MICROSCOPIC PUZZLE

MICROBES ARE THE MOST ABUNDANT LIFE FORM ON EARTH, BUT SCIENTISTS DO NOT AGREE ON HOW TO ORGANIZE THEM INTO SPECIES. BIOLOGIST FRED COHAN HAS SOME FRESH INSIGHTS.

BY WILLIAM HOLDER '75

PHOTOGRAPHY BY SUSAN CHASE COHAN

Well before Charles Darwin wrote *The Origin of Species,* the concept of "species" seemed clear enough. Put simply, species don't interbreed. Oak trees beget other oak trees with, at most, minor genetic changes over long periods of time. An acorn does not give rise to a swaying palm tree.

There are problems with this point of view. The red wolf in the southeastern United States, for example, has been considered a separate species, and has been the target of a huge effort to save it from extinction. But scientists in Canada disagree about whether the red wolf is actually separate; they argue it is simply an isolated population of a species that is plentiful in Canada. Whether or not these scientists are right, the controversy illustrates how the seemingly arcane science of defining species can have significant impact on public policy and expenditure of tax dollars.

The problem of defining species in the animal kingdom pales in comparison to the microbial world. Before dismissing microbes as too small to bother with, consider this: microbial life on earth swamps all other forms of life. Scientists don't have even a good approximation of the number of species of these single-cell organisms, present on the planet long before animal life, but estimates range as high as billions of species, orders of magnitude beyond any other life form.

Without microbes, life as we know it wouldn't exist. They are responsible for most photosynthesis on Earth and have a major impact on the global carbon dioxide balance. They fertilize plants, improve soils, and break down toxic waste. They form the base of the food chain, and they help us digest food (although sometimes they make us sick). In our bodies we have about 10 trillion human cells, but we carry 100 trillion microbial cells that live in and on us. The roles they play are largely a mystery.

As recently as 1987, scientists were aware of 13 major divisions within the microbial world (somewhat similar to phyla among animals, such as the chordate phylum, which includes the vertebrates and their closest relatives). More recently, scientists have uncovered upwards of 100 such divisions, known as "deep-rooting branches," many containing millions if not hundreds of millions of species.

Wesleyan biologist Fred Cohan collects sand from dunes in Death Valley. Collection of soil from different habitats is the first step toward characterizing the ecological diversity of the bacteria he is studying, *Bacillus*.

Within a single species, bacteria display genetic diversity unheard of in the animal world. Scientists consider *E. coli* to be a single species—it's the organism that lives in our gut but can wreak havoc if we consume the wrong strain. But some strains of *E. coli* have only 40 percent of their genes in common with others. Genetically, a human being has more in common with a stalk of broccoli than these strains have with each other.

Against the vast complexity of the bacterial world, scientists cannot agree on how to define species. Bacteria were once erroneously classified as fungi. As the ability of scientists to culture and grow bacteria in the lab improved, a system for naming species arose that was based on various cultures maintained in laboratories around the world. More recently, scientists have realized that this view was shockingly narrow; less than one percent of bacterial organisms can be cultured. The definition of species is again (or still) in play, and our understanding of how bacteria have evolved over time rests on our ability to distinguish among species. Professor of Biology Fred Cohan believes he has some fresh insights into this difficult problem.

As a site for research, Death Valley has some drawbacks. When Cohan was there with his then 10-year-old son, Willie, in early June of 2007, they were able to work for only two hours early in the morning before the blistering sun sent temperatures above 110 degrees. Environmental extremes, however, are advantageous to his research because Cohan argues that environment is a critical factor in distinguishing species among bacteria.

This view is fairly new. Cohan and his colleagues published a major paper just this past February in the *Proceedings of the National Academy of Sciences*. His argument is controversial, for while environment is certainly a determinant of which species exists where, that is different from saying that ecology can provide the theoretical underpinnings for understanding how the bacterial world is organized. Cohan argues this is so and is reflected in the DNA of microbes.

He was first led in this direction, not through a moment of inspiration in the lab or the field, but through his teaching.

"In my very first semester at Wesleyan," he says, "I was asked to teach a course in ecology, which I had not taken seriously before I came here. I was more of an old-school population geneticist, where evolution and ecology are totally divorced. In the process of teaching this course, I realized that there was a sensible ecologically based definition of bacteria that never would have occurred to me if I hadn't been teaching. People always talk about how working in the lab gives us insight that we can relay in our courses, but it works the other way, too."

Later, Cohan offered a graduate level course attended by a scientist at Wesleyan on a sabbatical leave, David Ward, professor of Land Resources and Environmental Sciences at Montana State University. "It was one of the most important professional experiences of my entire career," he says. "He organized my thinking about why population biology matters. He's a profound guy."

