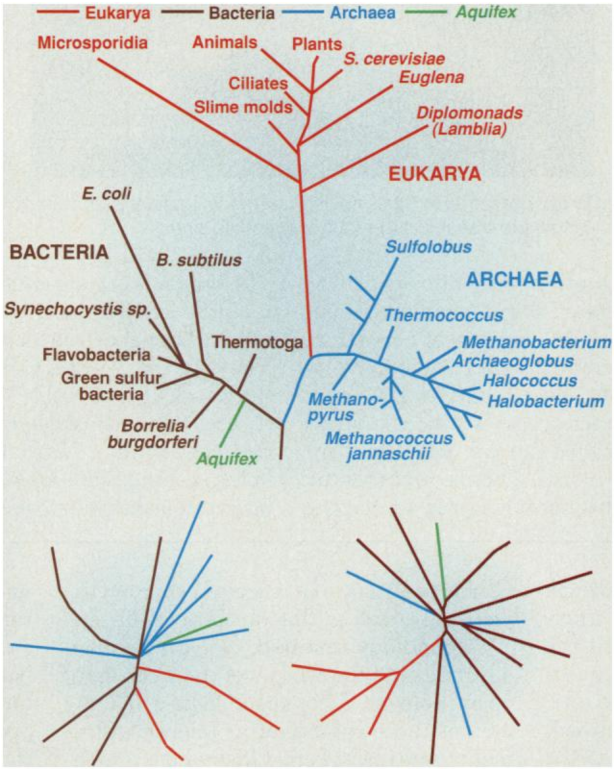
Genome Data Shake Tree of Life

New genome sequences are mystifying evolutionary biologists by revealing unexpected connections between microbes thought to have diverged hundreds of millions of years ago

Over the past 3 years, the deciphering of the complete genetic codes of more than a dozen microbes has opened the way to a whole new understanding of how bacteria live and cause disease. "It's like being in a candy store," says Richard Stevens, at the University of California, Berkeley. But on one front—the study of evolution—the information pouring out in the genome sequences has so far proved more confusing than enlightening. Indeed, it threatens to overturn what researchers thought they already knew about how microbes evolved and gave rise to higher organisms.



Shifting branches. Some gene analyses contradict the rRNA-based tree of life *(top)*. One puts *Aquifex* close to archaea *(left)*; another splits archaea *(right)*.

SOURCE: OTTO KANDLER

SOURCE: FELDMAN *ET AL.*

For more than 2 decades, systematic biologists, led by evolutionist Carl Woese at the University of Illinois, Urbana-Champaign, have been using the sequences of RNA from the ribosomes—the cell's protein-making factories—to classify bacteria. One of the stunning successes of rRNA analysis was Woese's identification of one group of microbes, now called the archaea, as a third kingdom of life, adding to the two kingdoms already known: the true bacteria, which like the archaea don't have cell nuclei, and the eukarya, including higher plants and animals, which do (*Science*, 2 May 1997, p. 699).

Since then, he and others have used rRNA comparisons to construct a "tree of life," showing the evolutionary relationships of a wide variety of organisms, both big and small. According to this rRNA-based tree, billions of years ago a universal common ancestor gave rise to the two microbial branches, the archaea and bacteria (collectively called prokarya). Later, the archaea gave rise to the eukarya. But the newly sequenced microbial genomes and comparisons with eukaryotic genomes such as yeast have been throwing this neat picture into disarray, raising doubts about the classification of all of life.

For one, because genes don't evolve at the same rate or in the same way, the evolutionary history inferred from one gene—say for rRNA—may be different from what another gene appears to show. "Before, people tended to equate rRNA trees with the [life history] tree of the organism," says John Reeve, a microbiologist at Ohio State University in Columbus. "From the whole genomes, you very quickly come across [genes] that don't agree with the rRNA tree."

Even more perplexing, the newly unveiled genomes often contain a mix of DNAs, some seeming to come from the archaea and others from bacteria. "Features of both bacteria and archaea are turning up in eukaryotes, and to a surprising degree," says Russell Doolittle, a molecular evolutionist at the University of California, San Diego.

Many evolutionary biologists are coming to believe that these mosaics arose because genes hopped from branch to branch as early organisms either stole genes from their food or swapped DNA with their neighbors, even distantly related ones. The genetic oddballs may simply mean that the branches of the tree of life intertwine, but that the basic shape is sound. But if the gene swapping was extensive enough, the true branching pattern may be quite difficult to discern. Worse, the tree's "base" may turn out to be indecipherable: a network of branches that merge and split and merge again before sprouting the modern kingdoms. It may be, Woese concedes, that "you can't make sense of these phylogenies because of all the [gene] swapping back and forth."

