GOING THE DISTANCE

HOW FAR WILL SCOTT STROBEL TAKE HIS STUDENTS TO FIRE THEIR INTEREST IN SCIENCE? TO THE AMAZON AND BACK.

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"Ain't no mountain high enough, ain't no valley low enough" could be Scott A. Strobel's theme when it comes to turning his students into scientists. There is almost no limit to how far he will go, even accompanying his undergraduates from New England to the heart of a South American jungle. But don't ask him to climb a tree.

A recent journey took Strobel, an HHMI professor at Yale University, and 15 undergraduates from a New York City airport to Lima, Peru, followed by a two-hour flight to Puerto Maldonado, a town in the Upper Amazon rainforest. Then they motored six hours by boat down the Madre de Dios River. A final two-mile slog through knee-deep mud, swarming insects, and pouring rain brought them to the base of an iboga tree (*Tabernanthe iboga*), an immense succulent whose upper branches disappeared in the dense canopy overhead.

Once there, student Daniel Vekhter, a wiry Yale junior from Buffalo, was near his prize. But it was up to him to do the climbing—more than 30 feet overhead and out on a limb stretching from the iboga's thick vine-draped trunk. He was after microorganisms that he planned to spend the coming summer and next school year studying, and that, he hoped, would form the beginnings of his career. With a wary Strobel and his classmates watching, the 20-year-old slung his collection bag over his shoulders, took off his boots and socks, grabbed a dangling vine, and began his ascent.

Vekhter's and his fellow students' Amazon adventure was a bioprospecting trip, the first offering of an experimental discovery-based science education program Strobel created for Yale undergraduates. The concept for the course won Strobel an HHMI professor's award, which provides him and Yale with $1 million in support over four years. Few undergraduates ever get the opportunity to learn by doing like Strobel's students. When they applied for the course, they knew they'd be trekking far from home, but they may not have realized just how far—as scientists—the experience would take them.

FIRING IMAGINATION

According to Strobel, most scientists can trace their professional start to a rewarding college research experience. But too many undergraduates who take on research projects abandon science after getting their diplomas. "Students grow discouraged because they have not experienced enough of science's excitement or opportunities," Strobel says. He asserts that some of the blame rests with the types of projects offered to undergrads. "It's the problem of ownership," he says. "All too often, we give undergraduates too small or too technical a piece of the scientific question to figure out how it fits into the equation." Instead of firing the student's imagination, such experiences often extinguish the desire to pursue science as a career.

Strobel wanted to see what he could do to change that outcome at Yale. "The idea is to give students control of scientific decisions," he says. "That's critical to the success of any research experience, regardless of the student's academic level." He devised an experiment in undergraduate scientific research for which, as he explained in the course description, "There is no lab manual." Students choose plants and microbes that interest them, pluck small samples from an untouched part of nature, and then bring them back to a campus laboratory for analysis using a variety of available techniques—many of which Strobel uses in his own research program, which focuses on basic biological processes including how RNA catalyzes protein synthesis and RNA splicing in the genome.

He drew inspiration from a program launched in 2002 by HHMI professor Graham F. Hatfull at the University of

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Pittsburgh. After collecting local soil samples, Hatfull's undergraduates and high school students isolate, sequence, and annotate mycobacteriophages—viruses that infect bacteria and are used by researchers to learn about the genetics of numerous diseases. "These students' projects give them a sense of intellectual ownership and project control," says Strobel. "It inspires them to see science as something they can do."

COMPLEMENTARY STRENGTHS

Scott Strobel had an inspiring teacher from very early on—his own father, Gary Strobel, a renowned plant pathologist at Montana State University. From age 2, Scott would spend days with his father in the biology laboratory. After majoring in biochemistry at Brigham Young University, Scott earned his doctorate at the California Institute of Technology studying site-specific cleavage of genomic DNA. His current interest in the biochemistry of RNA gelled during postdoctoral work in the University of Colorado at Boulder laboratory of Thomas R. Cech, now president of HHMI.

Though their styles differ, his father continues to inspire. Now 69 years old, Gary Strobel is lean and weathered with close-cropped hair. Unlike Scott, 43, whose experiments never require him to leave the laboratory, Gary's ventures into the biochemistry of natural products regularly take him to distant places. Several times each year, he travels to rainforests and other biologically diverse locations across the planet in search of endophytes—bacteria and fungi that live symbiotically on plants. The fascinating biology of these organisms led Scott to build his Yale course around them, and to persuade his father to lead the Amazon expedition.