Ward saw that his own views about ecological ordering of diversity were consistent with Cohan's view that ecology can be an organizing principle for understanding species in the microbial world. It's not the only one—the bacterial world is too large and variable for one model to explain all. But it's a compelling way of looking at the bacterial world "naturally," Ward says. It has spurred strong disagreement within the community of microbiologists. Many have held to a more conventional view of species, while others say that the exchange of genetic material among bacteria is so rampant that the concept of species hardly has any meaning at all. Ward says that Cohan has made deft attempts to defuse the controversy and has broadened the discussion about the meaning of species.

Part of the problem is that, for the most part, it's not possible to look at bacteria and pick out features that distinguish one species from another, even with a powerful microscope. Charles Darwin would have had a hard time with microbes. In a scientific article published in *Current Biology* last year, Cohan and Elizabeth Perry '06 wrote:

"We can imagine how evolutionary biology might have fared if Charles Darwin had arrived on the Galápagos Islands with the handicaps of a bacterial systematist. Would he have noticed 13 distinct finch species, each with a bill morphology adapted for consuming a different set of seeds or insects? Or would these birds simply have appeared as a flock of related organisms—all much of a muchness of finchdom?"

It's as though all of Darwin's finches looked exactly alike, yet unknown factors were separating the finches into different groups that were ecologically distinct and would not interbreed. So the question arises: How do you look for different species when you don't know what trait you are looking for?

The challenge of not being able to see differences among species has been surmounted, they say, but the field "still suffers deeply for not readily sensing the ecological differences among close relatives."

His trip to a canyon in Death Valley, and a subsequent one in May, illustrate his point about ecology. Cohan and his students gathered samples from soil in intimate contact with roots of different plant species, where bacteria live off plant exudate. They found three distinctly different types of *Bacillus subtilis* bacteria. One type lived in free soil, away from roots, another only with roots of juniper trees, and another proved to be a generalist that could live with free soil and with root soil of many different plants. In the nomenclature system he's developed, Cohan gives each *Bacillus subtilis* population a third "ecospecies" designation to indicate that each is a significant biological group.

Science magazine recently took note of his work, saying that some researchers "argue that similarity in lifestyle, not just genes, is the way to classify microbes….Cohan and others would like to do away with the microbial 'species' as the 'fundamental unit' of diversity. Instead, microbes would be divided into 'ecotypes,' based first on genetic relatedness and more finely on shared adaptations to a particular habitat."

The *Science* article described work that Cohan and colleagues have carried out in arid and semi-arid canyons in Israel. One study conducted in northern Israel near Haifa examined the forested north-facing slope of a canyon and the grassy south-facing slope. The researchers found 131 strains of *Bacillus simplex*, which could have been the end of the story without consideration of ecotype variation. But the study revealed that the strains fell into nine different ecotypes—arranged as north-facing and south-facing pairs. Similar work in the Negev desert also revealed ecotype complexity underlying the finding of 87 strains of *Bacillus licheniformis*.

To understand that more subtle variation, Cohan and Professor of Computer Science Daniel Krizanc have devised a computer algorithm called Ecotype Simulation. It looks at DNA sequence data and makes hypotheses about classifying bacteria into different ecotypes. Without knowing anything about a given bacterial group in advance,

