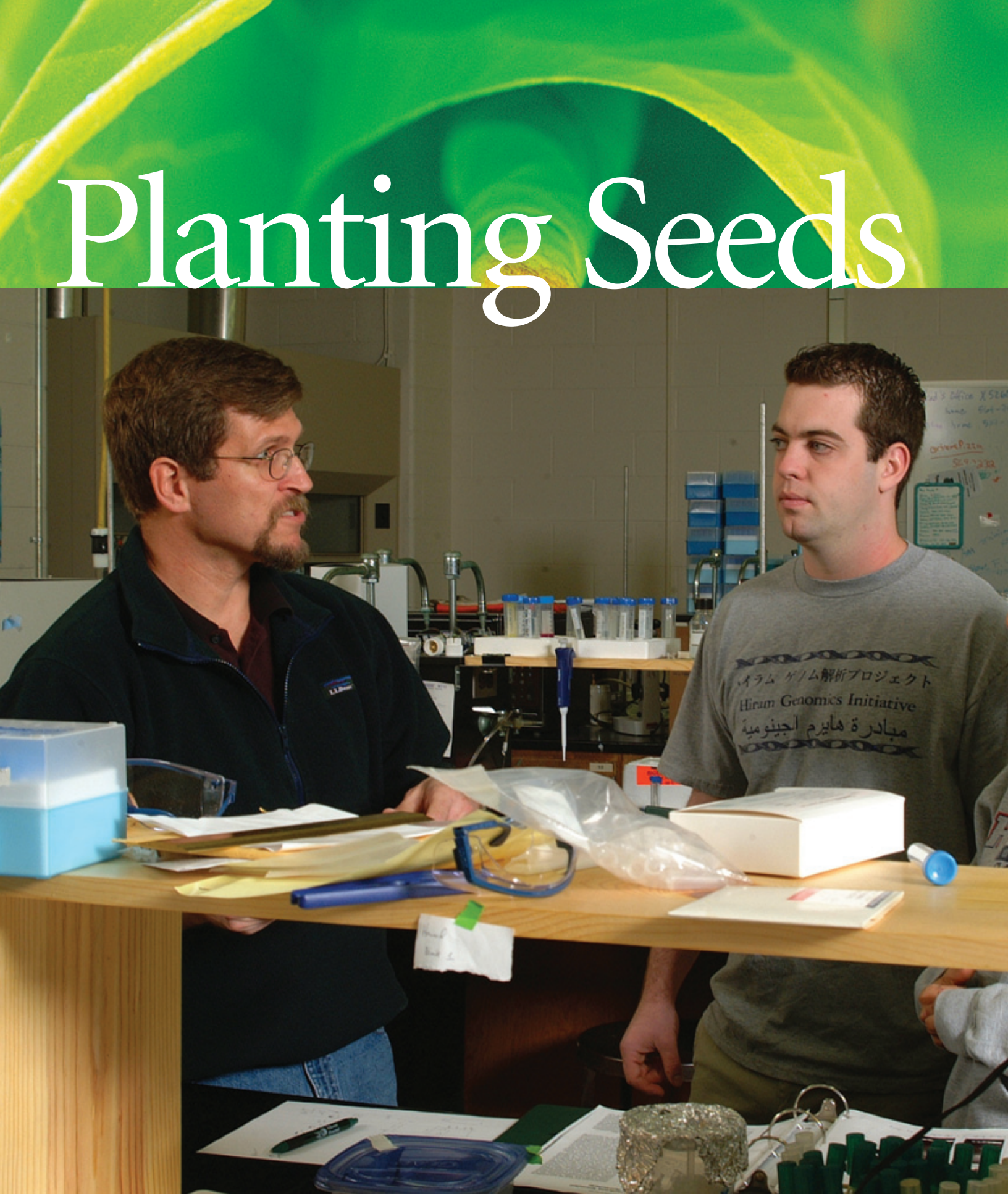
Planting Seeds



David Shoenfelt

"I love seeing the light bulb go on in my students."

Brad Goodner (left) is a biologist at Hiram College in Ohio. Goodner and his students study the genetic secrets of bacteria that can infect both plants and humans.

