures in molecular biology, and data analysis. The evolutionary tree diagram in Figure 3 shows our results. The tree is drawn with the "root," or common ancestor, on the left, and the "tips" of the tree, which are ribosomal gene sequences that represent individual mushrooms, on the right. Sequences in red type include eight

FIGURE 1

Chronology.

A chronology of my experience working in a molecular evolution lab at Clark University in Worcester, Massachusetts, during the summer of 2006.

Late-June/July	1) Field trips (over several days) to collect and identify mushrooms.
	2) Molecular biology procedures to extract and purify DNA, and amplify and sequence ribosomal RNA genes from mushrooms.
	3) Collaboration with other teachers during a two-day workshop to study fungi taxonomy and evolution (hosted by the Hibbett lab).
	4) Mycological Society of America meeting in Québec City, Canada.
August	5) Additional bench work in molecular biology.
	6) Analysis of DNA sequence data to estimate evolutionary relationships.
	7) Development of a draft field and lab-based curriculum for high school students, centered on the ecology, molecular biology, and evolution of fungi.
	8) Preparation of manuscripts to be submitted to a professional journal.

FIGURE 2

Procedures.

The following outlines the procedures in my molecular evolutionary studies of mushrooms; this set of methods that could be used with other organisms as well.

Method and Purpose	Brief Protocol	Required Time
Collection: Assemble a variety of organisms obtained in the field and prepare permanent research collections.	1) Locate and obtain field specimens noting the ecological context in which they are found. 2) Back in the lab, identify specimens with field guides. 3) Preserve samples by drying.	2 days
DNA Extraction: Isolate and purify the genetic material from an organism.	1) Pulverize pea-sized samples from specimen to break-up cell walls and membranes, thereby exposing its genetic material. 2) Clean DNA to remove cellular debris and pigments. 3) Check yield and purity of DNA using gel electrophoresis.	1 day
PCR Amplification: Generate many copies of a desired sequence of DNA (gene).	1) Amplify desired regions of DNA with the help of short DNA molecules called *primers,* along with enzymes and nucleotides (the building blocks of DNA). The reactions are performed in a machine called a *thermal cycler.* 2) Check amplification using gel electrophoresis.	1 day
DNA Sequencing: Decipher the genetic code of a gene from an organism.	1) Clean PCR products (DNA) of unincorporated nucleotides and other contaminants. 2) Sequence cleaned PCR products using enzymes, one primer, and labeled and unlabeled nucleotides, in a thermal cycler. 3) Clean sequencing reactions and run on an automated DNA sequencer.	2 days
Sequence Analysis: Edit sequences and estimate evolutionary relationships.	1) Edit output from the automated DNA sequencer to construct complete gene sequences. 2) Align gene sequences from different organisms in a data matrix. 3) Analyze the data matrix using computer programs that estimate the most likely pattern of evolutionary relationships based on differences among the DNA sequences.	1–2 days (or longer for large datasets)

new sequences that I generated. The other sequences, in black type, were downloaded from the GenBank DNA sequence database *(www.ncbi.nlm.nih.gov/Genbank/index.html)*. The branching pattern of the tree indicates genealogical relationships, and the (horizontal) lengths of the branches reflect the amount of genetic change that occurred along each branch (i.e., the number of mutations in the DNA sequence of the ribosomal gene).

I was particularly focused on a species called *Boletus longicurvipes*, a common but easily overlooked mushroom found in New England forests. As our phylogenetic tree shows (Figure 3), there is a cluster of eight closely related sequences (in the blue box in Figure 3) that are probably all *B. longicurvipes*. However, some of the sequences in the *B. longicurvipes* cluster are labeled with other names, including *B. rubropunctus* and *B. subglabripes*. These are erroneous names that were assigned to sequences deposited in GenBank, based on identifications made using morphology alone. These results show how difficult it is to identify fungi using morphology; even for professional mycologists (indeed, three of the misidentified sequences were deposited several years ago by Manfred himself, before he started to focus on the *B. longicurvipes* group). Using molecular data, I was able to improve our understanding of the limits of an ecologically important species, *B. longicurvipes*.

