

Conflict Over DNA Clock Results

The work of two researchers who, using the technique of DNA hybridization, reported an unexpectedly close genetic relationship between humans and chimpanzees, has been attacked as flawed

IN 1984 CHARLES SIBLEY and Jon Ahlquist, both then at Yale, surprised anthropologists and molecular biologists alike when they announced that they had resolved what had appeared to be an intractable question in human origins research. The question concerned the evolutionary relationship between humans and the two African apes, chimpanzees and gorillas. Ever since Morris Goodman of Wayne State University had demonstrated in the early 1960s that humans and African apes are genetically extremely closely related, molecular biologists had tried all kinds of ways to break the apparent evolutionary three-way split, or trichotomy, between these three species. Now, suddenly, the two Yale researchers—ornithologists by training—had succeeded where others had failed.

But Sibley and Ahlquist's announcement also provoked incredulity, because theirs was not the answer that had been expected: they said that according to their data from DNA hybridization experiments, humans and chimpanzees were each other's closest relative, with gorillas having diverged from them earlier. The more conventional view had been that chimpanzees and gorillas were each other's closest relative, with *Homo sapiens* standing apart from them.

As so often happens in science, once an erstwhile intransigent problem has apparently been cracked, many other researchers quickly come up with the same answer using several different approaches. Such was the case here, and the other techniques included protein electrophoresis, DNA restriction mapping, DNA sequencing, and conventional anatomical analysis. At the end of 1987 Sibley and Ahlquist themselves published a greatly expanded set of DNA hybridization results that appeared to make the issue pretty secure, at least in the eyes of some observers. For their pioneering work, Sibley and Ahlquist enjoyed a great deal of highly favorable attention, from both within and without the anthropological profession.

The whole episode was a vindication of the often highly controversial notion of using molecular data to build family trees, known as molecular phylogenetics, a hypothesis that was proposed just a quarter of a century ago by Emile Zuckerkandl and

Linus Pauling.

In recent months Sibley and Ahlquist have found themselves the center of attention once again, but this time for very different reasons. Two separate manuscripts—authored by Vincent Sarich, of the University of California, Berkeley, Carl Schmid, of the University of California, Davis, and Jon Marks, Yale University—have for several months been circulating widely among anthropologists and molecular biologists, challenging not only Sibley and Ahlquist's conclusions but also their method of analysis and the propriety of

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some of their data handling. Sibley and Ahlquist's reputations as scientists hang on the outcome of that challenge, as to some extent does the credibility of molecular phylogenetics in general.

Sarich and his colleagues have reanalyzed a small proportion of Sibley and Ahlquist's data, none of which has previously been made public, and conclude that the result not only throws doubt on the human/chimpanzee/gorilla comparison but also "calls into question everything else Sibley and Ahlquist have published." The reason for so sweeping a conclusion is that, according to

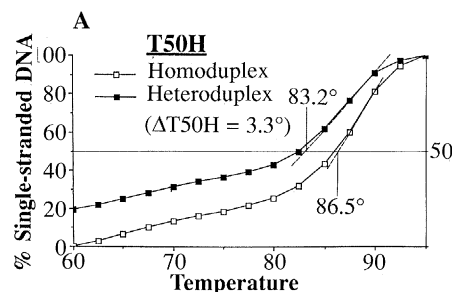
Sarich and his colleagues, the data "have apparently been subjected to manipulations of an unspecified nature."

One of the trio, Marks, recently wrote to Zuckerkandl, the editor of the *Journal of Molecular Evolution*, which published Sibley and Ahlquist's papers, demanding that the papers be retracted: "To fail to repudiate Sibley and Ahlquist's deceitful presentation to your reviewers of their means of collecting and analyzing their data . . . will be to the severe detriment of the journal, the field of molecular evolution, and to the scientific community in general." Zuckerkandl declined, saying that a paper can be withdrawn only at the authors' request, or by the editor if there is a case of established fraud. "In this case there is no such thing," Zuckerkandl told *Science*.