By Alison Davis

Step inside Brad Goodner's lab at Hiram College and you'll see the usual stuff.

There are computer screens, beakers and test tubes, moldy-looking things growing in culture dishes. And lots of people doing experiments.

The folks growing the slimy stuff in the dishes, working on the computers, and pouring liquids into the test tubes are not graduate students, lab technicians, or even Goodner himself. They are college students—on any given day, biology or chemistry majors, business students, psychology or computer science majors.

Many of them, such as sophomore Adam Ewing, treasure the chance to do research.

"I'm working on a part of something that nobody else has worked on before," says Ewing, a double-major in computer science and biology at this small college near Cleveland, Ohio. He is already planning to move on to graduate school.

Goodner's lab is a hands-on learning wonderland, and that's because the 42-year-old biologist is deeply dedicated to science, particularly to teaching it.

"I love seeing the light bulb go on [in my students]," he says.

But Goodner is also serious about pushing forward his research program, and he believes that college students are an important, even vital, part of that goal. Undergraduates not only help get the experiments done, he says, they also assist in other ways, such as growing the lab's expertise.

Ewing, for example, has acquired valuable knowledge in the area of bioinformatics, a science dedicated to sorting huge amounts of biological data and assigning meaning to it. He is currently applying some of this knowledge to the lab's research goals.

Goodner chooses his projects carefully, to accommodate the schedules and skills of his undergraduates as well as to satisfy his own scientific curiosity.

"I have to be interested in what I am doing, or it isn't worth it," he says.

What kind of science does Goodner find worth studying? The genetic secrets of microbes, particularly so-called plant pathogens. He wants to know how bacteria do their dirty work of causing infections that can lead plants to develop tumors.

Of Plants and People

Do plants really get tumors?

Yes, plants can and do get diseases, some of which cause the growth of external tumors such as those called crown galls on the trunks or branches of trees. One particular bacterium, *Agrobacterium tumefaciens*, injects a piece of its own genetic instructions into a wounded area of a leaf or bark. The inserted material contains a signal that tells the plant's cells to grow rapidly, divide, and form a tumor. Crown gall diseases are responsible for major economic losses in over 600 species of crops.

Brad Goodner (tree), David Shoenfelt (carrots)

Microbes such as *Agrobacterium* can infect a variety of plants, causing tumors.

Goodner is interested in more than the plants or their tumors. He wants to know how some plant pathogens can occasionally infect people. Microorganisms that can pull off this sort of trick are called opportunistic pathogens, and they can be fatal to people with AIDS and other diseases that weaken the immune system.

Over time, evidence has grown to suggest that *Agrobacterium* is among the plant pathogens that can infect animals and humans. Goodner has dug up more than 60 medical reports of opportunistic diseases caused by *Agrobacterium*. His interest lies in understanding the process by which this microbial menace makes its way into people with weakened immune systems.

How could the common soil microbe find access to sick people, you wonder?

"Everyone brings a potted plant to someone in the hospital or tracks in some dirt on their shoes, so *Agrobacterium* is often found on floors and other surfaces," Goodner says.

Most of us do not have to worry about dangers lurking in the soil of our houseplants. Healthy people deal with opportunistic pathogens like *Agrobacterium* with the immune defenses they are born with or by developing immunity during childhood after routine exposure to bacteria. Remember getting a cut while walking barefoot through the grass or consuming a "scrumptious" slice of mud pie?

But to an immune system that isn't working at full steam, otherwise harmless microbes can pose special problems. Goodner wonders if an aging population with more people who have weakened immune systems can bring into the spotlight many pathogens that don't normally cause trouble. Currently, he says, few doctors even know to look for them.

For example, researchers have spotted *Agrobacterium* in blood samples from infected patients only after the usual microbial suspects have been ruled out and a hospital lab technician has gone on to identify a strange microorganism growing in the culture dish, Goodner explains.

