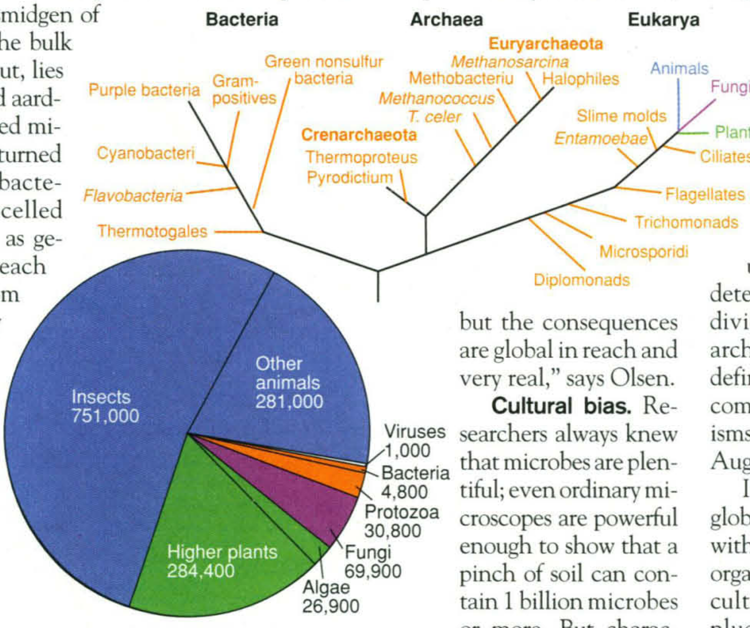
BIODIVERSITY

Microbiologists Explore Life's Rich, Hidden Kingdoms

To most people, biodiversity means plants, animals, or maybe insects. These are the organisms that taxonomists' tallies put at the top of the numbers game, with more than 248,000 described species of plants, 750,000 species of insects, and 280,000 species of other animals. But these counts are less a reflection of the true biological richness of life on Earth than of our ability to count what we can see, such as differences in the shapes of leaves and fins, and the colors of feathers.

Now, a new way of counting species is dethroning plants and animals. Using genetyping techniques that directly sample and compare gene sequences from different organisms, a new breed of biological classifiers is finding that the million-plus known plants and animals represent just a smidgen of life's genetic diversity. The bulk of the diversity, it turns out, lies not with azaleas, ants, and aardvarks but with single-celled microbes. Researchers have turned up dozens of groups of bacteria, archaea, and single-celled eukarya that are at least as genetically distinct from each other as animals are from plants, enough to qualify them as separate kingdoms of life. Such findings suggest that other such kingdoms and untold millions of individual species may still lie hidden in the microbial world. "It's like we're going into the Amazon basin for the first time," says David Stahl, a microbiologist at Northwestern University in Evanston, Illinois.



Dual view. Taxonomists' counts (pie chart) suggest that insects dominate the diversity game, but new analyses reveal that microbes are the real winners.

Although much of the work is still preliminary, already the gene surveys are turning up surprises. Scientists have found, for instance, that some bugs once thought to live only in extreme environments, such as hot springs, in fact, can make a living in decidedly nonextreme waters off southern California. The research also is challenging several long-held assumptions about microbial life, including the notion that many common bugs can be found worldwide.

This effort is bringing together two types of scientists who have rarely before collaborated—molecular biologists who study the inner workings of individual organisms, and microbial ecologists who study how communities of microbes work in their natural settings. Even before this staggering wealth of diversity came to light, researchers recognized that, as Gary Olsen, a microbiologist at the University of Illinois puts it, "microbes are really the movers and shakers in the world," providing nutrients that sustain all other forms of life. But with the new molecular techniques, these scientists finally are getting glimpses of how human impacts, ranging from oil spills to fertilizer runoff, may be perturbing microscopic communities. Such changes will not "necessarily [precipitate] the collapse of the biosphere today or tomorrow, but the consequences are global in reach and very real," says Olsen.

