

NEWS

Is It Time to Uproot the Tree of Life?

More genomes have only further blurred the branching pattern of the tree of life. Some blame shanghaied genes; others say the tree is wrong

A year ago, biologists looking over newly sequenced genomes from more than a dozen microorganisms thought these data might support the accepted plot lines of life's early history. But what they saw confounded them. Comparisons of the genomes then available not only didn't clarify the picture of how life's major groupings evolved, they confused it (*Science*, 1 May 1998, p. 672). And now, with an additional eight microbial sequences in hand, the situation has gotten even more confusing—so confusing that some biologists are ready to replace what has become the standard history with something new.

Many evolutionary biologists had thought they could roughly see the beginnings of life's three kingdoms: the ordinary bacteria; the Archaea, which are microbes best known for living in extreme environments; and the eukaryotes, which are all organisms, from yeast to people, whose cells have distinct nuclei. Comparisons of the genes encoding microbial species' ribosomal RNAs—the RNAs that make up the ribosomes, the small cellular structures where proteins are synthesized—suggested that life began with some sort of primitive bacteria. These then diverged into two branches, one leading to the modern bacteria and the other producing the Archaea and later branching again to produce the eukaryotes. When full DNA sequences opened the way to comparing other kinds of genes, researchers expected that they would simply add detail to this tree. But “nothing could be further from the truth,” says Claire Fraser, head of The Institute for Genomic Research (TIGR) in Rockville, Maryland.

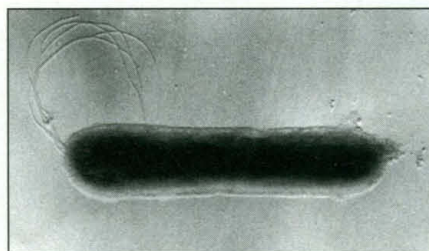
Instead, the comparisons have yielded many versions of the tree of life that differ from the rRNA tree and conflict with each other as well. Some put Archaea and bacteria together; others divide the Archaea into multiple groups. And microbiologists' response to this confusion is equally varied, as this year's Microbial Genomes III meeting, held in early February in Chantilly, Virginia, showed.

Some, pointing to evidence that microbes have swapped genes wantonly over evolutionary history, say that many of these genes are an unreliable guide to evolutionary history and the old RNA-based tree is still basically sound. But others think that it's time to uproot the old tree and are proposing candidates for new trees based

on specific features of the genome and cell structure. And still others worry that gene swapping has turned the tree of life into a tangled briar whose lineages will be next to impossible to discern. “There's so much lateral transfer that even the concept of the tree is debatable,” says André Goffeau, a geneticist at the Université Catholique de Louvain in Louvain la Neuve, Belgium.

A study in confusion

As a case study in how new data are muddying the evolutionary picture, consider *Thermotoga*, a heat-loving microbe discovered 13 years ago on Volcano Island off Italy. The sequence of one of the microbe's rRNAs puts



it on the bacterial branch of the evolutionary tree, just a bit higher than an ancient bacterium called *Aquifex*, sequenced last year by Diversa. But after TIGR scientists completed *Thermotoga*'s genome 9 months ago, TIGR's Karen Nelson and her colleagues compared gene sequences from the two microbes. They found, she says, that “there's no consistent picture [of] where these two organisms fall.”

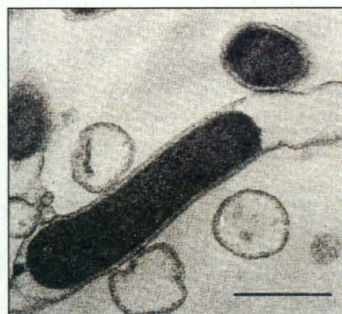
As she described at the meeting, Nelson first identified 33 genes that are found in both *Thermotoga* and *Aquifex*, as well as in an additional 10 bacterial species, four Archaea, and the eukaryote yeast. She then used the base changes in the genes of the various organisms to construct separate trees reflecting the evolutionary history of each gene. “We could find only three situations that supported the branching order [derived] from the ribosomal subunit,” she said. “It was impossible to say whether *Aquifex* or *Thermotoga* was more ancient.”

Some evolutionary specialists suspect

that such confusion is the result of rampant gene swapping. A gene acquired that way by one microbe would then look very similar to its counterpart in the donor organism, indicating their close kinship, while another gene could be very different in the two, possibly because it came from still another species. Thus, the histories inferred from the two genes would be contradictory, making the true history of the microbes difficult to discern.