Confounding genes

The just-completed sequence of the bacterium *Aquifex aeolicus*, which lives at near-boiling temperatures, embodies the problems that molecular evolutionists are now confronting. To assess *Aquifex*'s kinship to its fellow microbes, molecular geneticists Ron Swanson and Robert Feldman of Diversa Corp. in San Diego and their colleagues, who described the sequence in the 26 March issue of *Nature*, compared several of its genes with their counterparts in a range of species from the archaea and eukaryotes as well as other bacteria. The conclusion, Feldman reported in early February at the Conference on Microbial Genomes in Hilton Head, North Carolina, is that "you get different phylogenetic placements based on what genes are used."

The gene for a protein called FtsY, which helps control cell division, placed *Aquifex* close to the common soil microbe *Bacillus subtilus*, even though the two supposedly come from different branches of the bacterial tree. Even worse, a gene encoding an enzyme needed for the synthesis of the amino acid tryptophan linked *Aquifex* with the archaea. That wasn't the only anomaly the Diversa team found regarding the archaea, however.

Their analysis of the gene encoding the enzyme CTP synthetase, which helps make the building blocks of DNA, spread the archaea out among all the other organisms evaluated, suggesting that they may not be as coherent and distinct a group as the rRNA tree implies. "It points to caution in terms of interpreting the 16s [rRNA]," Feldman concludes.

The gene for FtsY and perhaps the gene for CTP synthetase might have been expected to tell a different story from the rRNA genes, because they have probably evolved at different rates. Woese picked the rRNAs to study because they are part of one of the cell's most basic activities—protein synthesis—

Direct Descendants From an RNA World

The newly sequenced microbial genomes are causing biologists to reexamine the "trees" showing the evolutionary relationships among living entities (see main text). Among other things, the new findings are challenging the consensus that eukaryotes, organisms ranging from yeast to human that have nucleated cells, evolved from archaea—one kingdom of nonnucleated prokaryotes—rather than from bacteria, the other prokaryote kingdom. But a team in New Zealand has made an even more radical proposal about that early stage of microbial evolution. In the January *Journal of Molecular Evolution*, microbial evolutionary biologists David Penny, Daniel Jeffares, and Anthony Poole of Massey University in Palmerston North suggest that eukaryote-like cells actually predated the prokaryotes.

The researchers began with the now well-accepted idea that the first life-forms lived in an "RNA world," where RNA not only stored genetic information in primitive cells but also catalyzed the chemical reactions necessary for life, jobs now done primarily by DNA and proteins. Then they reasoned back from what is known about RNA metabolism in current organisms to discover what those first life-forms might have looked like. "The primary evidence for an RNA world comes from the roles of RNA in modern cells; these are considered relics or molecular fossils from an earlier living system," Penny explains.

Their quest led them to picture a mythical microbe they call *Riborgis eigensis* (for ribosomal organism), the last organism before genetically coded protein synthesis evolved. As the New Zealand team describes it, *Riborgis* looked in some ways more like a eukaryote than a prokaryote. *Riborgis* had linear chromosomes, as do eukaryotes, instead of the circular ones seen in most archaea and bacteria. That was necessary, Penny says, because *Riborgis* had an unsophisticated system for replicating its genome, and linear fragments could be handled more easily.

But more important, *Riborgis* would have depended on RNA to carry out many functions, such as copying RNA or adding methyl groups to inactivate it. That reliance could have led to the evolution of RNA-protein particles similar to ribosomes and nuclear particles found in eukaryotes. This might have occurred, for example, if bits of *Riborgis* RNA happened to get translated into small amino acid sequences, and some of these eased the RNA's job by binding to the nucleic acid and stabilizing it. Over time, that advantage could then cause the RNA-based biochemistry to shift to a protein-based one like that found in modern prokaryotes. The protein-based systems are so much more efficient that Penny doesn't think the RNA-protein particles could have arisen later.

The New Zealanders can't explain how the nuclear membrane—a defining feature of the eukarya—could have arisen in their ancestral organism. But to Carl Woese, an evolutionist at the University of Illinois, Urbana-Champaign, who agrees with many of the ideas put forth by Penny and other proponents of the RNA world, that may be the wrong question anyway. Instead, he suggests, we should ask "how did the cytoplasm arise?" As Woese points out, for all anyone knows, the nucleus may be the true descendent of the primitive organism that gave rise to eukaryotes, with the cytoplasm forming as this organism evolved a way to enclose and control its local environment.

"The truth is we are all still arguing from ignorance and incomplete data sets," agrees Russell Doolittle, a molecular evolutionist at the University of California, San Diego, who calls Penny's ideas "food for thought." And although some argue that these events from 3 billion years ago will always be a mystery, Woese is optimistic. "Someday," he predicts, "the facts will come along" to tell us what happened.

