

## Evolutionary tree of major biological kingdoms constructed by team of UCSD researchers

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Like a genealogist seeking to build a family tree, a team of researchers from the University of California, San Diego has constructed an evolutionary tree that traces the lineage and origins of the major biological groups that have inhabited Earth in its 3.5 billion history.

What emerges is a time line showing when the planet's major lifeforms--including bacteria, protozoa, fungi, plants and animals--diverged from each other in history.

The results, published in the January 26 issue of the journal *Science*, shed light on one of the most fundamental questions in biology: when did the different forms of life appear on Earth?

"Common ancestry is what biology is all about," said Russell Doolittle, a professor of chemistry with UCSD's Center for Molecular Genetics and principal investigator of the study. Other members of the research team are Da-Fei Feng, Simon Tsang, Glen Cho and Elizabeth Little, all with UCSD's Center for Molecular Genetics.

Until recently, estimates of when two creatures last shared a common ancestor have relied predominantly on findings from the fossil record. Most large or macrofossils are restricted to the last 600 million years, however, while findings from smaller and more ancient fossils from single cell creatures--microfossils--generally are more difficult to interpret.

As a result, estimations of the divergence times of the major biological groups have remained elusive and often far apart.

For example, paleontologists and others generally believe that bacteria, or prokaryotes as they used to be called, appeared on Earth 3.5 billion years ago. Prokaryotes are characterized by the lack of a membrane-bound nucleus.

The appearance of eukaryotes, cells with nuclei and other complex architectural features, has been more difficult to pin down. Originally, paleontologists thought they evolved about 1.4 billion years ago. More recently, however, some scientists have postulated that prokaryotes and eukaryotes split very early after life itself began, perhaps more than 3.5 billion years ago.

The UCSD analysis now reports that the divergence between prokaryotes and eukaryotes was about 2 billion years ago.

Doolittle acknowledges that the team's results might generate some debate over which are the correct estimates. Nevertheless, he feels strongly about his results.

"The data and the data analysis for this research are very robust," he said.

The UCSD analysis relies on a the concept of a "molecular clock," which suggests that for any protein, rates of change are roughly the same over time in all lineages of living creatures. This implies that the differences among proteins or their amino acid sequences can be used to date the separation of all the major biological lifeforms.

In their study, Doolittle's team worked with 531 sequences from 57 different proteins, encompassing 15 principal groups of organisms. This includes nine animal subgroups, fungi, plants, slime mold, protists (eukaryotes that are not animal, plant or fungi), eubacteria (contemporary bacteria) and archeobacteria (a separate kingdom of prokaryotes distinct from eubacteria).

In the first part of their study, the researchers compared amino acid sequences among the different groups to find out how closely these sequences matched from organism to organism. For example, the UCSD team determined that plant sequences were more like animal sequences than fungal sequences. On the average, the sequences in plants and animals were 57 percent identical. By comparison, about 55 percent of animal and fungal sequences were about the same; also, the percentage of identical sequences in plants and fungi was about the same as those in animals and fungi.

However, further analysis of the data revealed that fungi sequences have been changing faster than sequences in plants and animals. As a result, the UCSD researchers concluded that fungi are more closely related to animals than animals are to plants.

To pinpoint divergence times, the researchers used the known fossil record to establish a baseline rate with sequences from vertebrate animals. They subsequently used that rate to extrapolate other divergence points.

"Scientists generally do not like to extrapolate," said Doolittle. "But in this case, we had no alternative."

Once having established that plants and animals diverged about a billion years ago, a time estimate generally agreed upon by others, the team was able to relate the divergence times of all the other major biological separations through a process known as phylogenetic analysis.

The results offered the following timetable for some of the major biological groups: plants, animals and fungi shared a common ancestor about 1 billion years ago; fungi and animals split from each other 965 years ago; protist lineages shared a common ancestor with eukaryotes about 1.23 billion years ago; prokaryotes and eukaryotes parted company about 2 billion years ago.

Doolittle said his analysis relies on the assumption that the rate of evolutionary change among protein sequences has been approximately constant among all organisms.

"If you look at the protein clock too closely over too short a distance, it tends to be erratic," he noted. "The trick is, you have to look over long periods with lots and lots of data that have been averaged."

"That's our contribution. We took vast numbers, by previous standards, and put them in a different way. We averaged them as opposed to looking at them individually, and this is what has led to these results. We took great pains to challenge the data ourselves."

Aside from when eukaryotes and prokaryotes diverged from a common ancestor, another problem suggested by the study revolves around the nature of the most primitive bacteria. Micropaleontologists believe their 3.5 billion-year-old fossils belong to a family of bacteria called cyanobacteria. But the new sequence data from UCSD

shows that the cyanobacteria have shared a common ancestor with all other existing bacteria a mere 1.5 billion years ago. This leaves unanswered what kinds of bacteria might have existed before that time.

While acknowledging there are some puzzling disagreements with current thought, the UCSD team says they are confident that their analysis has been conducted with great care. As in all scientific disputes, they say, more experiments and more thought likely will resolve the contradictory issues.

NOTE: ILLUSTRATIONS ARE AVAILABLE THAT HELP DEPICT THE STUDY'S RESULTS

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