DNA Reveals Surprises in Human Family Tree

The application of DNA-DNA hybridization to relationships among hominoids places humans with the chimps, while gorillas are separate

Every organism's evolutionary history is encrypted in its genes. For this reason, during the past 30 years, there has been considerable interest among molecular biologists and biochemists in using this information to reconstruct phylogenetic, or family, trees—an activity that traditionally has been the province of systematists who base estimates of genetic similarity on interpretations of morphological similarity.

Although the molecular approach to systematics can be applied in principle to any group of living organisms, it typically engages wider attention and stirs sharper controversy when the subjects of scrutiny are *Homo sapiens* and its closest relatives, the chimpanzee, gorilla, orangutan, and gibbon, which collectively are known as the hominoids. Thus, when Charles Sibley and Jon Ahlquist of the Department of Biology, Yale University, moved on from completing more than 20,000 DNA comparisons among the birds of the world (see box, page 1180) to measuring genetic distances among the hominoids, their work immediately provoked critical commentary.

Sibley and Ahlquist report that the gibbons were first to diverge from the hominoid family tree, 18 to 22 million years ago, followed by the orangutan, 13 to 16 million years ago, then the gorilla, 8 to 10 million years ago, leaving humans and chimpanzees sharing a common ancestor until they split some time between 6 and 8 million years ago (*1*).

The phylogenetic tree derived from the DNA comparisons therefore gives branching order and, with proper calibration, branching times. This dual aspect to molecular systematics—giving both branch order and branch length, or time—illustrates the great potential power of the technique and is based on the idea that the difference in the genetic profile between two species is a linear representation of the time at which the two diverged (of which, more below). Because morphological change is not necessarily directly related to genetic change, traditional systematics cannot automatically place a phylogenetic tree within a temporal framework.

The circumstances under which the human and African ape lines separated has been a matter of lively debate among anthropologists and, more recently, molecular biologists, for many years. The Yale scientists' statement on this question is interesting, for a number of reasons. First, they believe that their technique—namely, DNA-DNA hybridization—is inherently more powerful than other molecular approaches to systematics. Second, in contrast with most hominoid phylogenies derived from molecular biology, which typically show humans, chimpanzees, and gorillas in a three-way split, theirs clearly reveals two discrete branch points. And third, the notion that humans and chimpanzees shared a brief ancestry separate from the gorilla is unexpected and prompts speculation on the nature of the common ancestor for humans, chimpanzees, and gorillas.

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Some critics claim that Sibley and Ahlquist are wrong on all three points, and more. Others enthusiastically support the Yale team's claims.

It is not just Sibley and Ahlquist's version of the molecular clock that has its critics, however. The notion that the passage of time might be recorded in any regular, clocklike fashion in the genome has long been the butt of skepticism, for three reasons.

First, until recently, the date given for the human/African ape divergence based on molecular clocks has been much younger than the great majority of anthropologists were prepared to contemplate (see box, page 1182). Second, there appear to be sound reasons why molecular clocks simply should not work, including the selectionist notion that protein, and therefore gene, structure will come under differing pressures to change, both between species and at different times within the same species. And third, proponents' assertions that molecular clocks can be demonstrated as empirical observations apparently could not overcome the frank admission that, nevertheless, none could explain why they worked.

Ever since Morris Goodman of Wayne State University initiated a renaissance of molecular phylogeny in the early 1960's, a whole series of molecular clocks have been generated, which fall into two groups. In the first, and earlier developed, group are techniques that depend on differences in protein structure. Allan Wilson and Vincent Sarich of the University of California, Berkeley, pushed the microcomplement fixation and other immunological methods and spent a decade calibrating time with measured immunological distance. Electrophoresis and amino acid sequencing of proteins provided other productive approaches.

It became clear that, although different proteins apparently could tolerate different extents of mutation, and therefore changed at different rates, a clocklike quality could often be determined within each protein. It was not a metronome but an approximate, sloppy clock. And it consistently revealed the major outlines of the hominoid phylogenetic tree, but with the humans and African apes clustered in the unlikely arrangement of a three-way split.

Powerful though the various protein clocks were, their degree of resolution was inevitably limited because they are one step removed from the detailed structure of the gene. For instance, for every one point mutation of a nucleotide that caused an amino acid substitution in the encoded protein, there could be two "silent" mutations, which are not reflected in the amino acid sequence of the protein. This alone was a cogent argument for looking at the DNA itself, where more detailed information might be read.