Many endophytes produce natural chemicals that inhibit the growth of organisms potentially toxic to the plant host or provide it with some other evolutionary advantage. Endophytes' biological activity has also served human ends, providing a source of chemicals for pest control products, for example, and for many medicines. From cancer therapies to antibiotics, about 40 percent of all prescription drugs derive from natural compounds. Yet, very few endophytes on the Earth's 300,000 plant species have ever been characterized, even though nearly all plants serve as host to one or more bioactive microbes.

The senior Strobel has identified scores of previously unknown endophytes. Several have proven valuable, among them a fungus that produces the anti-cancer drug paclitaxel, more popularly known by its brand name Taxol, and a fungus that generates volatile chemicals that have proven useful for treating human waste.

Scott has accompanied his father on a few rainforest trips over the years, but those were nearly always family vacations, not bioprospecting trips. "That's my dad's type of science," he says. Sitting on a stool in a Yale laboratory sporting overalls and a wide grin, Gary looks like he would happily sleep in a tent in the wild. His son, wearing shirtsleeves, slacks, and a more serious demeanor, acknowledges, "I want to be in a comfortable bed at night." Yet for this HHMI research course, Scott says "trying to combine his brand of science with mine" made sense.

Cong Ma, now a Yale College senior from Williamsport, Pennsylvania, who participated in the HHMI research course, says that the two Strobels' different natures make them complementary, and highly effective, team leaders. "Gary is really encouraging and outgoing. Scott is a little

AND PROJECT CONTROL."

—SCOTT STROBEL

Scott Strobel (right) and his father, Gary Strobel, joined forces to bring their complementary styles and expertise to a discovery-based science course for undergraduates.

Lisa Kereszi

Tiny Plant, Grand Adventure

Kaury Eisenman focused her hunt on carnivorous plants. She hypothesized they would be inhabited by more endophytes than other plants because they eat insects that have traveled from plant to plant. The only carnivorous plant the graduate teaching assistant could find during the Amazon expedition, however, was the tiny *Drosera montana*. Although the mature plant could fit on the face of a quarter, each leaf the size of a pencil tip, Eisenman's discovery is providing five students in the class a veritable fungal treasure chest to explore.

Her hypothesis seems to have been borne out as she isolated three fungi that killed every test organism she exposed to them. She found the colored compounds the fungi produced in culture media "striking and awe-inspiring." Two of the fungi release a dark red substance while a third fungus produces a very bright yellow compound, or mix of compounds.

Undergraduate students have now taken over her potent fungi. They developed two bioassays in which extracts from both red fungi continue to prove highly active. A student noticed that the nearly invisible tips of the *Drosera montana*'s insect-catching appendages are red, possibly due to the presence of the red-producing fungi.

While Eisenman is returning to her graduate studies, she says, "I count myself very fortunate to have been a part of what has turned out to be a grand adventure start-to-finish." *—M.W.*

more reserved. But both are fun-loving and great characters. I wouldn't have picked anyone else to lead us."

All students participating in the program took a special spring-semester course, taught by Scott and visiting experts. It combined microbiology, pharmacology, plant science, ethnobotany (the study of plants in human culture), conservation, and intellectual-property law. After researching the indigenous plants and the culture of the Amazon, each student—and two graduate student teaching assistants—defined a theme that would guide his or her plant collection during the expedition. One student collected plants native peoples use to treat tuberculosis infections; another opted for plants likely to produce antioxidants. One aspiring scientist targeted carnivorous plants, while another sought plants used to treat wounds. Vekhter's iboga-tree extracts appealed to him because of their reputed properties for battling opioid addiction. "From a societal point of view," he says, "it would be great if one of our plants had value. That's something everyone in the class cares about."

A *DIFFERENT PLANET*

While many of their friends headed for spring-break beaches or home, the Yale undergraduates left their world behind. They voyaged to a place in the upper Amazon basin still largely free from the developed world's intervention. "It was one of the most remote places you can get to," says Ma. They hiked into an undisturbed, primeval forest with plenty to keep them on their toes: along with the biting bugs, he recalls walking past a small but potentially deadly pit viper, a venomous snake known as a fer-de-lance. He also witnessed a giant otter snagging and making a meal of a piranha. "It was amazing," he says.

Gary knows many of the world's rainforests intimately, yet even his knowledge counted as cursory within the overwhelming natural diversity and dense growth the group encountered. "It's not like going to a garden and picking flowers," he says. To scout out their plants within the seemingly impenetrable tangle, they relied on a guide, Percy Núñez, an Amazon-basin native and professional field biologist from Peru's National University of San Antonio Abad in Cusco with an encyclopedic knowledge of tropical botany.