Cultural bias. Researchers always knew that microbes are plentiful; even ordinary microscopes are powerful enough to show that a pinch of soil can contain 1 billion microbes or more. But characterizing the biodiversity of this thimble-sized rain forest, much less describing its ecological structure, was virtually impossible: Unlike macroscopic creatures, which can be categorized by their shapes, colors, and other features, microorganisms defied taxonomic breakdown. "Two microbes could be as different from each other as a grizzly bear from an oak tree, and you'd never know it," says Stahl.

To get some sense of microbial diversity, scientists have traditionally tried to isolate organisms from the field and then grow them up in culture. But only about 1% of bacteria can be grown in culture, says Ken Nealson, an environmental microbiologist at the University of Wisconsin, Milwaukee. This approach has given researchers a "horrendously skewed picture of the diversity of bacteria" and other microorganisms because it essentially ignores organisms that can't be cultured.

Scientists now are bypassing the petri dish and directly characterizing microbial diversity with sophisticated analyses of DNA borrowed from biology's molecular revolution. While these techniques have their origins several years back, "it's only now that this effort is beginning to enter [a period of] exponential growth," with many groups becoming involved, says Stahl.

The most widely used technique for gauging diversity relies on the sequence of about 1500 base pairs in a gene that is common to all cells. The gene codes for the so-called 16S subunit of the ribosome, a chunk of cellular machinery that assembles proteins. The 16S sequences of two related organisms—say, humans and chimpanzees—typically are quite similar, while those from two organisms that have been evolving separately for a long period—say, fungi and cyanobacteria—are more distinct. By comparing the 16S sequence of an unknown microbe to those of its unicellular brethren, researchers can gauge how closely it is related.

Because the 16S sequence evolves very slowly, the technique is good for making broad distinctions. For instance, University of Illinois evolutionist Carl Woese and his colleagues used this technique in the late 1970s to determine that the evolutionary tree of life is divided into three main trunks—bacteria, archaea, and eukaryota—a picture that gained definitive support last year when researchers compared the full genome sequences of organisms in each of the three domains (*Science*, 23 August 1996, p. 1043).

In recent years, researchers around the globe have been filling a common database with 16S sequences from thousands of other organisms, including microbes that have been cultured previously, as well as organisms plucked from backyards and arctic lakes, among other settings. While the main goal of this database, maintained by researchers at the University of Illinois, Urbana-Champaign, is to gauge the evolutionary relationships between organisms, it is also proving to be a handy catalog of microbial diversity. The database shows "it's much more diverse out there than we ever suspected," says University of Maryland microbiologist Rita Colwell.

Indeed, on land, virtually no two samples turn up with 16S fragment matches. For example, Erko Stackebrandt and his colleagues at the German Collection of Microorganisms and Cell Cultures in Braunschweig recently sequenced 16S fragments from microbes taken from an Australian forest soil and a German peat bog. Their analysis showed that neither of the two sets of more than 150 sequences included any previously recorded sequences, says Stackebrandt. In a related study, Michigan State University microbiologist James Tiege and his colleagues sampled similar soils in California, South Africa, Chile, and Australia for bacteria that metabolize a chlorine-containing compound called 3-chlorobenzoate and found that the organisms on each continent had different genetic makeups.

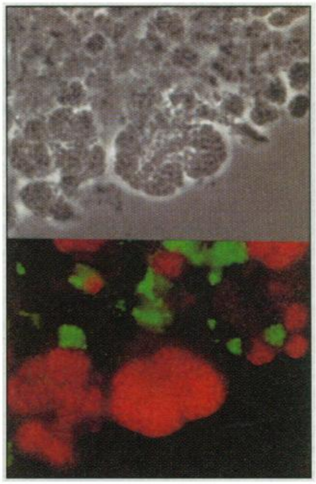
What is the evolutionary reason for all this diversity? It may simply be a function of the plethora of microhabitats in soil, says Edward DeLong, a microbiologist at the University of California, Santa Barbara (UCSB). For instance, while one side of a pebble may be exposed to oxygen, the other may not, thereby fostering the growth of aerobes on one side and anaerobes on the other.