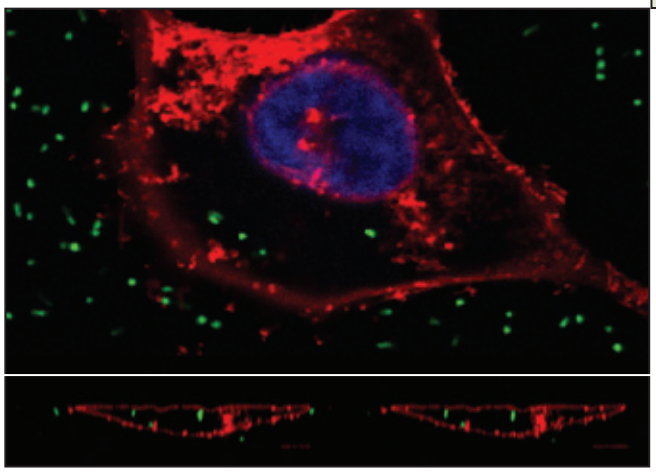
Further complicating the picture is the fact that more than one disease has been associated with an *Agrobacterium* infection. Medical reports have described cases of this bacterium causing an infection of the blood called bacteremia and infections of the muscles, eyes, and abdominal cavity. In each case, the infection site was the place where the bacterium gained access to internal body tissues through a wound, a surgical procedure, or an implanted medical device.

To date, Goodner has tracked down three of the *Agrobacterium* strains isolated from infected patients. He is busy trying to determine how these three versions of the microbe and the plant-infecting *Agrobacterium* strains are alike or different. One thing that stands out immediately is a genetic change: None of the human isolates of *Agrobacterium* contains a tiny circle of DNA called the Tumor-inducing, or Ti, plasmid, which is an essential ingredient of the *Agrobacterium* varieties that cause plant tumors.

And perhaps not surprisingly, none of the human isolates can infect plants.

In careful experiments with cultured animal cells, Goodner and his students have determined that the human-infecting *Agrobacterium* strains may be able to bust their way into cells (see photo, below). Researchers call pathogens that can perform this complicated maneuver invasive.

That's different from the strains of *Agrobacterium* that infect plants. The plant-infecting strains, Goodner explains, are never invasive. Rather, they sit on an open plant wound and "shoot things inside," like a piece of the Ti plasmid DNA that delivers instructions to the plant to make hormones that help the bacteria survive on the plant's exterior.

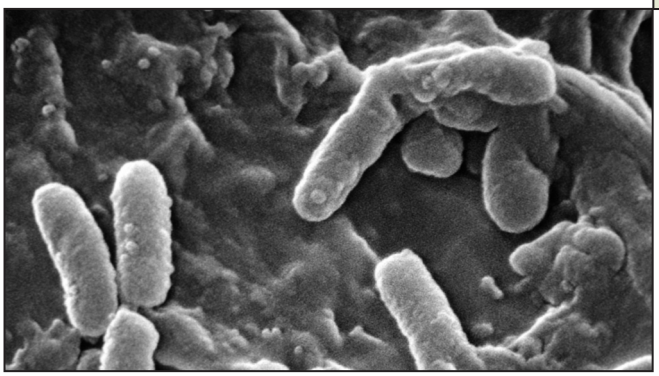


C. Crowe, S. Weeks

Agrobacterium (green dots) isolated from a patient infected with the bacterium can invade mouse cells cultured in the lab. The bottom picture is a microscope's view through the side of the same cell, showing *Agrobacterium* inside.

Jacks of All Trades

Some pathogens—by definition, microorganisms that cause disease—have the uncanny ability to infect a wide range of living things. For example, one notorious opportunistic pathogen, *Pseudomonas aeruginosa*, can infect plants, insects, and humans. In burn patients, *P. aeruginosa* is the most common cause of a life-threatening condition called sepsis, and it is the leading cause of lung infections and death in people with cystic fibrosis.



CDC/Janice Carr

Pseudomonas aeruginosa is a notorious bacterial pathogen that can infect plants, insects, and humans.

Slight changes in the chemical "spellings" of microbial genes can also endow microorganisms with the ability to thrive in different places within the same host. The strains of the *Streptococcus* bacterium that can give us "strep throat," for example, are genetically different from the *Streptococcus* strains that cause scarlet fever or other serious infections like toxic shock syndrome.

