

NEWS

Confusing Kinships

Understanding microbial evolution and ecology rests on a solid classification system, but coming up with one is difficult

ALONG THE PARCHED SLOPES OF A CANYON in Israel, in salty pools in Spain and Algeria, and on countless petri plates in laboratories around the world, a scientific debate is playing out: how to classify microbial organisms accurately. Not only are bacteria and their ilk amazingly diverse, but genes cross species lines so frequently that researchers argue whether microbial species exist at all. At stake is much more than the esoteric record-keeping of taxonomists, says R. Thane Papke, a microbiologist at the University of Connecticut, Storrs: “This is about our fundamental understanding of evolution.” Without rigorously categorizing diversity, “we’re really stuck.”

The problem is that prokaryotes, the single-celled organisms without a nucleus, are promiscuous. Instead of one cell splitting into two genetically identical daughter cells, over and over, most take part in a global orgy of gene swapping, passing genes between different taxa. This spells trouble for traditional systematics, built as it is on the assumption that organisms’ genes faithfully reveal their common ancestry. Whereas most genes in a particular microbe do come from its direct ancestor, many may not, making lines of descent difficult, if not impossible, to describe (*Science*, 1 May 1998, p. 672).

This is not the first time that microbial systematics has been disrupted. The tremors began 4 decades ago when DNA sequence became the gold standard for classifying organisms, revolutionizing our understand-

ing of how microbes fit into life’s grand scheme. Now, the availability of whole genomes and DNA sequence from complete microbial communities is shaking up the field anew. “An earthquake is coming for microbial systematics,” says Hans-Peter Klenk, a microbiologist at the German Collection of Microorganisms and Cell Cultures (DSMZ) in Braunschweig. And after the dust settles, what microbial “species” will look like is anyone’s guess.

What’s in a name?

Classifying microbes has never been easy. Well into the 20th century, bacteria were considered members of the fungi, themselves erroneously classified as plants. At the first International Microbiological Congress in Paris in 1930, scientists decided that microbes needed their own scheme. At that time, members of the new Commission on Nomenclature and Taxonomy called microorganisms “in part plants, in part animals, and in part primitive.” They therefore concluded that these single-celled creatures belonged with neither.

From then on, microbiologists assigned names to the organisms they found wriggling and dividing under their microscopes based on the few characteristics that could be reliably observed. Whether microbes irreversibly soak up a dye called crystal violet designated them as Gram-negative—such as our common gut inhabitant, *Bacteroides fragilis*—or Gram-positive, such as

Sex in the salt pond. The search for genetically isolated microbial special in hypersaline pools like this one near San Diego, California, revealed rampant gene swapping among species.

the food-poisoning microbe *Clostridium botulinum*. The *Bacillus* species were distinguished by their need for oxygen and ability to form spores. Others, such as *Streptococcus pneumoniae*, earned their monikers based on the diseases they caused. “But everyone understood that these species definitions were rather arbitrary,” says Klenk, because there was no way to confirm that they reflected genetic relatedness. Nonetheless, “it was a practical system that could help microbiologists know what they were talking about.”

And help it did. Microbiology exploded during the second half of the 20th century, transforming every field it touched, including medicine, agriculture, ecology, and even geology. The enterprise was built on an ever-growing microbial family tree and “type cultures” of each microbial species, representative batches kept in laboratories around the world. Type cultures made it possible for researchers to replicate and build on previous experiments, says Klenk.

By the late 1970s, there were some 40,000 type cultures. “And that’s when we had our first big shock,” says Klenk. Using the newly available tools of molecular biology, scientists compared DNA sequence from different microbial species and found that “we were completely wrong about evolutionary relationships.” The prokaryotes split, some staying in the familiar Bacteria and others shifting into Archaea, single-celled organisms that are superficially similar to

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bacteria but whose genetic architecture is more like our own.

Starting in 1980, the naming of microbial species went in for a complete overhaul by the International Committee on Systematics of Prokaryotes. The standards for type cultures were made far more rigorous, says Klenk, calling for DNA sequence data and more thoroughly documented isolation. The 40,000 type cultures were pared down to about 4000—growing since then to 6800.