This phenomenon is becoming more apparent with each new genome. The *Thermotoga* genome, for example, confirmed a suggestion made last year by Frank Robb and Dennis Maeder, microbiologists at the University of Maryland Center of Marine Biotechnology in Baltimore. Based on the partial *Thermotoga* genome, they proposed that the microbe has many genes in common with Archaea, and now that idea has been borne out. Because the two aren't supposed to be closely related, the likely explanation is that one somehow acquired genes from another, possibly because those genes were once part of mobile genetic elements capable of inserting into foreign genomes. Other researchers recently buttressed the case for such microbe-to-microbe transfers by finding what appears to be evidence for a recent transfer—evolutionarily speaking—within the past 100 to 1000 years (see sidebar).

Indeed, microbes can apparently even appropriate genes from “higher” organisms. At the microbial genome meeting, for example,



Who's older? Whether *Aquifex* (top) predates *Thermotoga* (bottom) depends on which of the microbes' genes are compared.

Kira Makarova of the Uniformed Services University of the Health Sciences in Bethesda, Maryland, and her colleagues reported that the genome of the bacterium *Deinococcus radiodurans* contains several genes previously found only in plants. Thus it appears that these genes somehow got transferred to the microbe. And evolutionary biologist Winston Hide of the University of the Western Cape in Bellville, South Africa,

reported that his team found that *Mycobacterium tuberculosis*, which causes tuberculosis, has taken on at least eight human genes. This is apparently beneficial to *M. tuberculosis*, as the genes encode proteins that help break down the hydrogen peroxide that some white blood cells use to kill foreign invaders. They could thus help the bacteria fight off host defenses.

Experts differ, however, on what such gene transfers mean to researchers trying to trace evolutionary relationships. Some, such as Goffeau, wonder whether they make tree-

building difficult, if not impossible. These researchers point out that the transfers may have gone on even more extensively in life's early days, when the molecular machinery for replicating and processing genetic material was still universal and organisms had not yet evolved ways of getting rid of foreign DNA. If so, tree building may be a meaningless exercise. The genomes of modern microbes may be mosaics of genes from different organisms rather than descendants

of any single early form of life, and thus not even the ribosomal genes reflect true historical relationships.

Yet a few evolutionary biologists question whether the gene swapping has been as widespread as some researchers are now suggesting. TIGR's Owen White points out that the conclusion that *Deinococcus* has picked up plant genes is based primarily on researchers' inability to find those genes in any other microbes. But he cautions that the

genome sequences currently available represent "a mere sliver" of all microbial genomes so that the genes may actually exist in a much broader range of organisms than researchers now realize. If so, the "plant" genes in *Deinococcus* might just be relics of genes lost from most other microbes. That might also hold for other apparent transfers.

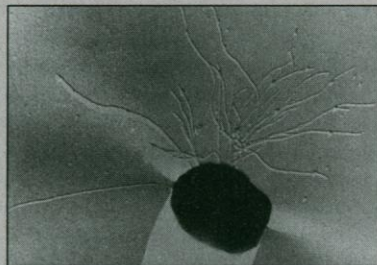
And Peer Bork, a biochemist at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, argues that even if there has been a lot of lateral gene transfer, it might still be possible to detect the outlines of the tree of life. In a study published in the January issue of *Nature Genetics*, Bork and EMBL's Berend Snel and Martijn Huynen looked for the percentage of genes in common among 13 fully sequenced genomes, including those of bacteria, Archaea, and the eukaryote yeast. They found that each microbe shares between 77% and 17% of its genes with another organism. They didn't try to estimate what percentage of those genes had transferred in from elsewhere.

But based on those percentages, the researchers concluded that gene swapping would not displace any organism from its designated place in the existing RNA tree of life, with the kingdoms of the Archaea and eukaryotes branching off from a common ancestor, much as is now envisioned. That's because close relatives on that tree, such as *Escherichia coli* and *Haemophilus influenzae*, have higher percentages of genes in common than do organisms further apart, a finding that supports the microbes' current positions. "Gene transfer happens quite frequently at all levels," says Bork. "But there is still a strong [evolutionary] signal in the gene content."

New trees needed?

Despite supportive data based on the relative gene contents in the genomes, some researchers are not convinced that the tree of life based on ribosomal genes is correct. Other types of gene comparisons and analyses of cell morphology make other evolutionary scenarios seem just as—if not more—likely, they suggest. Radhey Gupta, a molecular biologist at McMaster University in Hamilton, Ontario, for example, focuses on two different microbial features.

One feature involves small insertions or deletions, which he calls "indels," in the coding regions of genes. When an indel exists between two conserved stretches of coding sequence, Gupta says, it almost certainly has been retained through evolutionary time, as it's highly unlikely that two unrelated organisms would have the exact same alteration. Such indels can therefore be used to trace gene lineages. Gupta also takes into account the structure of the cell



DNA swap. Did close quarters enable *Thermococcus* (bottom) and *Pyrococcus* (top) to exchange genes?

