

# RELATIONSHIPS OF LIVING SPECIES: CALIBRATING MOLECULAR CLOCKS

In the early 1960s, Emile Zuckerkandl and Linus Pauling sequenced the amino acids of the hemoglobins of several different species and counted the differences. In a significant show of insight, they juxtaposed their results in biochemistry with the history of life from paleontology. The findings surprised them: the differences in the amino acids were roughly proportional to the estimated geological time since these species had a common ancestor!

**Choose six very light colors and begin at the top right. Color the matrix, which shows the hemoglobin amino acid differences among six species. At some of the intersections of the colors from two species, the number of amino acids that differ in the two hemoglobins is recorded. Leave these squares uncolored. At the other intersections, combine the two light colors.**

Using the matrix we can compare species. Follow the human row until it intersects with the horse row at 18. Zuckerkandl and Pauling found 18 amino acid differences between human and horse hemoglobins. Similarly, comparing human to mouse they counted 16; comparing human to bird, 35; human to frog, 62; and human to shark, 79. The three mammals differed from each other by roughly 20 amino acids (human to horse, 18; human to mouse, 16; and horse to mouse, 22). Mammals and sharks differ in almost 80 amino acids (79, 77, 79).

**Using the same colors, color the family tree on the left.**

Zuckerkandl and Pauling insightfully observed that the number of amino acid differences among these species corresponded in rough proportions to geological time (as illustrated) when they juxtaposed these two lines of evidence. Primate (human), horse, and mouse lineages were thought to have originated about 70 mya, birds about 270 mya, frogs (amphibian origins) about 350 mya, and sharks (cartilaginous fish origins) about 450 mya.

Hemoglobin was acting like a molecular clock! Nobody expected biomolecules to change at a somewhat regular rate over hundreds of millions of years. Many paleontologists criticized molecular researchers for "assuming" such clocklike behavior. To the contrary, steady rates of change are not an assumption, but, based on observations of the data, are now confirmed by more studies on other protein and DNA molecules.

In their first application to human evolution, molecular clocks demonstrated that Ramapithecus could not be a member of the human lineage.

**Color the traditional family tree of ape and human ancestry and the position of Ramapithecus and Australopithecus.**

A fossil species from Pakistan and Africa called Ramapithecus was thought to be the earliest human ancestor. Ramapithecus fossils were as old as 14 million years, and their teeth in some ways resembled human teeth. Many anthropologists therefore assumed that the divergence of the human lineage from apes occurred more than 20 mya. Anthropologists did, however, agree that the then-known 2-million-

year-old Australopithecus was an early human (hominid) ancestor. The separation times of gibbons, orangutans, chimpanzees, gorillas, and human were estimated to more than 20 mya, but there was no way to confirm the time.

