

Researchers Trade Insights About Gene Swapping

Genes that move between species play by rules that microbial experts are just beginning to discern

When research labs began churning out the genome sequences of a multitude of microbes in the late 1990s, microbiologists got a big surprise: Many organisms seem to be swapping genes with abandon from strain to strain, even across species. Astonishingly, for example, about 25% of the genome of the gut bacterium *Escherichia coli* turns out to have been acquired from other species.

The realization that gene swapping, or horizontal gene transfer as it is called, is a common phenomenon has thrown the field into a tizzy. The implications, says microbiologist Matthew Kane of the National Science Foundation in Arlington, Virginia, "are very, very broad." Borrowed genes can spread antibiotic resistance from one pathogen to another or help an organism survive new or stressful conditions. And it happens often enough to alter the dynamics of microbial communities and even affect the course of evolution. For systematists trying to figure out the relationships between different organisms, however, gene transfer causes a big headache: It blurs the boundaries between species, making it difficult to determine where organisms belong on the family tree.

Last month, about 50 microbiologists, bioinformaticists, microbial ecologists, and evolutionary biologists met to take stock of what they know and need to know about gene transfers.* To date, they've learned quite a lot about where and when microbes take in new genes and demonstrated the phenomenon in laboratory experiments. They are now trying to document it in the field and beginning to discern rules that determine where and when genes move.

Nonetheless, the conclusion from the meeting was a sobering one: "We've been working on this for a decade, but we still have many outstanding questions," says conference co-organizer Barth Smets, an

environmental engineer at the University of Connecticut, Storrs. Researchers are making progress, but they need better techniques for growing microorganisms in the lab and new ways to detect and monitor gene transfer both in the lab and in field studies. Improved computational tools for squeezing more gene-transfer information from newly sequenced genomes will help, points out co-organizer Tamar Barkay of Rutgers University in New Brunswick, New Jersey. But, Kane adds, "when you encounter such a revolutionary new way of looking at life on Earth, understanding the implications will take time."

Genomic revelations

Microbiologists have known for decades that certain pathogens share genes that protect

retrieve and discard it or incorporate segments into their own chromosomes. Conjugation—a cell's version of sex—can also lead to genetic exchange when two bacteria come in contact with each other. Viruses that infect a cell sometimes pick up host DNA as they replicate, carrying it to the next bacterium they infect. Finally, independent pieces of bacterial DNA, called plasmids, can enter foreign cells and—if they survive the cell's defense mechanisms—set up residence separate from the host's genome.

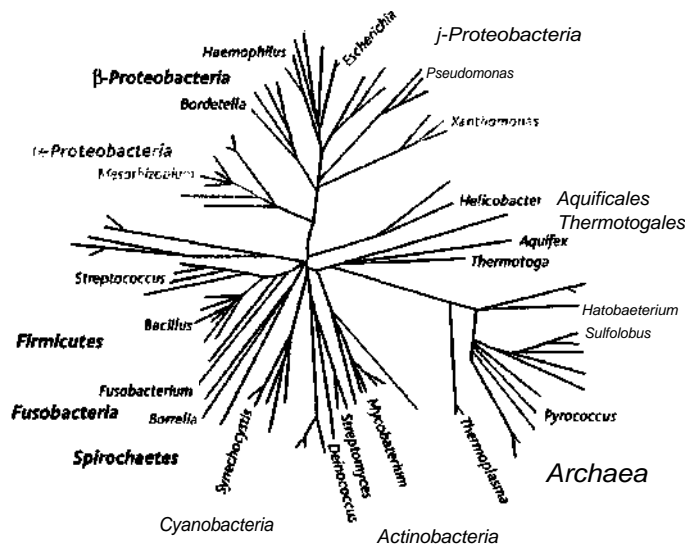
Researchers have discovered several factors that determine whether, and under what conditions, genes are likely to move. Using bioinformatics, James Lake, an evolutionary genomicist at the University of California, Los Angeles, has looked for the frequency of gene exchange in bacteria from different temperature, acidity, pressure, and oxygen regimes. He also tested to see if genome size or base composition affects the likelihood of an exchange. Based on studies of 20,000 genes in eight free-living microbes—including the bacteria *E. coli*, *Bacillus subtilis*, *Aquifex aeolicus*, and the archaean *Methanococcus jannaschii*—he found that microbes from similar environments are more likely to swap DNA. Similarly, "big genomes exchanged genes with other big genomes," he reported at the meeting.

Jeffrey Lawrence of the University of Pittsburgh, Pennsylvania, points to accumulating evidence that organisms do limit gene exchange to microbes on nearby branches of the family tree, probably because their chromosomes share certain characteristics. Genes appear to be exchanged between species with similar chromosomal structures; where replication stops on a particular species' chromosomes, for example, can limit what genes can be incorporated into that genome.

Others are finding that external factors make gene transfer possible. Conjugation, says Soren Sorensen of the University of Copenhagen, Denmark, works

best in dense microbial communities. And plasmids can only get into a target cell that has the proper proteins on its surface.

A gene's function also helps determine its mobility. Three years ago, Lake's computational survey of known genomes showed that so-called informational genes, such as those whose proteins are involved in RNA production and related functions, usually stay put. But genes that code for



Microbial fellows. Distinct branches on the microbial family tree could be the result of patterns of shared gene transfer (as indicated by different colors) as well as common ancestries.

them against antibiotics. But gene swapping was considered rare until about 7 years ago, when researchers began to compare the sequences of microbial genomes. They noticed that sometimes an organism's genome had DNA that didn't seem to belong.