But DNA studies have continued to muddy the microbial waters. “In some ways, the more genetic data arrives, the less clear things get,” says Christophe Fraser, an epidemiologist at Imperial College London.

One problem revealed by DNA is the vastness of microbial diversity, says Fred Cohan, a microbiologist at Wesleyan University in Middletown, Connecticut. “All of microbiology was based on what we could culture in the lab,” with heavy emphasis on pathogens. But lab-culturable bugs turn out to be “certainly less than 1%” of living microbes. As technologies improved, ever more DNA sequence has been harvested from environmental samples, representing all the microbial genetic material in a pinch of soil or milliliter of seawater. The realization that “a soil community contains tens of millions of bacteria” is “humbling,” says Cohan.

Sequencing of whole genomes has presented microbiologists with an even more daunting challenge: Microbes have an active “sex” life. Scientists have long known that genes can move between microbes; the spread of antibiotic-resistance genes since the 1950s is a case in point. “But what’s surprising is how frequent and widespread it turns out to be,” says Fraser.

For traditional species to be well-defined, their genes need to flow vertically, from parents to offspring and nowhere else. But among microbes, genes can move along a bewildering variety of routes between genomes: sliding through bridges between cellular membranes, hitchhiking inside viruses, or even getting

sucked up from the environment as naked fragments. That means that any given microbe can have a large number of “parents” from many different species. But “if the genes are moving freely, then how can you nonarbitrarily define the relations between different microbes?” says Papke. “Do you really have species at all?”

The earthquake begins

Yet Fraser and others haven’t given up on classifying microbes. They think there are other ways to define “species.”

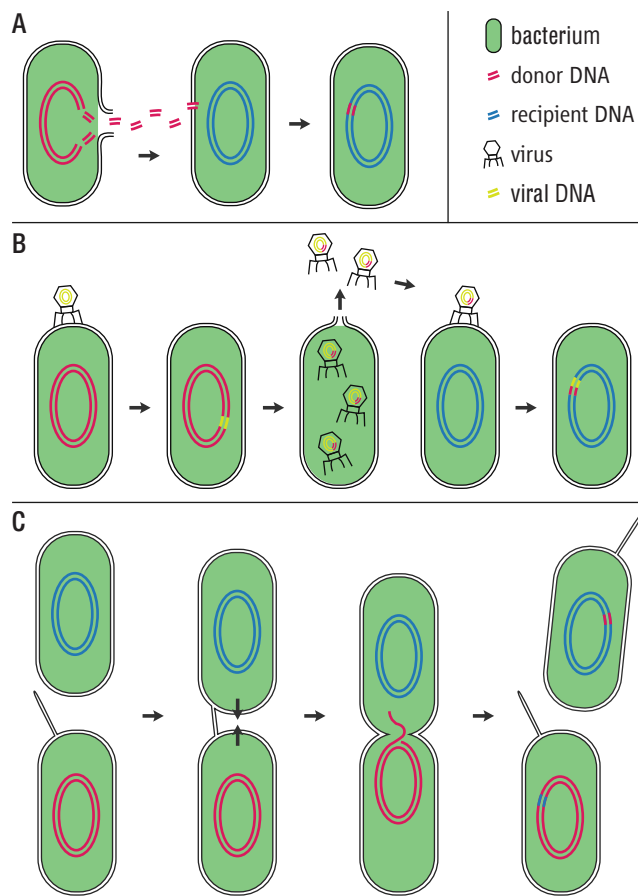
Take recombination, for example. In plants and animals that have no choice but to reproduce sexually, recombination happens every generation: Matching strands of DNA on chromosomes line up and swap segments, producing offspring with a shuffled deck of genes from each parent. This can happen within microbes, too. When foreign DNA finds its way inside a microbe’s cell membrane, it has a chance of lining up with

a similar sequence and swapping segments with the genome. Such recombination events happen infrequently, but when they deliver a more useful version of a gene, the recombined genome can even sweep through a population to become the norm. DNA sequencing of many microbes from the same population has revealed that recombination is much more common than was ever thought.

