

Ticks in the Tocks of Molecular Clocks

Comparing the DNA, RNA and proteins of different species may reveal the entire tree of life, but obstacles are emerging

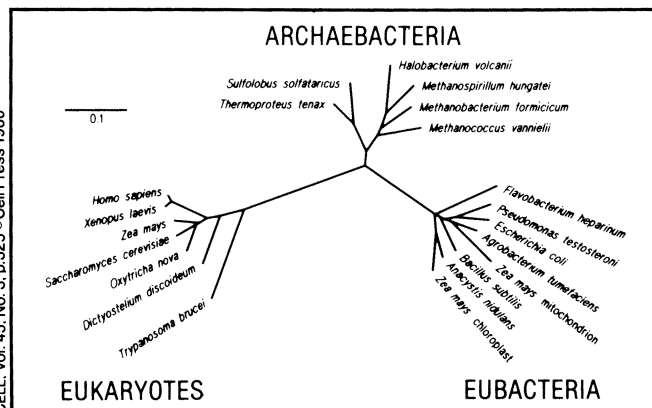
By IVAN AMATO

Billions of years ago, the first living assemblage of matter arose out of the primordial ooze, at once the Adam and Eve of the millions of species living today. Not until the 19th century did Charles Darwin and Alfred Wallace bring into view how the creative power of evolution could start from such a lonely beginning and yield such a diversity of living forms. Yet remaining concealed until recent decades were the microscopic evolutionary sagas played out by the molecules of life found in every cell.

In 1965 Emile Zuckerkandl and Linus Pauling, both then at the California Institute of Technology, proposed that proteins could serve as molecular clocks that measure the evolutionary distance between different species. They theorized that the more these molecules differ between two organisms, the more ticks have ticked on the molecular clock and the more time has passed since the organisms diverged from a common ancestor. Evolution biologists ever since have been using molecular clocks based on various proteins and other biological molecules such as DNA and RNA.

Early users of molecular clocks had hoped that the method might yield the grand tree of life, showing how the millions of living species are related. But today, biologists are more cautious with their expectations as they learn about the peculiarities of molecular timepieces.

For years, biologists have known that molecular clocks are not Swiss made. Over the billions of years during which they have been running, molecular clocks at times have run slow or fast. According to some biologists, "neutral mutations" — those changes in molecular structure that ultimately have no bearing on natural selection — occur at an average rate, which varies significantly only in studies involving "short" spans of evolutionary time. This "neutrality theory" asserts that over long periods of time, variations in clock rates get washed out and the molecular clock is effectively accu-



An outline of a master tree of life derived from studies using a molecular clock based on ribosomal RNA.

rate enough to be used in evolutionary studies.

But recent findings threaten to undermine the neutrality theory — at least for some applications — and leave the molecular clock on a thin theoretical foundation. Research from a number of labs is showing that clocks based on different molecules tick at different, and often varying, rates. For instance, clocks based on a particular molecule such as ribosomal RNA sometimes run at different rates for different species. Moreover, the rates of DNA clocks based on different cellular sources of DNA can differ within the *same* organisms.

Wesley Brown and Lisa Vawter reported evidence in the Oct. 10 SCIENCE that clocks based on DNA from the mitochondria of sea urchins and a variety of primates, including humans, chimps and Old and New World monkeys, run at rates that differ from those of clocks based on DNA from the cell nuclei of these species. The researchers, both at the University of Michigan in Ann Arbor, point out that the different clock rates imply different times at which pairs of species diverged from a common ancestor. Yet such evolutionary divergences are one-time-only affairs.

Roy J. Britten of Caltech in Pasadena came to a related conclusion in a study published in SCIENCE eight months earlier. He found that the rate of DNA evolution

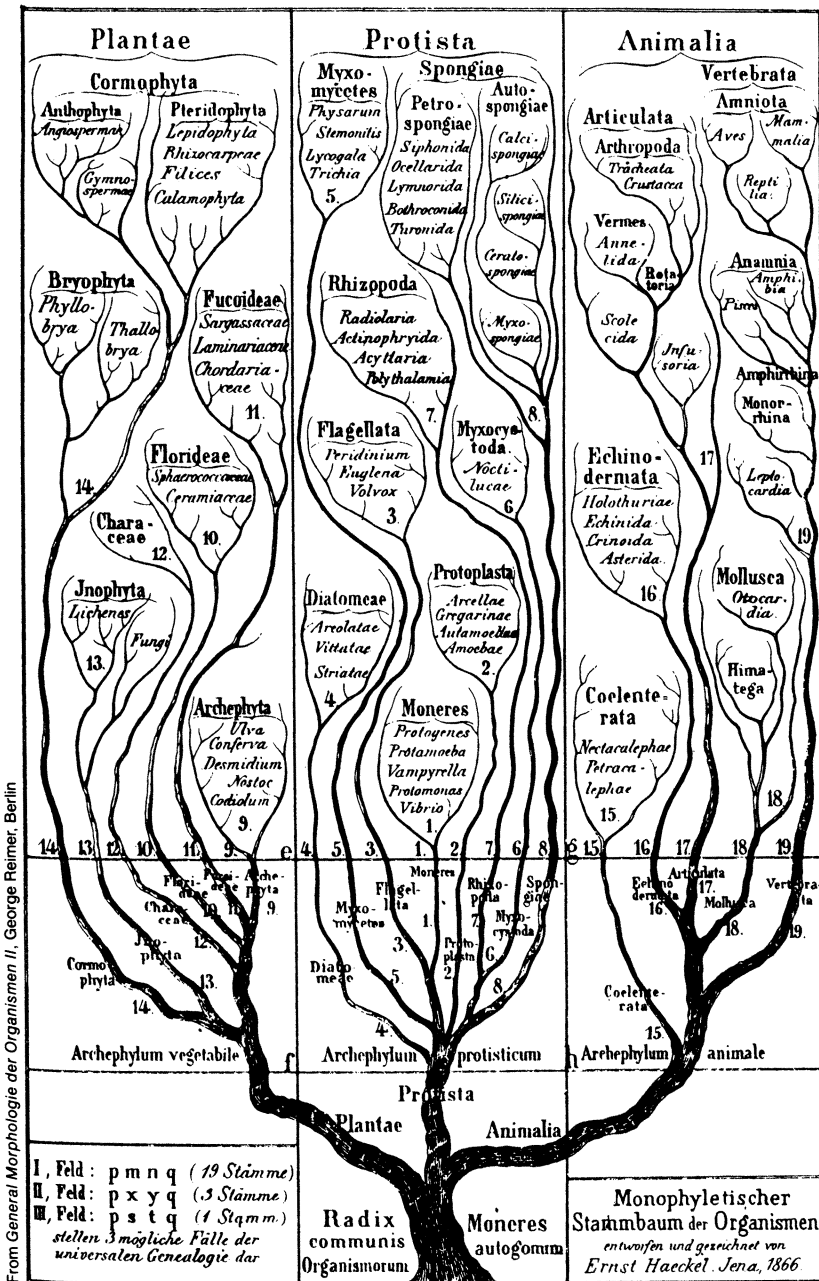
differs by a factor of 5 between higher primates and some bird lineages, on the one hand, and rodents, sea urchins and fruit flies, on the other.