In any case, this rich diversity evident in land-based studies casts doubt on a basic tenet of microbial ecology—that common microbes travel widely, whether as spores or in dormant states via wind or water. If, as the studies suggest, bugs are endemic to specific regions, the total number of microbial species could be staggering, says Jim Staley, a microbiologist at the University of Washington, Seattle. Still, microbes may prove to be more cosmopolitan than these preliminary surveys suggest, says Stackebrandt. Even though he and his colleagues didn't find exact matches in their samples from Australia and Germany, they did find many that were related, he says. He adds that his team may be encountering a sampling problem. Perhaps, the number of species in each environment is so great that the chance of spotting overlap between two samples is slight.

Microbes in the ocean, by contrast, may be more cosmopolitan—perhaps because seawater mixes relatively freely. Stephen Giovannoni, a marine microbiologist at Oregon State University in Corvallis, and colleagues recently sampled Atlantic and Pacific waters, and found that of 440 different bacterial genes sequenced, 86% come from just eight phylogenetic groups. But other studies point to a different conclusion. Staley and his University of Washington colleagues sampled waters in ocean waters of the Arctic and Antarctic, and although they found some similar organisms, "we couldn't find any that were the same species," says Staley.

A few surprising faces are showing up in these diverse crowds of microbes. One remarkable discovery is that a new class of organisms, known as crenarchaeota, exist in Pacific and Atlantic ocean waters. Crenarchaeota were long thought to live exclusively in extreme environments, such as hot springs where most are anaerobes and depend on sulfur for energy production. In 1992, however, two research groups—Jed Fuhrman and his colleagues at the University of Southern California in Los Angeles, and DeLong, then at the Woods Hole Oceanographic Institution in Massachusetts—isolated a 16S ribosomal sequence from crenarchaeota from several sites off southern California, Oregon, and Massachusetts. And 2 years later, DeLong led a team along with UCSB microbiologist Barbara Prézelin that found that these organisms account for as much as 30% of microbes in the Antarctic oceans. "That really opened people's eyes that [the crenarchaeota] are not just for hot springs anymore," says Susan Barns, an environmental microbiologist at the Los Alamos National Laboratory in New Mexico.

Indeed, the crenarchaeota have begun turning up everywhere. In a flurry of reports since December, teams have reported finding crenarchaeota in Illinois marsh sediments, Michigan lake sediments, and Wisconsin soil. The abundance of these organisms suggests that they may play a key role in the cycling of nutrients through the biosphere, says DeLong. But because cool-living crenarchaeota have yet to be cultured, researchers are still struggling to figure that out.



Form and function. New probes reveal ammonia-metabolizing (red) and nitrite-metabolizing (green) bacteria. Traditional microscopic view *(top)* of same sample.

MOBARRY *ET AL., APPLIED AND ENVIRONMENTAL MICROBIOLOGY* **62**, 2156 (1996)

It is on precisely this question—the ecological function of microbial movers and shakers—that a 16S sequence analysis can fall short. By itself, sequence data do not tell researchers much about how an organism makes a living in a natural setting. Still, sequence similarities between newly discovered organisms and microbes with well-characterized biochemistries can sometimes hint at the function of newcomers. For instance, in a 1994 paper in *Applied and Environmental Microbiology*, Tiege and his colleagues reported isolating a 16S sequence from an organism found at the bottom of a 25-meter-deep oil-drilling site in Michigan. When the researchers compared the sequence to others in the 16S database, they found that their organism was closely related to a nitrogen-fixing bacteria isolated from plant roots in Pakistan. When the researchers then tested their organism, they found that it too could fix nitrogen.

As critics of this approach point out, not all organisms with genes for some function are necessarily performing it in the real world. Fortunately, 16S analysis is not the only tool in the molecular toolbox. Stahl and others also are working with several other techniques that they hope will give them a window on not just what newly discovered microbes are doing but how they fit into microbial communities. One scheme relies on fluorescent or radioactive probes designed to bind only to the ribosomal DNA of select organisms. By adding these probes to field samples, researchers can track particular microbes so as to learn about their preferred environments and neighbors.