Borrowing—Genes—From Microbial Neighbors

Microbiologists have known for some time that certain pathogenic bacteria and some soil microbes swap genes, which shuttle from microbe to microbe as part of mobile bits of DNA called plasmids. Such gene transfers account, for example, for the rapid spread of resistance to antibiotics. But recent work suggests that the transfers may be even more common than thought, particularly in the deep evolutionary past, allowing microbes to exchange a wide variety of different genes—and complicating biologists' efforts to trace microbial evolution (see main text). Indeed, as new work presented at the Microbial Genomes III meeting shows, these widespread gene transfers have not been confined to the evolutionary past but are going on today, even in supposedly ancient organisms, called Archaea, whose genomes have been thought to be long fixed.

The work comes from Frank Robb and Jocelyne DiRuggiero of the University of Maryland Center of Marine Biotechnology in Baltimore and their colleagues. For the past 3 years, they and Robert Weiss's group at the University of Utah, Salt Lake City, have been sequencing the genome of *Pyrococcus furiosus*, a member of the Archaea that thrives in the 100°C water off Italy's Volcano Island. The researchers wanted to see how the genome of this species would stack up against those of two other pyrococci, *P. horikoshii*, isolated from hot springs on the ocean bottom in Japan, and *P. abyssi*, which came from volcanic vents in the waters off Fiji. But along the way, they found an unexpected link to a far more distant relative.

The first hint of the connection came when DiRuggiero and Winfried Boos of the University of Konstanz in Germany found that *P. furiosus* has the same gene for transporting the sugar maltose as does an archaeon called *Thermococcus litoralis*, which happens to live close by in slightly cooler waters, 85°C rather than 100. The Pacific cousins of *P. furiosus* have no such gene, and at first, Robb recalls, Boos and DiRuggiero "thought they had made some terrible mistake," such as contaminating their *P. furiosus* DNA with *T. litoralis* DNA.

Further work confirmed that the link was real—and even stronger than it had seemed at first. The maltose-transporter gene in *P. furiosus* contains a stretch of 16,000 bases that is almost identical to a sequence in the *T. litoralis* gene. The researchers found identical runs of some 6000 bases in some regions of the shared section, and overall there were just 138 base differences between the two species. Because the sequences are so similar, Robb suspects the entire sequence was somehow transferred from one to the other, possibly with the help of transposons—mobile DNA elements—that flank the DNA in *Pyrococcus*, and that the transfer occurred within the past 1000 years, perhaps even within the past 100 years.

Researchers have come to expect aggressive gene acquisition in pathogenic bacteria that are trying to stay virulent. "But Archaea are supposed to be ancient, slowly evolving," Robb points out. The finding that they traffic in genes today "should consolidate the idea that [early lateral transfers] could have actually happened."

—E.P.

CREDITS: (TOP) G. FIALA AND K. STETTER; (BOTTOM) M. KESSEL/NIH AND R. HOLLEY-SHANKS/COHB

membrane. The result is a drastic reorganization of the classic tree in that Gupta finds a fundamental split among the bacteria. In addition, he divides the Archaea between the two bacterial branches instead of placing them in a separate kingdom.

Gupta begins with the supposition that the most primitive organism resembled today's gram-positive bacteria (which are so called because they are dyed by the Gram stain). Based on the indels present or absent in other genes coding for highly conserved proteins, such as those involved in DNA synthesis, Gupta then came up with a scenario for how other organisms arose.

In his view, one group of these gram-positive bacteria eventually gave rise to the salt-tolerant Archaea and to the modern gram-positive bacteria, while some descendants of a second group eventually split into two lines, one of which became the gram-negative bacteria, while the other includes the Archaea that today thrive in methane- or acid-rich environments.

This picture is supported, Gupta says, by the presence in all the gram-negative bacteria

(*Science*, 13 March 1998, p. 1633), Gupta then suggests that eukaryotes arose when an archaeon fused with a gram-negative bacterium. The archaeon became the nucleus, while the gram-negative bacterium contributed most of the rest of the cell's components as indicated by the bacterial nature of the modern eukaryotic cell's membranes.

His theory got a lukewarm reception at the meeting, however. "It's an interesting hypothesis," says TIGR's Fraser. "But I don't think the story is anywhere near that simple." Nor does mathematician Hervé Philippe, who with microbiologist Patrick Forterre of the University of Paris-Sud has tentatively put forth an even more radical scenario. These researchers propose that a eukaryote-like cell, rather than a prokaryote, is the last

RNA world, where RNA, rather than DNA, was used to carry life's instructions. And compared to prokaryotes, eukaryotic cells have many more genes involved in processing the RNAs that may be relics of that RNA world.