–E.P.

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and thus are unlikely to change radically. He hoped they would serve as a slow, steady clock. But genes not involved in such core activities, including those for FtsY and CTP synthetase, may evolve fast or slowly, depending on the different conditions microbes live in. "Each gene has its own history," says Feldman.

If differences in the way genes evolve account for some of the disparities between the patterns Feldman and his colleagues traced, the real tree of life might be worked out by overlaying different gene trees to come up with a consensus. "The sum of all these trees makes up the organism," suggests biochemist Dieter Söll of Yale University in New Haven, Connecticut.

But the tree Feldman derived from the gene for the tryptophan synthesis enzyme implies a more insidious problem: the possibility of widespread gene swapping among organisms, which could make arriving at a consensus tree quite difficult. Few researchers think *Aquifex* is kissing cousin to archaea, in spite of the similarity of their genes for this enzyme. The enzyme might be a relic from the ancestor common to both kingdoms, which has evolved unexpectedly slowly since then. But more likely, at some point, *Aquifex* took on the archaeal gene, substituting it for its own version, a process called lateral transfer.

Gene swapping

Not too long ago, attributing an unusual result to lateral transfer would have raised quite a few eyebrows. For years molecular evolutionists tended to use this idea to excuse irregularities in their attempts to construct phylogenetic trees, when actually their methods were at fault. But the microbial genomes have made the idea respectable.

> **"I think it's open whether the three domains [of life] will hold up."**
>
> **—Robert Feldman**

In one case last year, for instance, W. Ford Doolittle, an evolutionary molecular biologist with the Canadian Institute for Advanced Research in Halifax, Nova Scotia, scanned the genome of an archaeon called *Archaeoglobus fulgidus*, newly sequenced by The Institute for Genomic Research (TIGR) in Rockville, Maryland, with a computer program that searches new genomes for resemblances to genes in existing databases. The scan turned up an enzyme called a reductase that was much more like the reductases seen in bacteria than like comparable enzymes in other archaea and eukaryotes—supposedly the closer relatives of *A. fulgidus*.

Similarly, an unusual gene that Söll, Yale biochemist Michael Ibba, and their colleagues first spotted in the genome of the archaeon *Methanococcus jannaschii* has since turned up in the Lyme disease pathogen, a spirochete called *Borrelia burgdorferi*. Because spirochetes are thought to be descendants of bacteria that had a different version of the gene, "we believe [it] arose from a lateral transfer," Söll says, in which the spirochete took up an archaeal gene and lost its original one.

Conversely, other researchers are finding archaealike genes in microbes classified as bacteria. Take *Treponema palladium*, the spirochete that causes syphilis. After Steven Norris of the University of Texas School of Medicine in Houston, working in collaboration with TIGR scientists, completed the *Treponema* genome last year, they noticed that its DNA contains genes for two particular ATPases—enzymes that break down adenosine triphosphate, often to release energy—known before to exist only in the archaea. And *Treponema* also has other genes that look suspiciously archaeal in origin, he reported at the microbial genomes meeting.

Revising history

In an upcoming issue of *Trends in Genetics*, Ford Doolittle proposes a new mechanism for this kind of gene swapping. He suggests that early eukaryotes may have gotten a significant part of their genomes from genes picked up by their predecessors from their food. As he puts it, "You are what you eat."

Assuming that the current tree of life is correct, he asks, how else can one explain Russell Doolittle's conclusions that 17 of 34 families of eukaryotic proteins that date back to early cell evolution look as if they come from bacteria, while only eight show a greater similarity to archaea, the supposed ancestor of eukarya. Terry Gasterland's team at Argonne National Laboratory, outside Chicago, Illinois, has made a similar finding: Twice as many yeast nuclear genes match up with bacterial genes as with archaeal genes.

Although some modern bacteria are quite adept at taking up new genes—many pathogens develop antibiotic resistance this way—the successful incorporation of genes from food bacteria into eukaryotic genomes would be accidental and infrequent. But "we've got hundreds of millions of years for it to happen," Ford Doolittle points out. Also, these genetic morsels are consumed with each meal, so that an incoming gene can have many opportunities to get into the genome and replace its native counterpart. In contrast, once a native gene happens to get removed from the host's genome, "it's lost forever," he adds. Over evolutionary time, these processes would favor the loss of native genes and their replacement with borrowed ones.

Woese thinks gene swapping was rampant even among life's earliest organisms. In his view, the organisms that lived before archaea, eukarya, and bacteria went their separate ways lived communally. "It was more like a consortium," Woese says of this very early world. The ability to make use of a neighbor's genes would have proved an important advantage, he asserts.