In addition, Francisco J. Ayala of the University of California at Davis last summer reported in THE JOURNAL OF HEREDITY that an enzyme called superoxide dismutase appears to evolve at different rates depending on which species are compared.

Brown, Vawter, Britten and Ayala all suggest that such variations imply that the mechanisms of molecular change are themselves subject to evolution. And they say this introduces a feedback loop that greatly complicates the use of molecular clocks.

Brown and Vawter argue that the lack of precision and synchrony in clock rates is sufficient grounds for rejecting the possibility of using a single molecular clock to work out the entire tree of life. In addition, Ayala says pitfalls lie in wait for those who assume molecular clocks are steady tickers and who then draw evolutionary inferences under this assumption. Without a good theory about why molecular clock rates vary, he says, there is no way to determine "the probable error of evolutionary dates and of phylogenies" that come from such inferences.

Biologists can test specific molecular clocks for accuracy by comparing phylogenies, or the genealogies of organisms, inferred from molecules with phy-



The first evolutionary tree of life, drawn up by the early Darwinian Ernst Heinrich Philipp August Haeckel in 1866.

logenies derived through other evidential avenues. In a celebrated 10-year study of bird genealogy published in 1984, Charles G. Sibley and Jon E. Ahlquist, both then at Yale University, compared DNA from more than 1,500 species and worked out nearly the entire genealogy of over 9,000 species. The DNA clock they used worked well in their research because bird species are closely related taxa. And their findings are robust because they drew upon, and cleared up, a number of ambiguities in the anatomical, morphological and fossil evidence associated with the bird species. But for comparing taxa that are quite distant, such as bacteria and buffalo, the DNA clock falls short, say Sibley and other biologists.

For comparisons of widely divergent taxa or of closely related species that diverged hundreds of millions of years ago, some biologists are lobbying for a molecular clock made out of RNA from ribosomes. In a review in last May's CELL, Norman R. Pace and Gary J. Olsen of the Institute for Molecular and Cellular Biology in Bloomington, Ind., and Carl R. Woese from the University of Illinois in Urbana-Champaign claim that ribosomal RNA (rRNA) clocks "have revealed the outlines of the master phylogenetic tree relating all known life-forms." Ribosomes are subcellular construction sites at which proteins are assembled.

This "master" tree, which has three main branches from the "progenote" — the organism from which allegedly all life

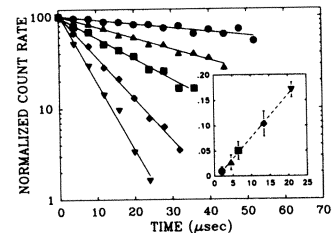
is derived — differs from ones often found in introductory biology texts. The rRNA clock makes it clear, Pace says, that the eukaryotes, animals whose cells have nuclei — have as ancient an origin as prokaryotes, organisms whose cells have no nucleus (SN: 5/3/86, p.280).

Pace identifies the rRNA clock as fit for studies that get at the "big picture" or at the larger branchings of the evolutionary tree, which occurred billions of years ago. But porcupines and people, which diverged from a common ancestor within the last several hundred million years, are nearly the same animal when compared using an rRNA clock, Pace says. The ticks on the rRNA clock are few and far between. To work out evolutionary branchings that have occurred in relatively short periods of time — a few hundred million years or so — other molecular clocks have to be used, he says.

Most evolution biologists now agree that no single molecular clock is going to answer all of their questions. Ayala argues that biologists first must learn more about how and why molecular clocks vary in order to build a theory about their molecular clockworks. Such a theory might allow scientists to synchronize their many molecular clocks, each one fit to answer a limited set of evolutionary questions. And this could lead scientists now wandering in the complex molecular forest to the one tree they seek — the tree of life that shows even how bacteria are related to giant redwoods and humans. □

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