Future directions

This project was fascinating, but I can imagine that some of my students might find taxonomic research on fungi to be rather arcane. So, how can I translate my adventure at Clark University into an engaging classroom experience for my students? First, I realize that it is up to me to communicate to my students that cryptic organisms such as fungi and bacteria are important to the functioning of

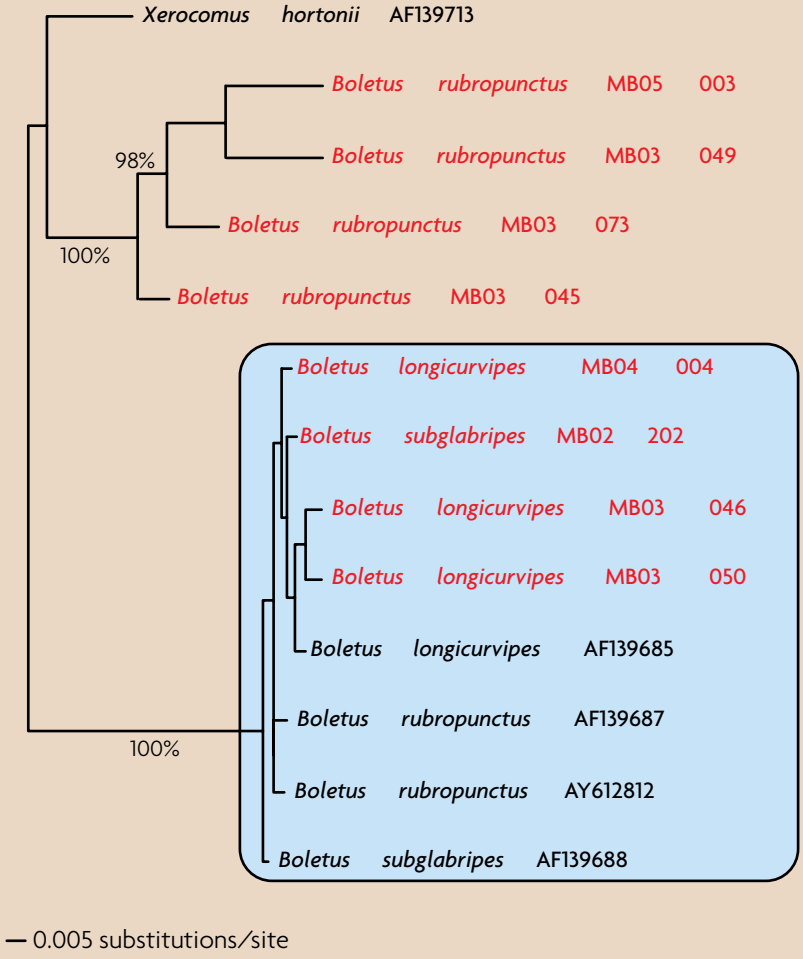
ecosystems. I also need to show students that at the most fundamental levels, the biology of a mushroom is not so different from that of plants or animals. For example, all three have ribosomes that function in the same way during protein synthesis (this is just one reason why yeast, a single-celled fungus, is a useful model system for studying cellular processes in humans). Practically speaking, analyzing DNA from a mushroom is really no different than analyzing the DNA of a human, a bacterium, or an oak tree. With this knowledge I can confidently facilitate experiments so my students can analyze DNA from diverse organisms collected on our school's nature trail. Perhaps most importantly, students should become aware that molecular DNA and RNA evidence is revolutionizing our understanding of taxonomy. My RET experience allowed me to share this exciting new research with my students.

For the next two years, I will continue to collaborate with David and Manfred through my high school biol-

FIGURE 3

Phylogenetic Tree of Boletes.

Left: A phylogenetic tree of boletes, based on ribosomal RNA gene sequences. The sequences in the blue shaded area are all in a single lineages, and are similar in sequence, suggesting that they are all one species, *B. longicurvipes*. The sequence data were analyzed with an algorithm called *Neighbor Joining*. Numbers along the branches are from a bootstrap analysis, which measures the strength of support for the lineages (the maximum possible value is 100%, values below 70% are not shown). Red type = new sequences, with collection numbers. Black type = sequences downloaded from GenBank, with GenBank accession numbers. **Right:** Images of bolete fruiting bodies. [A–C by M. Binder; D by Raymond Boyer, Champignons de Sept-Îles, reproduced with permission.] For further explanation, see the text.



Tips for Finding RET Opportunities.