Members of this consortium may even have had different genetic codes. But the organisms that outlasted the rest would have been those that could make use of their neighbor's genes to adapt to changing conditions, says Woese. Over time, this advantage "ensured that the [DNA] code was universal," he says, because those not able to read DNA-based genes could not survive as well as those organisms using DNA.

This prehistoric commune might have worked well for early life, but it adds to the challenge for biologists trying to make sense of it all. With each descendent from this community "having taken up different things from the ancestor, you won't be able to draw clear trees," Woese points out. He still has faith, however, that organisms roughly followed the patterns of evolution seen in changes in rRNA and that the three kingdoms will remain intact.

However, the existence of so many genes that seem out of place has led some researchers to question whether eukarya descended from archaea. These researchers are also wondering whether archaea really are distinct from true bacteria, noting that although archaea were once considered limited to extreme environments, they are also turning up in the milder surroundings favored by true bacteria (*Science*, 24 April, p. 542). "I think it's open whether the three domains will hold up," says Feldman.

Despite the current ferment, however, Woese and others are confident that eventually a consistent picture of microbial evolution will emerge—even if what it might look like is uncertain, and even if its base is the mix of communal organisms Woese envisions. Within a year, some two dozen more genomes will be complete. At the same time, new software programs are refining researchers' ability to trace the heritages of different genes and discover more links between the three kingdoms. All this, says Texas's Norris, "will lead to a much better understanding of evolution as a whole."

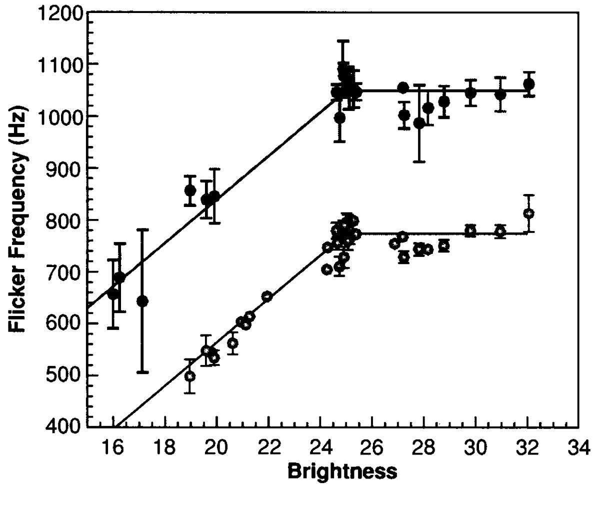
–Elizabeth Pennisi

ASTROPHYSICS

X-ray Flickers Reveal a Space Warp

If Earth had no atmosphere and no mountains, a satellite could orbit the planet at treetop level without falling. But Einstein's theory of gravity predicts that a very dense body, such as a black hole or a neutron star, bends space so steeply that objects orbiting closer than a certain point would slide catastrophically inward. Now x-ray signals from a distant neutron star have offered the first strong evidence for this smallest stable orbit. The findings, presented at a meeting of the American Physical Society in Columbus, Ohio, last week, offer a rare test of Einstein's theory in a strong gravitational field. They also offer new clues to the workings of these x-ray beacons.

Neutron stars are only about 20 kilometers across, but they contain more mass than our sun. (Someone standing on the surface would weigh over a trillion kilograms.) In 1996, NASA's Rossi X-ray Timing Explorer satellite picked up rapid-fire x-ray flickers coming from some of these distant heavyweights. Most astronomers believe that the fastest flickers come from a long stream of material that spirals down to the star's surface from the innermost edge of a spinning "accretion disk," producing a splash of x-rays. The x-rays emerge from the point where the material spills into the star. That point moves around with the accretion disk, so the x-rays rotate past Earth like a beam from a lighthouse.



Topping out. Two sets of x-ray pulses track the speed of material orbiting a neutron star. The pulses brighten as the material creeps inward, but a frequency cutoff reveals a closest possible orbit.

SOURCE: W. ZHANG ET AL., ASTROPHYSICAL JOURNAL LETTERS

The flicker frequency rises whenever the inner edge of the disk creeps closer to the star and whips around it even faster. This may happen when a big chunk of material happens to fall in, throwing up a flare of x-rays but blocking some of the neutron star's radiation, which keeps the disk at bay.

Hoping to spot the innermost orbit, astronomers spent a year watching a neutron star called 4U 1820-30 with the Rossi satellite. The group, from the NASA Goddard Space Flight Center in Greenbelt, Maryland, clocked the frequency of the beacon and watched its brightness, which told them how much material was falling in. As expected, when the