The techniques of recombinant DNA technology have, during the past half-dozen years or so, produced DNA clocks based on restriction enzyme mapping and nucleotide sequencing. In spite of the potentially enhanced resolution, particularly when applied to mitochondrial DNA, which evolves ten times fast-

Some Avian Puzzles Solved

In the 10 years since they embarked on an overhaul of avian systematics using the tools of molecular biology, Charles Sibley and Jon Ahlquist have completed more than 20,000 DNA-DNA hybridization tests on 1600 species, which represent all but three of the 171 bird families recognized by traditional classification. Convergent evolution is an ever-present snare for evolutionary biologists who try to judge relatedness of species by similarities in morphology: it simply is not always readily obvious whether identity of structure is the result of common ancestry or common adaptation. This problem is particularly acute with birds, which is why Sibley and Ahlquist's application of the DNA hybridization clock has been so useful in uncovering many understandable but erroneous classifications (*1*).

The endemic passerines (songbirds) of Australia are a striking example. This diverse group was thought to have been assembled by waves of immigrations from Eurasia, which was a perfectly reasonable suggestion before the idea of continental drift became an established fact. According to the molecules, however, this group—which includes lyrebirds, bowerbirds, various wrens, honeyeaters, babblers, crows, and so on—are the diverse products of an extensive adaptive radiation from a single lineage, which began close to 60 million years ago. Prior to the beginning of this adaptive radiation, the continent had apparently been scrubbed clean of songbirds, which some people interpret as the result of the putative asteroid impact at the end of the Cretaceous, 65 million years ago. Once the Australian passerine radiation was under way, nothing avian got into or out of the continent for almost 20 million years, until the crows (corvines) began a northward expansion, which produced an interesting pattern. Today there are some 16 genera of this group in Southeast Asia, 12 in North America, and 14 in Europe, which indicates a long-established dispersal. By contrast, crows are relative newcomers in Africa, where there are three genera, and in South America, which has just two genera.

Australia had, of course, experienced a similar endemic radiation of mammals, but as these were readily recognized as marsupials rather than placentals, zoologists did not become ensnared in the same kinds of taxonomic traps that caught the ornithologists.

The most controversial result—that is, before the hominoid data came through—concerns the relationships among starlings, which are natives of the Old World, and mockingbirds and thrashers, which are New World inhabitants. Traditionally, starlings have been said to be related to crows and other members of the corvines, while mockingbirds and thrashers were aligned with thrushes and wrens. When the DNA hybridizations indicated that starlings and mockingbirds are in fact each other's closest relative, many ornithologists began to have doubts about the validity of Sibley and Ahlquist's technique. The compilation of more data, which confirm this most heterodox suggestion, has now proved persuasive (*2*). During their forays into this problem, Sibley and Ahlquist came across some serological data produced in 1961, which tied starlings and mockingbirds together as closest relatives. The results had been largely dismissed as the product of an unreliable technique.

In addition to revealing the relationship between these Old and New World birds, the DNA hybridization data indicated that they had separated some 25 million years ago, which is puzzling at first sight. Sibley and Ahlquist explain this by pointing to paleobotanical data that shows habitable territory throughout the northern part of the globe during the balmy days of the Oligocene. With the onset of deteriorating climates by the beginning of the Miocene, 25 million years ago, the common ancestor of the starlings and mockingbirds would have been pushed southward, into the New World on one side of the Atlantic and the Old on the other, where it adapted and radiated in different directions.—**R.L.**

References

1. C. G. Sibley and J. E. Ahlquist, *Curr. Ornithol.* **1**, 245 (1983).
2. ———, *Auk* **101**, 230 (1984).

er than nuclear DNA, none of these clocks has, according to their practitioners, unequivocally been able to break the human/chimpanzee/gorilla trichotomy.

When Sibley and Ahlquist started on a third DNA clock—DNA hybridization—they were building on what in many ways is a conceptually and technically simple method, which had first been developed in the early 1960's and applied to hominoids at least twice.

The technique's first attribute is the scale of the genetic comparison. Clocks based on proteins, for instance, are effectively matching about 1000 nucleotides in one species with the homologous set in another. With DNA mapping or sequencing methods, the scale of comparison may jump to something like 17,000 nucleotides, which is already impressive and clearly offers a great deal more information. The DNA hybridization method, however, pushes the scale at least another five orders of magnitude because it matches one organism's entire genome with another's genome. Mammalian genomes contain on the order of 2 billion nucleotides, but the hybridization technique scrutinizes only the "single copy" DNA, which constitutes about 60 percent of the genome, the remainder being repeated sequences.