### **Color the 1960s molecular family tree.**

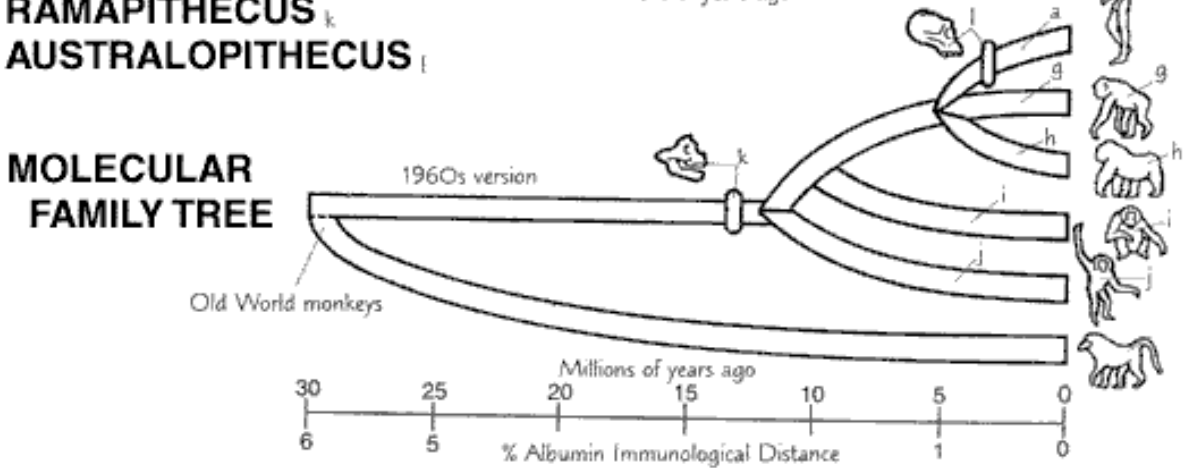
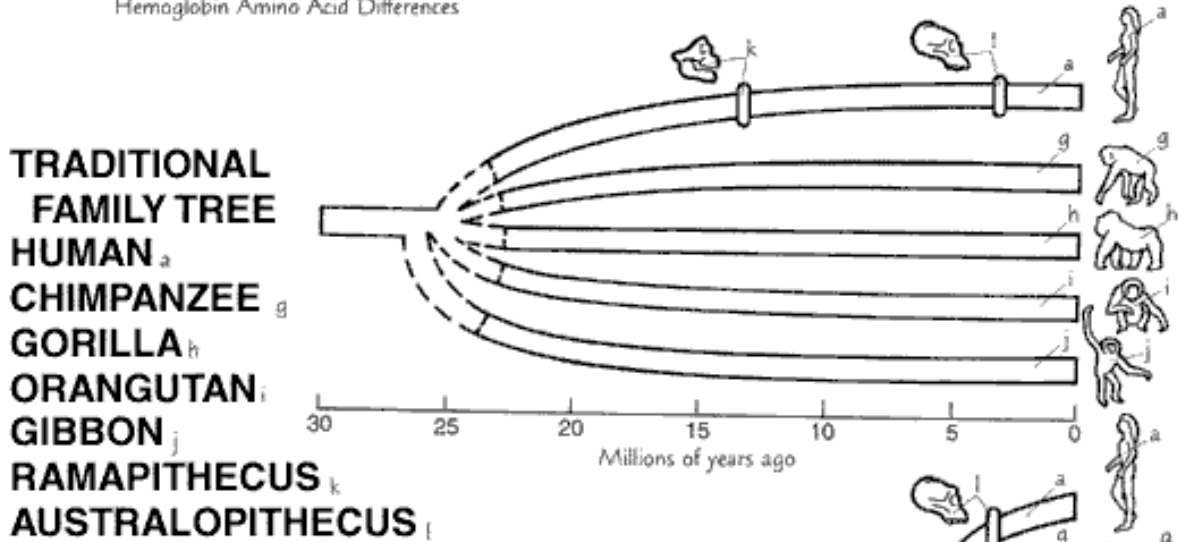
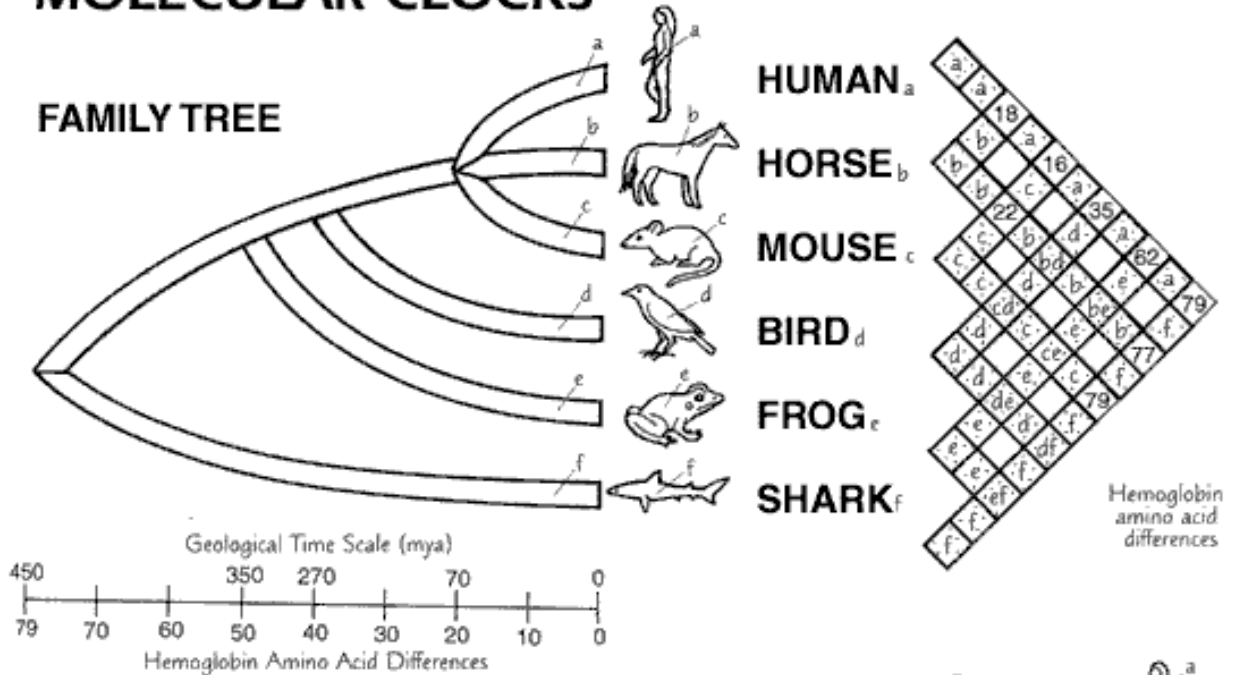
To estimate the time when humans and apes had separated from a common ancestor, Vincent Sarich and Allan Wilson used albumin immunology. Albumin is the main serum protein of all vertebrates, including primates, and can be extracted from blood samples. Sarich and Wilson injected the albumin of humans, apes, and monkeys into rabbits and obtained antisera, just as Nuttall had done 60 years earlier. They improved on Nuttall's methods by using a more quantitative technique called complement fixation, instead of precipitation.