Whatever the organism in which a microbe makes its home, a complex relationship exists between the microbe and its host. Some bacteria, like the one that causes ulcers in people, live happily in the intestinal tract for years. *Agrobacterium* can survive in a plant tumor for decades. It's possible, Goodner says, that microorganisms use common tricks to enable them to shift from host to host, and he is hoping his research may unlock some of these secrets. How do strains become different? How do pathogens take advantage of different conditions?

"These are questions we still don't have the answers to," he says.

Goodner thinks that the *Agrobacterium* strains found in sick patients have undergone a number of genetic changes to adapt to a new environment (the human body), since those strains grow better at warmer (body) temperatures, and their DNA is sprinkled with sequences that confer resistance to the weaponry of humans: antibiotic medicines. He is currently pursuing what outcomes those genetic changes may have on the function and behavior of *Agrobacterium*.

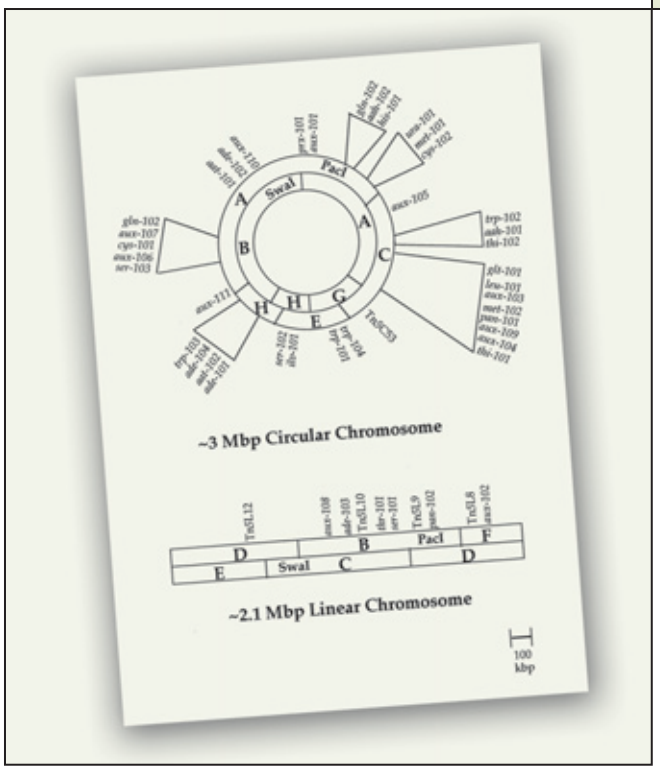
To begin to examine these possibilities, Goodner and his students are combing through the microbe's genetic code, looking for genes that direct the production of factors important for communicating with a host cell, or perhaps for stealing some of the host's nutrients.

So far, Goodner and his students have unearthed one potentially interesting set of genetic instructions within the *Agrobacterium* genome, those that direct the production of powerful chemicals called polyketides and nonribosomal peptides. These bacterially produced poisons are known to serve as signals between interacting species, and they can be lethal to a microbe's enemies:

other bacteria, fungi, or plants. Several medicines, such as the antibiotic erythromycin and the cancer drug doxorubicin, are polyketides. Getting a handle on the roles polyketides might play in making *Agrobacterium* a versatile and effective infectious agent may reveal important details about the pathogen and point to ideas for new therapies.

In the Beginning

Having worked on *Agrobacterium* as a graduate student, Goodner left it behind to concentrate on plant development, first as a postdoctoral researcher and then as a new faculty member at the University of Richmond in Virginia. However, he returned to *Agrobacterium* and has never regretted the decision because it led to a significant research achievement. He and his students led one of two large research teams that published the *Agrobacterium* genetic code in one of the biomedical research world's major journals, *Science*.



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It all started in 1996 when Goodner, while preparing to teach a new course, saw a research article about *Agrobacterium* hinting that the microbe had two chromosomes, not one, as scientists had believed for years. Goodner decided to "do a few experiments" within the course to begin to address this perplexing observation. Those few experiments led him to change his research focus and to a subsequent 2 years of work done by students both within courses and in independent research projects.