Because of the very large number of nucleotides being compared, each of which is effectively a single data point, the DNA hybridization technique immediately commands a statistical robustness not readily achieved by other approaches.

More important, however, is the fact that, by involving every piece of homologous single-copy DNA between two species, the technique automatically addresses one of the strongest criticisms of molecular clocks in general. Namely, it is highly unlikely that any particular gene or genetic unit will tick clocklike and uninterruptedly for very long periods of time. The rate of fixed mutation may be fast at some periods, slow at others. Moreover, genomes are in a dynamic state of flux, with many factors complicating any attempt to measure the changes in a precise way. The complexity has so far defeated virtually all mathematical essays to describe potential patterns of mutation. It is, in essence, a black box. But, argue Sibley and Ahlquist, this is no impediment to the DNA hybridization technique because the very large number of nucleotides in the box ensures that fluctuations away from the average in one direction will be matched by fluctuations in the opposite direction. The result, they say, will be a uniform average rate of change, which is

likely to be the same in all classes of animals.

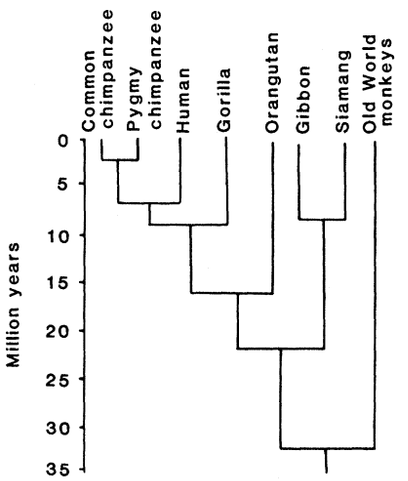
If Sibley and Ahlquist are correct in their assumptions, the criticism that molecular clocks are unreliable—because rates of change within individual genes is uneven—is resolved because the very large numbers allow it to be ignored. This is because the range in the rates of nucleotide substitution is narrow compared with the enormous total number of nucleotides in the genome. The Yale researchers have so far shown the uniform average rate of evolution to be verified in more than 20,000 DNA hybrid pairs among bird species, but just one direct test among mammals. The system is so tight, they say, that deviations in the numbers produced are the result of experimental error, not of differences in the average rate of DNA evolution.

The actual procedure of the technique is very straightforward. After the excess repetitive sequences are removed from DNA preparations of two species that are to be compared, the single-copy material is sheared into 500 nucleotide-long pieces, mixed together, and allowed to reassociate, forming heteroduplexes. The more similar are the two sets of DNA, the more strongly bonded will be the hybrids formed. Even DNA strands that are identical will dissociate when heated to a high enough temperature, usually in the region of 100°C. Strands that have some nucleotides mismatched begin to dissociate at lower temperatures. Molecular systematists get a measure of the difference in nucleotide sequences between two species by noting the difference in temperatures at which the homoduplex and the heteroduplex dissociate by 50 percent, a figure known as the delta $T_{50}H$. One delta $T_{50}H$ represents about 1 percent difference in nucleotide sequence.

This figure must of course be converted to a measure of time, which Sibley and Ahlquist have done by resorting to biogeography. They argue that as the South Atlantic Ocean became an impassable barrier some 80 million years ago, a measure of the delta $T_{50}H$ between the ostrich in Africa and the rhea in South America should calibrate the DNA difference. By so doing, they calculated that a delta $T_{50}H$ of 1.0 = ca. 4.5 million years. A series of five other avian comparisons based on biogeographic separations give similar calibrations. The one mammalian test—based on fossil evidence for the origin of the orangutan, 13 to 16 million years ago—gives a similar calibration. Sibley and Ahlquist plan to extend tests of calibration by examining geographically separated groups of frogs, freshwater fish, lizards, and rodents.

Application of the time calibration to the hominoid DNA hybridization data gives the phylogenetic tree mentioned earlier. The Yale results are different from those obtained from most other molecular clocks, not only in clustering the human and chimpanzee, separate from the gorilla, for 2 million years, but also in having a relatively early divergence (6 to 8 million years) between humans and apes. Most of the protein clocks are in the region of 4 to 6 million years for this split, and some mitochondrial DNA data have been interpreted to show it as late as 2.5 million years.