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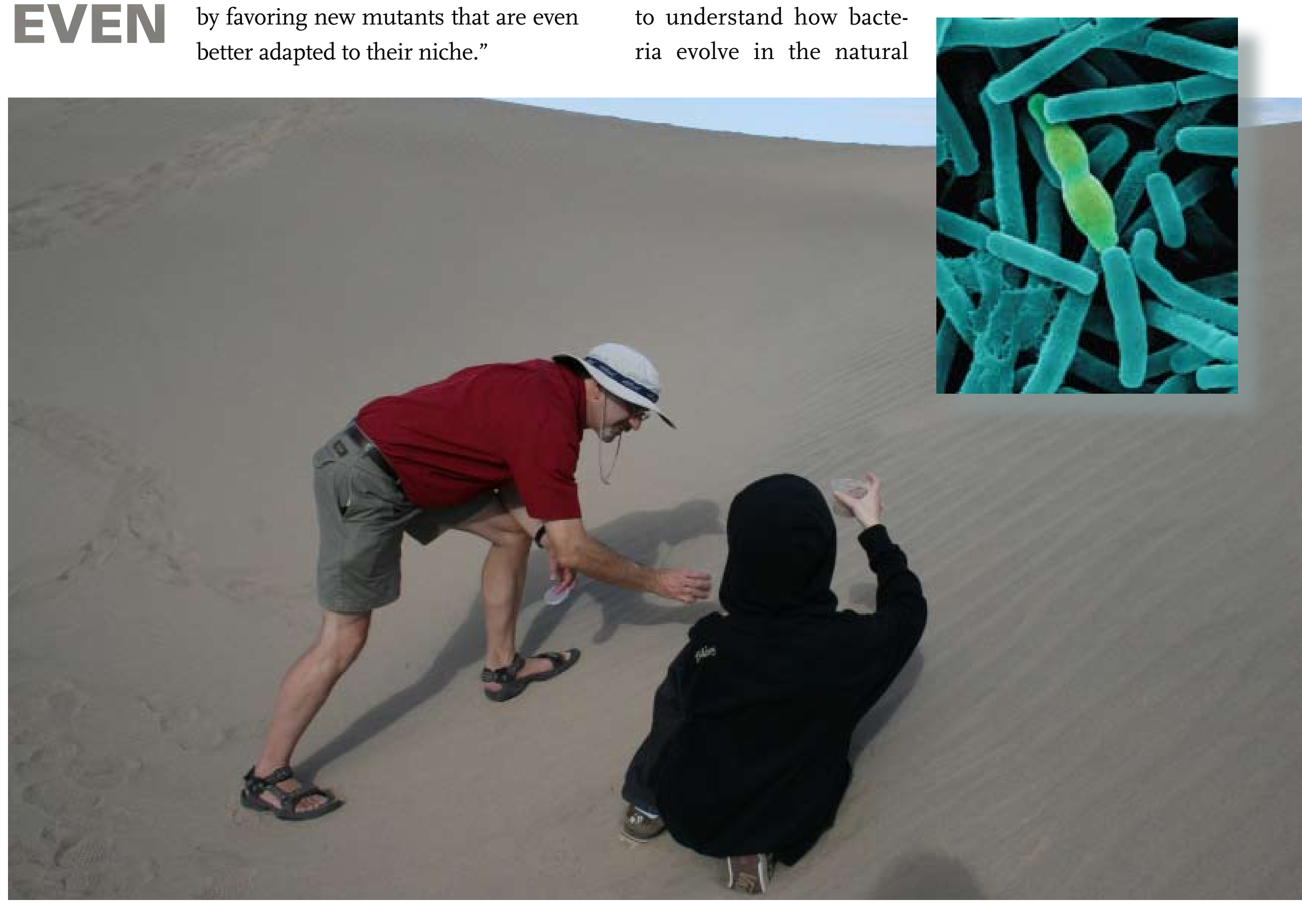
they can make predictions that certain seemingly identical bacterial strains will occupy different ecological niches and exist as distinct species.

Using the computer program, along with information about the actual environment of each microbe, the team determined that the bacteria fell into as many as 30 distinct ecotypes across all the canyons studied. Cohan argues that these ecotypes are the fundamental units of bacterial diversity that reveal the true complexity of bacterial adaptations.

"Fred has done a lot with limited resources," says Jacek Majewski, a former PhD student who is now an assistant professor at McGill University specializing in genomics. "His big contribution was the theoretical concept for how to define a bacterial ecological population. Also, he had the idea that you could use DNA sequencing to distinguish bacteria even when the technology for doing this was primitive compared to today. He was able to make interesting theoretical predictions."

This work may sound abstract. Its importance, however, is suggested by a $5-million NSF grant obtained by Cohan and a group of colleagues at other institutions to apply these methods to identifying bacteria in hot springs at Yellowstone National Park. The work is challenging because most of the bacteria they find cannot be cultured in the lab. Some of the ecotypes are distinguished by living in hot spring water that differs only slightly in temperature: 60 degrees Celsius versus 62 degrees, for example. Others are separated by only millimeters of space, but that's sufficient to create a different environment.

An article in *Scientific American* noted that the Yellowstone work provides a proving ground for Cohan's contention that microbes "are not just a blur of variation but clusters adapted to particular ecological niches. Natural selection keeps their clusters from blurring by favoring new mutants that are even better adapted to their niche."



For the future, Cohan suggests that understanding variations in ecotypes may have some important practical applications. For instance, a strain of bacteria might produce an enzyme with useful medicinal or industrial properties, but scientists may wish to examine naturally occurring variations of the enzyme to see if there are useful differences in its properties. Different ecotypes of the bacteria would be a good place to look for such variations. Or, in the field of public health, ecotypes of known pathogens may have slightly different characteristics that make them more easily transmissible. Studying these could give scientists early warning of possible future epidemics.

Ecotypes might also be useful in the study of global warming. The spread of an ecotype better adapted to warmer temperatures could suggest that an environment is undergoing a climate shift.

Cohan would of course be happy to see the value of his work on ecotypes be validated by novel uses. But for the time being he is content to use ecotypes as a tool to understand how bacteria evolve in the natural world. "I've always been interested in species," he says, "and how they cross a threshold that allows a group to go on a different evolutionary path as a new species."

A family adventure—Cohan's son, Willie, helps him collect bacteria-containing sand from the top three millimeters of the south-facing slope of a dune.

Do you have an opinion about this topic? Please write us at letters@wesleyan.edu.