In eukaryotes and Archaea, he says, the complex interactions between the proteins encoded by the genes needed to process the genetic information would have limited the ability of the genes to change. But those constraints would be less severe in bacteria, which somehow lost a few of these genes. The bacteria could have then evolved faster—and as a result they end up at the bottom of the tree when they don't deserve to be there.

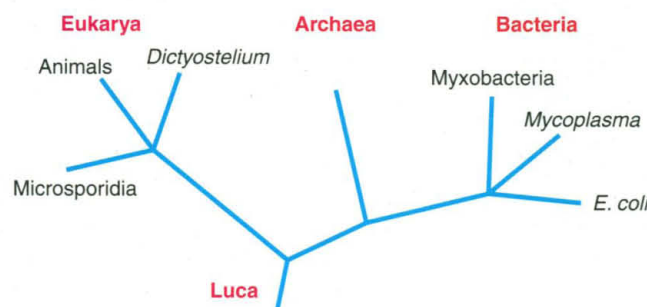
This new tree of life pleases some researchers, particularly those who already have reason to suspect that the current tree might be misleading. For example, Maryland's Robb says he's often suspected that the placement of the hyperthermophiles, microbes that live at extremely high temperatures, toward the bottom of the tree might be an artifact resulting from assumptions about how fast these microbes evolve and on their initial discovery in extreme environments reminiscent of what early life might have experienced.

That placement was based in part on the finding that the DNA in the ribosomal genes of the organisms has lots of guanine and cytosine bases, an indication that their genomes had been around long enough for certain bases to become overrepresented. But it turns out that these bases tend to dominate in the genomes of organisms living in extreme environments, as they help stabilize DNA (*Science*, 8 January, pp. 155, 220). Thus, these DNAs do not necessarily tell the true story of evolutionary relatedness. "A lot of what Philippe is saying makes sense to me," Robb notes.

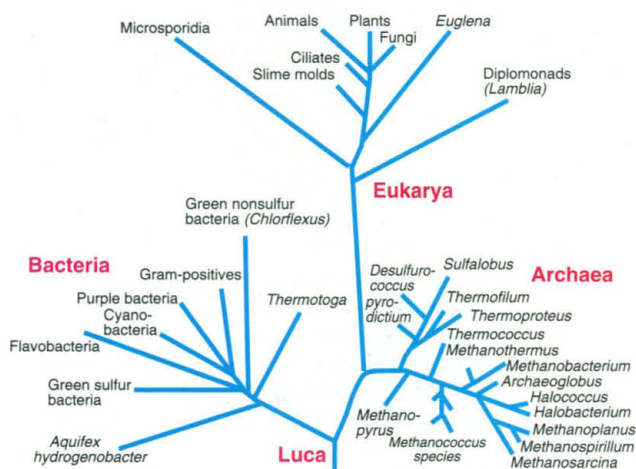
Nonetheless, like Gupta, Philippe has his skeptics, as many researchers think it's too early to reach any firm conclusions about early evolution from the new genomes. "We just don't have that high a resolution for ancient [events]," says EMBL's Bork. To try to resolve the issue, both he and Philippe advocate not only looking at more genomes but also working to understand better how genomes change through time.

Fraser agrees that more needs to be learned about the molecular mechanisms that underlie evolution. At this point, she says, the only thing that can be said with any certainty "is that the story is by far more complicated than is suggested by the ribosomal DNA tree."

—ELIZABETH PENNISI



Tree turmoil. Analysis of recent genome sequences suggests that the tree of life based on ribosomal DNA (left) may need rerooting as shown above.



examined of a particular 26-amino acid insert that is not found in the gram-positive organisms or in the Archaea. The insertion must have occurred, he says, after the gram-negative bacteria diverged from their gram-positive ancestors. Other support comes from the proportions of the bases guanine and cytosine in the genomes of these microbes, he notes. The salt-loving Archaea and many of their close gram-positive cousins belong on one major branch because they have a high proportion of these bases, while the other Archaea and gram-positive bacteria, with a low proportion, belong on the other.

In keeping with other recent evidence

in their rates of evolution. (The results are also in press in *Molecular Biology and Evolution* and in the *Journal of Molecular Evolution*.) As a result, organisms that have a lot of the faster evolving genes tended to appear more ancient than they really are, confusing the trees. And Philippe says bacterial genes are the faster evolving ones, causing bacteria to fall to the tree's base.

But that's actually where eukaryotes belong, he says, basing this conclusion on an examination of the genes involved in the synthesis and use of the genetic information in the various types of organisms. He notes, for example, that many researchers think that the earliest organisms lived in an