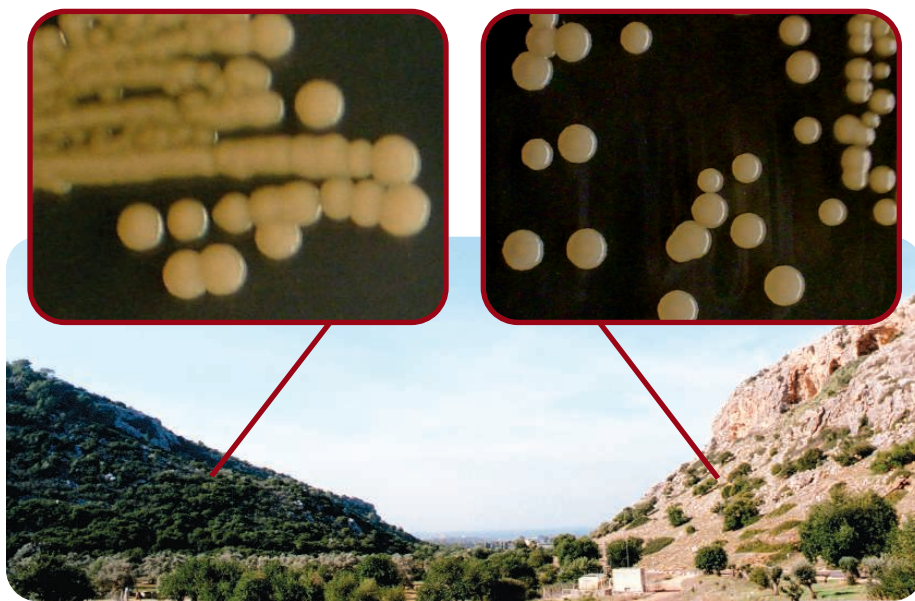
But the “crucial insight” is that the frequency of recombination depends on the kinship between the donor and recipient microbes, says Fraser. The more closely related two microbes are—and hence, the greater the similarity between their genomes—the greater the chance that recombination will happen. When the microbes are too different, “the recombination rate drops off steeply,” he says, effectively blocking gene flow. “If we can define this threshold, then that could be a rigorous way to define microbial species.”

Last year, Fraser and colleagues used computer simulations to study microbial evolution (*Science*, 26 January 2007, p. 476). The results suggested that when the rate of recombination is high enough, genetically isolated groups can emerge that are “analogous” to traditional species, he says.

Recent evidence for Fraser’s view has come from a study of real-world microbial recombination (*Science*, 11 April, p. 237). In this case, a species is being lost. A team led by Martin Maiden, a microbial geneticist at the University of Oxford, U.K., studied two species of *Campylobacter* bacteria. The genomes of *C. coli* and *C. jejuni* share only 86.5% of their most conserved DNA sequence, due to millions of years of adaptation to different wild host animals. But in the 10,000 years since the advent of agriculture, the two species have been living together cheek-by-jowl in farm animals, and there, recombination is on the rise, blurring what in other environments is a clear species line. Because recombination is occurring nearly 20 times faster in the genome of *C. coli*, that genome is becoming ever more



DNA highway. Genes have many options for moving between different microbes, including getting slurped up as fragments from the environment (A), hitchhiking inside retroviruses (B), and getting swapped with similar sequence on a foreign genome after a cellular tryst called conjugation (C).



Defined by lifestyle. In this Israeli canyon, researchers have divided one bacterial “species” into “ecotypes.” *Bacillus simplex* ecospecies Graminifolius (left) prefers the grassy southern slope, whereas *B. simplex* ecospecies Sylvaticus prefers the dry and sunny northern slope.

like *C. jejuni*’s, and Maiden predicts that the two will eventually become indistinguishable.