Both Wilson and Sarich are impressed



The hominoid family tree

Compared with many molecular clocks, the DNA clock places the human/African ape split quite early, 6 to 8 million years.

by the potential power of the hybridization technique. But Sarich considers that the average rate of DNA evolution in mammals is faster than in birds, which would mean that the estimate of human/ape divergence time would have to be reduced, so coming closer to the 4.5-million-year date he favors from microcomplement fixation techniques. Sarich is not prepared to believe that morphologists are able to identify orangutan affinities in the Pakistan and Kenyan material that is said to be the beginning of that group, and so dismisses Sibley's only mammalian calibration.

Wilson, by contrast, thinks that DNA evolution may well be the same in different groups, although he has reservations about this. He notes, for instance, that while mammalian genomes appear to contain many pseudogenes, which are nonfunctional and tend to diverge rapidly in sequence, birds appear to have very few. If pseudogenes represent a significant proportion of the mammalian genome, this might bestow a tendency to faster evolution compared with birds.

A more serious criticism of Wilson's is on Sibley and Ahlquist's choice of 80 million years as a calibration point for the separation of the ratite birds, such as ostrich and rhea. He argues that, although the African and South American continental masses were indeed separate at that time, there would have been a very long period during which passage from one landmass to the other would have been possible, even to flightless birds, mainly because West Africa and Brazil would have been sliding past each other rather than simply separating. Wilson states that there is fossil evidence to support this suggestion and guesses that Sibley's calibration might therefore be wrong by a factor of 2. Such an adjustment would, again, bring the separation time of humans and apes from the DNA hybridization data in step with Wilson's own results from mitochondrial DNA.

Sibley and Ahlquist's response to these last points are that they have six independent calibrations on avian data judged across three biogeographic events, which range from 80 to 40 million years. The calibration is the same in all cases and seems to indicate a linear average rate of DNA evolution through time. On the question of different rates between different groups, Sibley and Ahlquist reiterate that this is a statistical, not a biological, issue.

Sibley's enthusiasm for the power of the numbers is infectious, but Alan Templeton, of Washington University, St. Louis, has remained immune. A year ago he published a statistical analysis (*2*) of hominoid mitochondrial DNA data produced by Wilson and his colleagues. Although they were unable to break the human/chimpanzee/gorilla trichotomy with these data—and still cannot—Templeton reported that he was able to do so. His tree clustered the chimpanzee and gorilla, with the human line splitting off first.

When Sibley and Ahlquist published their DNA hybridization data, Templeton again applied himself to statistical analysis. He used a procedure called the Q-statistic, which E. C. Pielou had developed for analyzing paleoecological similarity matrices, on the DNA hybridization data and concluded that of the two phylogenies—his and Sibley and Ahlquist's—the Yale version was favored, but only weakly.

More important was Templeton's application of *t*-tests to the various human/chimpanzee/gorilla pairwise comparisons. The *t*-test on the human/chimpanzee, human/gorilla distances showed the Yale phylogeny to be statistically signifi-

The Dethroned Ape

When, almost 20 years ago, molecular biologists first came up with dates for the divergence between humans and African apes, they ran slap into a formidable obstacle: their date, of 4 to 5 million years ago, was but a fraction of that supported by most of the anthropological establishment, which was 20 to 30 million years ago. For the anthropologists there was a perfectly respectable first human ancestor, dated at 15 million years old, in the fossil record. This ancestor, *Ramapithecus*, was represented principally by fragments of upper and lower jaws. Ergo, the molecules simply could not be right.

Ramapithecus had first been unearthed in India in the early 1930's, but it was not until the 1960's that its fortunes rose in the anthropological circles, principally through the efforts of Elwyn Simons and David Pilbeam, both then at Yale. These and other scientists saw in the jaw fragments features that appeared to betray the fossils' incipient humanity. These features included robustness and shape of the mandible, small canines compared with those of apes, and thick tooth enamel, which is characteristic of later hominids, the australopithecines, and modern humans. The small canine was particularly important because it was part of the "package" of adaptations that has dominated stories of human origins for so long: to wit, a bipedal ape steps out onto the arid, hazardous savanna to become a stone weapon–wielding, large-brained hunter.