The result was a landmark research paper defining clearly that, yes, *Agrobacterium* did have an extra chromosome. The team published a genetic and physical "map" describing the newly discovered genetic characteristics of *Agrobacterium*.

"The second chromosome looked really different, as if it came from somewhere else. We wanted to know more about it, so it became our own little 'mini' genome project," he says of the team effort he embarked upon with his students.

Goodner and his students published a genetic and physical "map" (left) that revealed the existence of *Agrobacterium*'s extra chromosome.

Scientists at Cereon Genomics, a division of Monsanto Company, took notice. At the time, the company had begun an effort to decode the complete genome of *Agrobacterium*. Their interest in the microbe lay in its use as a powerful resource for producing transgenic plants. Some researchers are producing novel medicines, such as edible vaccines, by inserting certain genes into plants with the help of *Agrobacterium*.

"It was clear that Brad's [*Agrobacterium* genetic] map was going to be an extremely useful tool for us," says Steven Slater of Monsanto Protein Technologies. Slater approached Goodner at a scientific conference and asked him if he was interested in pursuing a collaboration that included the students' effort.

According to Slater, Goodner's students were instrumental in carrying out several steps of the project, including creating methods to decode the genome and filling in gaps in the partially completed sequence of genetic code. Slater and his fellow company scientists co-published the *Agrobacterium* genome sequence with Goodner and his student team.

Slater is quick to note that, for him, a major draw of the collaboration was its educational value, so much so that he is doing it again. Slater and Goodner are already working together to decode another microbial genome, *Sphingomonas elodea*, and Slater thinks the setup could be emulated by others.

Another key step in the *Agrobacterium* genome project to which Goodner's students made an important contribution was the process of "annotation," a sort of checks-and-balances process in which gene sequences are verified and matched to potential cellular functions.

These efforts spun off many additional projects that are currently being followed up either by Goodner's students or by Slater's team.

One such effort was launched by student Adam Ewing. He single-handedly created a "proteomics" database that he and Goodner hope will serve as a clearinghouse for the entire *Agrobacterium* research community. Proteomics is the large-scale analysis of all of the proteins encoded by an organism's genome. Ewing's database is a novel tool that will enable researchers to search for proteins encoded by *Agrobacterium* genes based on the proteins' predicted chemical properties, not just on their gene sequences.

Can-Do Attitude

Goodner strongly opposes the notion that "undergrads can't do research." In his lab, they do all the work, with guidance and support from him. Students co-author scientific publications along with Goodner, and they present their projects at national scientific conferences, where they are sometimes the only undergraduate attendees.

Above all, Goodner says, "They learn how to solve problems."

"You don't want to leave college saying, I wonder if…" Goodner says of the advice he gives to students considering doing research projects. "If it's interesting, do it now, while you're an undergraduate."

In the 9 years Goodner has had his own lab, he has supervised about 50 students. They have gone on to obtain graduate degrees; attend medical, veterinary, or law school; or pursue many other careers, such as journalism or business. By and large, his students have been successful in getting interviews and jobs.

"Everybody wants to hire a problem solver," he says. ■

Junior Explorers

Brad Goodner's experience in getting undergraduates into the research act set the stage for extending teaching beyond his own lab and classroom. Last year, he and a few biology department colleagues launched the Hiram Genomics Initiative (HGI), an outreach program involving Cleveland-area high school students. The goal of the effort, supported by seed money from Hiram College, is to invite high schoolers to design and conduct experiments in collaboration with the college students at Hiram. Linking pieces of the HGI project together is an internal journal, *Genome Explorer*, in which students communicate their research results and hone their critical thinking and writing skills. Working together, the students learn how to generate a genetic and physical map of a genome or how to interpret a genomic sequence, which Goodner describes as looking like "someone aimlessly banged away at a typewriter with only four keys (A, C, G, and T)."

"It was nothing like any other method of study I had experienced in high school," says college freshman Tim Lukianowicz, who participated during his senior year at Benedictine High School in Cleveland. "In no other class was I able to integrate what I learned with real-life studies." —*A.D.*