But other tests of recombination as a gold standard for microbial species have not yielded such clear results. Papke was part of a team that studied microbes in three hypersaline pools, two in Spain and a third 250 kilometers away in Algeria. The salt-loving microbes isolated in each pool, all members of the genus *Halorubrum*, should represent separate species. Yet among the 153 strains tested, there was a bewildering degree of recombination—even between “species” in pools separated by the Mediterranean, Papke and his colleagues reported last August in the *Proceedings of the National Academy of Sciences (PNAS)*. Considering their blush-worthy promiscuity, “we’ll just have to accept that microbes evolve in ways that don’t allow them to be pigeonholed into species,” says Papke.

The eco-challenge

But some researchers argue that similarity in lifestyle, not just genes, is the way to classify microbes. Even with DNA flowing willy-nilly, microbes pigeonhole themselves into coherent groups by adapting to different niches, says Cohan: “The key to understanding microbial diversity is ecology.” Cohan and others would like to do away with 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.

To demonstrate the existence of ecotypes among real-world microbes, Cohan and a team led by David Ward, a microbial ecologist at Montana State University in Bozeman, have studied *Bacillus* bacteria from a group of arid canyons in Israel. The bacteria have adapted to the canyons’ various microenvironments, says Cohan, from the harsh, dry northern slope to the relatively mild, lush southern slope, and a periodically flooded streambed between. The team isolated 218 bacteria from these locations, all members of the genus *Bacillus*, and sequenced the DNA of five genes from each. On the basis of their highly conserved 16S ribosomal RNA gene sequences, bacteria from one canyon divided neatly into two species, *B. simplex* and *B. licheniformis*. But stopping there “would not be informative,” says Cohan, because the sequences of other genes revealed substantial diversity between bacteria with identical 16S sequence. That diversity is driven by adaptation to different microhabitats, says Cohan.

So Ward and Cohan’s team devised a method called “ecotype simulation” to categorize the canyon microbes based on lifestyle. First, they generated a phylogenetic tree based on variation in the four marker genes that were different in the various bacteria. Then, the simulation comes up with putative ecotypes: It divides the bacteria into clusters of genet-

ically similar individuals, all the while checking to make sure that the similarities are due to adaptive changes and not to chance. Finally, the researchers use what is known about the actual environment of each microbe—solar exposure, moisture, soil type, plant resources—to test whether the predicted ecotypes correspond to various microhabitats.

The team identified as many as 30 distinct ecotypes across all canyons studied. Rather than species, these are the “fundamental units” of microbial diversity, the team concluded in a paper published in *PNAS* 19 February. Cohan says they are now “preparing to propose” some of these ecotypes, such as *Bacillus simplex* ecospecies Graminifolius, to the systematics community for formal recognition.

Ward and Cohan aren’t the only researchers using ecology to make sense of microbial diversity. A study of marine microbes led by Martin Polz at the Massachusetts Institute of Technology in Cambridge found that groups of bacteria of the same species occupy different parts of the plankton community, and even during specific seasons (see p. 1081).

But an ecology-based classification faces an uphill battle for acceptance. “I just don’t see how ecotypes can work,” says Papke. He thinks gene swapping is so frequent among microbes that recombination, not ecological adaptation, is the main cause of diversity. Ecotypes “still have a way to go,” agrees Ford Doolittle, a microbiologist at Dalhousie University in Halifax, Canada.

Doolittle is pessimistic that anything better than a “compromise solution” can ever be achieved for microbial systematics. When conditions are just right, microbes “may cluster into what we could all agree to call species,” says Doolittle, on the basis of either “ecotypes” or rates of recombination. “But there is no reason to suppose that conditions will often or even ever be right and thus no reason to suppose that there must be ‘fundamental units’ of bacterial diversity.” Traditional systematics requires such units, he says, “but needing something to be true does not make it so.”

And how will all this affect day-to-day microbiology? “I expect species names and type cultures will continue,” says Klenk. Papke agrees: “We need to be able to have a conversation.” But as for the “fundamental unit” of microbial diversity and what, if anything, is represented by microbial species, “we’ll probably need philosophers to sort that out.”

—JOHN BOHANNON