Stahl, Wisconsin's Nealson, and colleagues, for example, are using a variety of such probes to link specific organisms in lake sediments with their function. Researchers have long known that microorganisms tend to form layers in many lake sediments, based on, among other things, the compounds they rely on to produce chemical energy. In Lake Michigan sediments, for instance, microbes that use molecular oxygen for respiration lie in the top centimeter of sediment. Other microbes that use nitrate, manganese, iron, sulfate, and CO_2 can be found in successively deeper layers.

But while this general layering is well described, little is known about which species occupy each layer. Now, however, by adding radioactive and fluorescent probes to samples of lake sediment, the Stahl team is beginning to identify the organisms living in the layers. Although the work is still in its initial stages, it already has turned up one surprise, that a plethora of crenarchaeota live in the top sediment layer, again suggesting that these organisms are aerobes well adapted to nonextreme environments.

In another approach, Gary Sayler and his colleagues at the University of Tennessee, Knoxville, are trying to infer the function of microbes by determining which of their genes have been switched on. To do so, the researchers scour their samples for messenger RNAs (mRNAs), the gene transcripts that tell a cell to construct particular proteins.

The researchers are especially interested in observing how extraordinarily diverse microbial communities change after an environmental assault, such as an oil spill. With their analytic technique, they have been able to track in soil and water samples the activity of organisms that break down oily, hydrocarbon compounds, such as naphthalene. In normal soil, the researchers found that mRNAs for genes for naphthalene degradation are expressed only at low levels. But following an oil spill, the level of these mRNAs rose as much as 10,000-fold, suggesting that oil-chomping organisms were displacing a host of other types of organisms.

The tools also are providing insight into the structure of these mini–rain forests. University of Illinois microbial ecologist Lutgarde Raskin and her colleagues, for example, are using fluorescent probes to study the associations between organisms that break down nutrients in waste-water treatment plants. In such plants, as in many more-natural environments such as soils and sediments, numerous types of microbes work in close association. In waste-water plants, microbes often clump together to form granules about 1 millimeter across, says Raskin, and with their probes, she and her colleagues have shown that these granules have a consistent structure, with organisms that get their food from the nutrient-rich water located on the outside and species that feed on each others' waste products lying in successive rings toward the center.

By all accounts, such studies are causing a fundamental shift in the discipline of microbiology. By giving researchers the means to study specific organisms in the environment, "molecular methods are creating a wonderful bridge" between molecular biologists and environmental scientists, says Sayler. Many researchers believe that this type of cooperation will be critical to deepening researchers' understanding of how microbial species control broad biogeochemical processes, such as the cycling of carbon, nitrogen, and sulfur. "The biosphere is dominated by microbial processes," says Olsen, and global change, ranging from the buildup of greenhouse gases to new land uses, may be altering these processes.

"We don't yet know a lot [about the effect of such changes on microbial processes] ... although the field is progressing rapidly," says Woese. And for many researchers, that progress spells excitement. "No one could have convinced me 10 years ago that [microbiology] would be this exciting today," says Staley. "The next decade is likely to be one of the best yet."

–Robert F. Service

PHYSICS

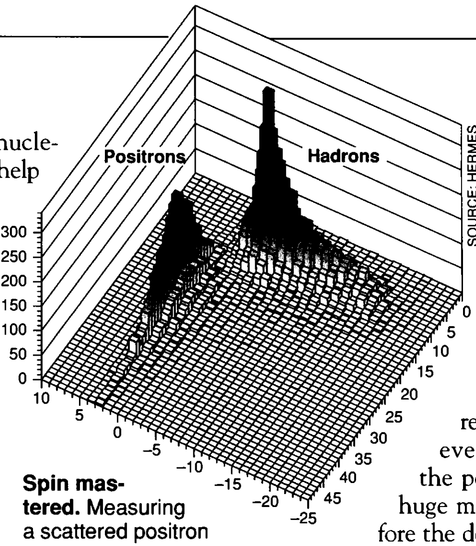
Subatomic Spin Still in Crisis

A new detector on the HERA positron-proton collider in Hamburg, Germany, has confirmed that there really is a crisis in particle physics—and physicists are delighted. The crisis is a decade-old conundrum about the toplike spin of protons and neutrons. These particles are composed of quarks, which themselves spin, but the quarks don't contribute nearly enough spin to explain the total. Results announced last week from the new detector, called HERMES, have confirmed the shortfall—and thus shown that HERMES's new spin-probing technology is working properly. That's welcome news, say physicists, because HERMES, as the most advanced experiment in the field, could eventually track down the missing spin.