They gradually realized that gene transfer is a widespread phenomenon that occurs in a variety of ways. Sometimes a dying bacterium spits out its DNA, and other bacteria

* "Horizontal Gene Flow in Microbial Communities," 14 to 16 June, Warrenton, Virginia.

proteins involved in, say, building amino acids are more peripatetic.

Often, genetic drifters help defend against a suddenly hostile environment—and that can make them valuable to a variety of species. When conditions deteriorate, "it makes a lot of sense to try to scavenge DNA from your neighbors," says Sørensen. "Horizontal gene transfer facilitates a fast microbial adaptation to stress." In support of this, he and his colleagues have found higher-than-suspected transfer rates among microbes living in nutrient-poor environments, where sharing genes may be key to survival.

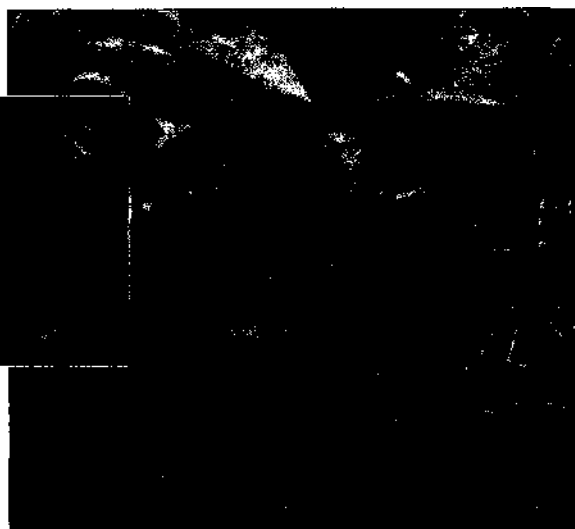
Mobile genes don't just help a community survive. They also provide the grist for evolutionary innovations. According to a calculation that Lake reported at the meeting, gene exchange speeds the spread of new traits by a factor of 10,000. Once a critical gene improves survival, it "can spread like wildfire," quickly becoming part of many microbes' genomes, says Lake. In contrast, bacteria that have to adapt on their own, without the help of mobile genes, would need 10,000 years to come up with the right gene—too slowly to do the stressed organisms any good.

Clarifying gene transfer's chaos

Although mobile genes help microbes survive, they complicate the work of microbial systematists. Species are defined by their genomes: Each has its own unique set of genes, distinguishable from those of other species. But the definition breaks down if gene swaps are common. Systematists then can't easily piece together microbial family trees—a necessary first step to truly understanding the microbial world. "We may have to revolutionize our species notion" and perhaps change the way relationships among microbes are determined, suggests Daniel Drell, an immunologist at the Department of Energy.

Lake has, however, published in the April issue of *Molecular Biology and Evolution* a new computational approach to building microbial trees that may get around some of these classification problems. It rests on a new understanding of which genes are likely to move, focusing on those that form an organism's core genome and tracing those back through time.

Even better, says Lawrence, is to define microbial groups according to who shares



Chasing gene transfers. Gene swaps are much easier to demonstrate in a petri dish (*inset*) than in a natural environment.



Reach put and touch. Bacteria can exchange genes by extending threads to one another that make conjugation possible.

genes with whom. This alternative view sidesteps the need to define species and ancestral lineages and instead tends to put together microbes with similar physiologies. For example, soil organisms might be on one branch, whereas those that process methane are on another. This approach seems to work for the most part, and the resulting branches closely parallel the traditional view of microbial relationships. "Thus they reflect more than ancestry; they reflect the domains of gene exchange," says Lawrence.

Fieldwork

While Lake, molecular evolutionary biologist Johann Peter Gogarten of the University of Connecticut, Storrs, and others rely primarily on bioinformatics to probe horizontal gene transfer, others are taking an experimental approach. Eugene Madsen, a microbial ecologist at Cornell University in Ithaca, New York, demonstrated just how quickly a useful gene can spread in a bacterial culture. The organic com-

pound naphthalene is often toxic, except for organisms that have a gene that is involved in degrading it. In the lab, Madsen mixed *Pseudomonas* and *Burkholderia* bacteria that lack the gene with soil containing bacteria that can degrade naphthalene and put them on media containing naphthalene. It took fewer than 24 hours for the bacteria that lacked the gene to acquire it and thrive in this hostile environment.

As helpful as such lab studies are, they tell only part of the story, because many microbes cannot be grown in the lab. Furthermore, laboratory findings don't always hold up in the natural environment, says Sørensen. When Madsen tried to demonstrate the rapid spread of the naphthalene-degrading gene in the environment, for example, he struck out.

Madsen works in South Glens Falls, New York, at a coal-tar waste site where local bacteria degrade naphthalene. He knows that the bacteria must be acquiring this ability by horizontal gene transfer because many different microbes isolated from the site carry an identical gene for breaking down this organic chemical, and the gene has become quite prevalent in the tar pit even though the pit has been contaminated for only about 50 years, he says. Yet when Madsen added bacteria that couldn't degrade naphthalene to soil full of microbes that could, he was unable to detect the gene in the introduced bacteria even after 9 days.

Part of the problem, says Ian Pepper of the University of Arizona, Tucson, is that "we don't really know how to look at gene transfer [in the wild]." But that could soon change. Sørensen has come up with a way to track gene transfer in the field using fluorescent tags and cell-sorting technology. And other such technologies are in development. Madsen and Sørensen hope these technologies will help them understand how and why genes move around in the environment—information that should provide insight into how genes in genetically modified organisms might escape. Ultimately, they hope to put that information to use in harnessing mercury- or other pollutant-degrading genes for bioremediation.

With ever more microbial genomes being sequenced, "we're starting to learn enough about horizontal gene transfer that maybe we can begin to lay it out," Lake says. "We're really starting to understand it; it's not such a black box."

—ELIZABETH PENNISI