Sarich and Wilson found that the albumins of humans, chimpanzees, and gorillas (the hominoids) differed by 1%; each of them differed from the albumins of Old World monkeys by 6%. To turn this information into a clock, Sarich and Wilson picked an event from the fossil record that paleontologists agreed on and used it to calibrate their molecular findings. The event they chose was the divergence of the hominoids and Old World monkey lineages, and the estimated time, 30 mya. Therefore, the 1% difference among the African hominoids represents one-sixth of 30, or 5. As you can see, Sarich and Wilson were then able to deduce that humans, chimpanzees, and gorillas had a common ancestor about 5 mya. A 14-million-year-old Ramapithecus could not have been a hominid!

When these findings were first reported in 1967, they were controversial. Many paleontologists insisted that Ramapithecus' dental morphology proved it to be hominid. They denied the validity of molecular clocks and rejected a 5-million-year ape-human divergence as being too recent. Subsequently, more molecular data supported the idea of a 5-million-year divergence. Fossil bones were discovered that indicated Ramapithecus was a tree-living ape, not an upright walking human. Hominid fossils 3 to 4 million years old are very like chimpanzees. The 5-million-year estimate for the common ancestor of apes and humans fits with the current hominid fossil record.

Fossils tell us when and where ancient ancestors lived, and what they might have looked like. Molecular data, on the other hand, provide quantitative information on species relationships and estimates of when in the past the lineages diverged. These two kinds of information, paleontological and molecular, are complementary, not contradictory, and both are essential for reconstructing evolutionary history.

# MOLECULAR CLOCKS



– adapted from *The Human Evolution Coloring Book*, 2d ed., by Adrienne L. Zihlman.  
Produced by Coloring Concepts Inc. New York: HarperCollins, 2001.