To find RET opportunities, it is essential to develop a relationship with a scientist in your area who is performing NSF-funded research. The PI, not the teacher, requests the RET supplement (via the sponsored research office at the PI's institution). Organizations such as MABT can help make connections between K–12 teachers and researchers. However, teachers should not hesitate to contact researchers directly to inquire about RET possibilities. While PIs are under no obligation to support RET projects, they are increasingly receptive, in part because one of the two major criteria for evaluating NSF proposals is "Broader Impacts," which can be enhanced by outreach efforts to the K–12 community (the other criterion is "Intellectual Merit" of the proposed research). By participating in an RET project, a teacher can actually improve the PI's chances of obtaining highly competitive NSF funding.

To locate researchers in your area who have active grants, use the NSF awards search page *(www.nsf.gov/awardsearch)*, which allows you to search current awards by state, organization, keywords, and NSF program areas. Search results will include links to an abstract of the research and the name of the PI. Be aware that some of the grants listed may not be eligible for RET supplements, such as grants to support doctoral dissertations, equipment purchases, or scientific meetings. If you find an interesting current research project, contact the PI and ask if he or she would be willing to apply for a RET supplement. Most experienced PIs will already know about the program, but be prepared to explain your personal goals and interests. Supervising a new lab member is labor-intensive, and not every lab will be able to support a RET project. However, others may be excited to share their ongoing research with K–12 educators.

Some universities and other research institutions have established formal RET programs for teachers in a variety of sciences, including astronomy, chemistry, and other fields. Below are a few examples:

- The National Radio Astronomy Observatory: *www.gb.nrao.edu/epo/ret.shtml#Anchor-Wha-52608*
- Materials Research Science & Engineering Centers: *www.mrsec.org/ret*
- Columbia's Summer Research Program for Science Teachers: *www.scienceteacherprogram.org/indexorig.html*

ogy classes at Acton-Boxborough Regional High School (ABRHS). This fall, students will once again use DNA sequence data provided by David's lab. However, I will apply for a grant from the Massachusetts Biotechnology Council (MBC) that would allow me to purchase molecular biology equipment for my school. If the grant is successful, students at ABRHS will be able to perform DNA extraction and PCR, and set up the DNA sequencing reactions themselves. My honors and Advanced Placement Biology students would use this equipment to study a limited set of molecular techniques and concepts. In addition, a more ambitious summer course is being planned for the summer of 2009. The three-week course, Molecular Evolution and Systematics, would involve participants both in the field and lab. Students would analyze family trees they generate from their research and discuss the results in the context of scientific papers they read. High school applicants entering their junior and senior year, who completed a year of both biology and chemistry, would be solicited from the greater Boston/Worcester area. We will still need to collaborate with David and Manfred to run the sequencing reactions, using the automated DNA sequencer (a very costly piece of equipment) at Clark University. The MBC favors proposals in which teachers have a practical plan for using the proposed equipment in their curriculum. I believe that my background gained through the RET program will improve my chances of obtaining an MBC award, and enable me to develop a research-oriented curriculum for science students at ABRHS.

The NSF RET program enabled me to spend a productive summer learning how I can help students understand how ecology, molecular biology, and evolution are often integrated in research science. As Milt Johnson, a physics teacher from South Mountain High School in Arizona and former RET participant emphasizes, "science teachers need to do science as well as teach science." The best way to keep up in science is to just do it! ■

Brian Dempsey (bdempsey@mail.ab.mec.edu) *is a biology teacher at Acton-Boxborough Regional High School in Acton, Massachusetts; David Hibbett* (dhibbett@clarku.edu) *is an Associate Professor and Manfred Binder* (mbinder@clarku@edu) *is a Research Fellow, both in the Department of Biology at Clark University in Worcester, Massachusetts.*

Acknowledgments

The workshop and RET project described in this article were supported by two NSF grants, including the Assembling the Fungal Tree of Life project (award DEB-0228657, PI, David Hibbett) and the Boletales phylogeny project (award DEB-0444531, PI, Manfred Binder). Many thanks to members of the Hibbett lab who assisted with this project, including Jason Slot, Brandon Matheny, Kelly Hallstrom, Andy Wilson, Shlomit Klopman, Chris Ewald, Deborah Robertson, and Ms. Boyer and Raymond Archambault for their permission to use Raymond Boyer's photograph.

Editor's note

For additional information on the RET program, visit *www.nsf.gov/funding/pgm_summ.jsp?pims_id=13439*.