"People will mainly be excited by the fact that the experiment is working," says physicist Richard Milner, spokesperson for the HERMES collaboration. HERMES replaces the solid targets of past experiments with a gas of simple atoms, polarized so that their nuclei spin in the same direction. The arrangement allows polarized positrons (antimatter counterparts of electrons) from HERA's circular beam to pass through the target time after time, yielding plenty of clean data on the spinning innards of nucleons—protons and neutrons. "They made a major accomplishment in proving this new technique works," says Yale University physicist Vernon Hughes.

With HERMES, physicists should eventually be able to probe how much spin is carried by elusive components of nucleons such as gluons (force particles exchanged by quarks) and so-called strange quarks, which don't normally inhabit nucleons but can briefly pop into existence and then vanish. A resolution to the spin crisis could also open new insights into the theory that describes how quarks and gluons behave, called quantum chromodynamics (QCD). The surgical precision of QCD's equations is difficult to translate into ironclad predictions about the interiors of nucleons, so "we need all the help we can get from experiments," says Massachusetts Institute of Technology theorist Robert Jaffe.

To tease out the sources of spin, physicists make two measurements, one with the spins of the target nucleons aligned with those of the probing particles, and the other with the target spins reversed. Differences in the numbers of particles scattered hold clues to how spin is distributed in the nucleon. Several major experiments, including the Spin Muon Collaboration at CERN, the European center for particle physics, and projects at the Stanford Linear Accelerator Center (SLAC), have fired polarized probe beams at targets such as solid ammonia or butanol. Solid targets yield more collisions—but also a lot of "noise" from unpolarized nucleons, says HERMES team member Klaus Rith.



Spin mastered. Measuring a scattered positron beam and identifying other debris (hadrons) reveal the neutron's spinning interior.

SOURCE: HERMES

A decade of studies at SLAC and CERN has shown that instead of carrying about 60% of a nucleon's spin, as QCD implies, the quarks account for just half that amount. Now, analyses of the early HERMES data confirm that quarks carry about 25% to 30% of a neutron's spin, estimates Milner—"about a factor of 2 lower than you would expect" from theory.

Physicists now hope that HERMES's innovative technology will allow it to show where the extra spin is coming from. The gas target—a cloud of spin-polarized hydrogen or helium—yields a cleaner signal than the solid targets of past experiments, and a clever polarization scheme exploits the natural alignment of HERMES's high-energy positron beam.

As Milner explains, the spin axes of the positrons end up pointing across their flight path as they are accelerated in HERA's 6.4-kilometer ring. Spin studies need probe particles that spin along their direction of travel, however, so HERMES twists the positrons' spin with a huge magnet placed just before the detector. After the detector, a second magnet restores the positrons to their original orientation for experiments on HERA's two other detectors.

Next week, HERMES physicists will start building a new detector element that will identify particles dislodged from nucleons in the gas by the impinging positron. "The [structure of] those residues ... reflects important information about the spin of the original proton and neutron and the way it was put together," says Jaffe. Particles called kaons, for example, betray the presence of strange quarks inside the nucleon. To identify and analyze these fragments, whose low energy makes them hard to detect, the new instrument will record the Čerenkov light (the optical equivalent of a bow wave) generated when charged particles enter the counter moving faster than the speed of light in the material.

HERMES won't rule the spin field for long. CERN and SLAC both plan new experiments that will also be able to identify emitted particles. But HERMES, true to its name, may bring the first news that could end the spin crisis.

–Andrew Watson

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