One interesting irony in the tale of *Ramapithecus* and the advent of molecular clocks is that the rehabilitation by Simons and Pilbeam of the one coincided in time with the development of the other. For 10 years the molecules were outclassed, or at least eclipsed.

By attrition, the molecular data began to have an impact on anthropologists' thinking, though few would admit it at the time. There began to be closer scrutiny of what exactly was the nature of the characters displayed in *Ramapithecus* and its relatives: were some of them perhaps primitive, and could not therefore be interpreted as signs of divergence in the hominid direction? Some characteristics simply vanished, having been more in the minds of the observers than in the fossils observed. For most anthropologists, the end of the reign of *Ramapithecus* came with the discovery in 1979 and 1980 of facial remains of a slightly larger relative, *Sivapithecus*. Peter Andrews, of the British Museum, and Pilbeam, recognized in *Sivapithecus* characters that allied it with the orangutan, which split off from the hominoid stem long before the human/African ape divergence. Because of the resemblances between *Ramapithecus* and *Sivapithecus*, if the one was to be shunted off in the orangutan clade, then so was the other. End of the Indian ape as the first hominid. Clearly, robust jaws and thick enamel were primitive features, as far as hominids were concerned. And the molecular divergence dates began to see more favorable exposure in the anthropological literature, with the older dates, such as those from the DNA hybridization technique, finding most favor of all.

Pilbeam and his colleagues began to think of the robust-jawed, thick-enameled creatures as a coherent group, the ramamorphs. But, as more and more Miocene ape material is coming under scrutiny, there appears to be a greater diversity than once was imagined. The ramamorphs, says Pilbeam, are no more.

Meanwhile, the molecular clock—or, more properly, clocks—is becoming an established part of anthropological thinking. So much so that Vincent Sarich, who, together with fellow Berkeley biochemist Allan Wilson, did so much to champion the eloquence of the molecules, now fears that molecular clocks, once heresy, are now in danger of becoming the dogma that the fossils once were.—**R.L.**

Additional Reading

1. *New Interpretations of Ape and Human Ancestry*, R. L. Ciochon and R. S. Corruccini Eds. (Plenum Press, New York, 1983).
2. *Primate Evolution and Human Origins*, R. L. Ciochon and J. G. Fleagle Eds. (Benjamin/Cummings, Menlo Park, Calif., in press).
3. D. Pilbeam, *Sci. Am.* **250**, 84 (March 1984).

cant. However, the same test on gorilla/human and gorilla/chimpanzee favored his phylogeny, which, he says, reveals an internal inconsistency. Templeton says that as the chimpanzee/gorilla clustering, with the human line separate, is the only phylogeny that is consistent with all the data, it is to be preferred over the Yale version. Moreover, he says, as the comparison of sequences in the mitochondrial DNA technique represents a direct measurement of something biologically meaningful (character states), whereas DNA hybridization is in some ways a blind amassing of numbers, the former approach should be given more weight.

Ponderings upon the most appropriate statistical analyses of all these results continues by both the Yale and Washington University protagonists, each side recruiting comment from others. Richard Holmquist, of the University of California, Berkeley, suggests, for instance that, if the *t*-tests are indeed appropriate, then their outcome in the two sets of pairwise comparisons might be saying something about the nature of the DNA clock. He is impressed by the consistency with which the bird DNA data fit with the various biogeographic events, but considers that the problems with the hominoid analyses might indicate that the constant rate hypothesis is false or that, in some lineages at least, time is not proportional to delta $T_{50}H$ values.

Sibley's argument that the only possible source of error is in experimental procedure will be tested by the further accumulation of hybridization data within the hominoid pairs.

If the Sibley and Ahlquist tree is the correct one, then one is tempted to make a small speculation about the nature of the common ancestor between humans and African apes. As both the chimpanzee and the gorilla are knuckle walkers, which split off separately from the hominoid stem, it becomes slightly more probable than not that the common ancestor also was a knuckle walker. Such a suggestion has been made in the past, principally by Sherwood Washburn, of the University of California, Berkeley, and his school, but it has never been especially popular. Most anatomists see nothing in ancestral human anatomy that would support this view. Sarich, however, in preliminary studies, believes he has found some indication of knuckle-walking ancestry in the metrics of the wrist of living hominoids.—**ROGER LEWIN**

References

1. C. G. Sibley and J. E. Ahlquist, *J. Mol. Evol.* **20**, 2 (1984).
2. A. R. Templeton, *Evolution* **37**, 221 (1983).