Shouts of "I found my plant!" or calls over the walkie-talkie of "We found your plant!" came frequently as the students spied their prey, recalls Strobel. They used machetes and plastic bags to cut and collect small stems and leaves.

Most specimens had to be collected from upper portions of plants, where endophytes are more likely to be found in abundance and less likely to be contaminated by soil microbes. Hence, Vekhter's high climb up the iboga tree—where he did find the endophytes he was after. Other students collected samples from high branches using clippers attached to long poles. "The way Scott is making us get our scientific chops is really unique for undergraduates," Vekhter says of their adventures.

By the end of the two weeks, the students had found more than half the plants on their lists. For Gary Strobel, it was his most fruitful bioprospecting trip ever. The group returned to Yale with

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samples of around 300 plant species, many of which he had never seen before. All became reference species in the Yale University Herbarium, one of the nation's oldest and most extensive plant-specimen collections, and in Núñez's herbarium at the university in Cusco.

GETTING DOWN TO IT

Upon returning to Yale, the young researchers prepared their specimens for bioactivity screening. They dissected them, culturing minute bits in plates containing growth medium as well as test organisms placed a couple of inches away. The students then waited to see what activity might emerge when the endophytes and their bioactive products proliferated and encountered the test organisms, which included a variety of plant pathogens as well as human-infecting bacteria and fungi. Among them were *Candida albicans* (a cause of many fungal infections, some now resistant to treatment), *Escherichia coli* (responsible for many types of infection), and *Bacillus subtilis* (widely used by researchers as a model organism).

When an endophyte or its products showed visible activity, the students put them through a battery of tests to isolate the source of their bioactivity. DNA samples from several microbes were sent to a Yale laboratory for sequencing. In several cases, the resulting data revealed bacteria and fungi whose genomes differ significantly from any known organism in GenBank, the annotated online collection of all publicly available DNA sequences maintained by the National Institutes of Health. Michelle Schorn, a junior from San Diego, is one of the students who found a novel bacterium. As a result, she says she expects, "to write a paper identifying it and what compounds are in it. Having a publication would be really exciting."

The students probed their microbes further. They grew them in liquid culture and extracted metabolites to look for the biologically active components. The crude extracts were also screened using a variety of biochemical assays. Those steps might include separating out individual molecules using chromatography, studying structure through crystallography, and applying other laboratory techniques to look for interesting and potentially beneficial properties.

As the students dug into their projects, Scott challenged them, "Anything your mind can imagine, go for it. Figure out the taxonomy and what is known. Look beyond that and see what's new. Then you have to decide what you're going for."

Kathleen Fenn may have already discovered something truly new—and potentially medically beneficial—in the rainforest. She collected a stem sample from a *Capirona decorticans* tree. She hypothesized that it might prove useful after she learned that Amazonian native tribes apply capirona-based concoctions for a variety of medicinal purposes including treating fungal infections, diabetes, and wounds. Back in New Haven, Fenn isolated a strain of pink-colored bacteria from it, which when cultured formed pink crystals on top of the test colonies. A Yale crystallographer identified those crystals as 2,4-diacetylphloroglucinol (DAPG), a broad-scale antibiotic produced by some strains of *Pseudomonas fluorescens* bacteria. Fenn's preliminary analysis shows that the bacterial strain she isolated produces DAPG on a scale several times greater than any previously reported. She plans to continue her research in hopes of demonstrating that this strain exhibits unprecedented efficiency in antibiotic production—and to learn more about its properties.

Fenn, like several other students in the class, intends to collaborate with various Yale laboratories to see if her molecules have any promising disease-modulating properties. In fact, a Yale School of Medicine scientist has already invited Schorn to screen the extracts she found using his cellular assay for potential Alzheimer's disease treatments. The undergraduates, most of whom expect to continue working on their projects during the current school year, are already thinking like advanced medical investigators, says Strobel.

For him, the results prove something he was unsure of at the start. "We're seeing that undergraduate students can go from a jungle trail to complete chemical characterization of a natural product in less than four months," he says. "That's astounding." But much more important to him is the hope that the course has "inspired the scientific imagination" of his students, giving them a "feeling of empowerment to control the direction of an inquiry."

With the HHMI grant, Strobel will repeat the Yale course three times, taking his classes to new locations in search of endophytes. The final outcome of Strobel's experiment in science education may not be known for years to come. For Vekhter, who plans to study his iboga endophyte extracts through his senior year at Yale and intends to "stay in science," the results are already clear. As an undergraduate, he says, "Usually you get plugged in" on a professor's research project, "but here we get to do our own thing." ■