What began as an ostensibly objective examination by Sarich and his colleagues of the power of the technique of DNA hybridization in general, and of Sibley and Ahlquist's work on humans and African apes in particular, has quickly degenerated into a rather personal conflict, with big egos on both sides. As Roy Britten, of the California Institute of Technology, told *Science*, "Those manuscripts [by Sarich and his colleagues] are not scientific articles, they are weapons with political purposes."

All kinds of rumors abound, and there are many researchers who make no attempt to hide their delight that Sibley and Ahlquist may be wrong in their conclusions about the human/African ape relationship: the more orthodox chimpanzee/gorilla association has many supporters. There are many others who, because of a commitment to a particu-

Measures of distance. Figure A shows the T50H measure, in which the DNA melting from the hybrid is plotted completely, and includes the difference in initial overall hybridization between the homoduplex (same species) and the heteroduplex (different species). B shows the T_m measure, in which the initial difference in hybridization is corrected for. C shows the T_{mode}, which shows differences in maximum melting temperature.



lar approach—cladistics—to building evolutionary relationships, would be happy to see Sibley and Ahlquist's brand of molecular phylogenetics discredited. And there are still others who, because of Sibley's rather immodest personal style, are happy to join in with what some characterize this episode as, specifically, "Sibley bashing."

There are, however, some real issues here. The first has to do with how good the technique of DNA hybridization is in extracting evolutionary relationships among species by effectively comparing the entire genetic components of those species. Second, associated with this is the choice of statistical analysis of the raw data from the DNA hybridization experiment, an apparently arcane business that nevertheless raises strong, opposing opinions.

Third, there is the question of the availability of raw data—in this case a series of radioactive counts for each species comparison—as opposed to the statistic derived from them: except in the early days of their work, Sibley and Ahlquist have published only the numbers derived from the raw data, not the raw data themselves. The last and potentially most explosive point is the nature of Sibley and Ahlquist's data manipulation that Sarich and his colleagues recently exposed: is it scientifically valid and does it affect the phylogenetic conclusions?

Schmid, a chemist, has long argued that DNA hybridization is not as simple a technique as its proponents claim, both in the experimental procedure itself and in the inferences that can be reached on evolutionary relationships. In essence you line up the DNA from the two species and measure (indirectly) the degree to which the sequences differ: the bigger the difference, the greater the evolutionary distance between the species. In fact, the sequence comparison—the hybridization process—is fraught with potential perturbations, including factors such as the preparation of the DNA itself and the kinetics of the interaction between different DNA molecules.

The procedure is as follows: You obtain what is called tracer DNA from one of the

species to be tested, by cutting the DNA into lengths of about 500 base pairs and separating the double-stranded molecule into single strands. A key part of the procedure is to remove those sequences that appear in multiple repeats, theoretically leaving behind only unique sequence DNA.

This single-stranded tracer is made radioactive and then added to a large excess of single-stranded DNA from the second species to be tested, known as the driver. The mixture is allowed to anneal, during which process tracer sequences that are sufficiently similar to driver sequences will form double strands, or hybridize: the more similar sequences are to each other, the more tightly they hybridize. The duplex formed during annealing is then subjected to step-wise increases in temperature (from about 60°C to 90°C), which progressively shakes the double-stranded fragments apart, a process that is monitored by the number of radioactive counts lost at each step.

In theory, if tracer and driver DNAs come from the same species, all the tracer will hybridize to the driver, and a high temperature is required to release all the radioactive tracer. In species that are genetically diverged, and therefore have somewhat different DNA sequences, duplex formation will not be quite as strong or as complete, and a lower temperature is required to release all the tracer fragments. The reduction in the initial degree of hybridization and the lowering of melting temperature is assumed to be a measure of the evolutionary distance between the species.

In practice, for reasons that remain unclear, initial duplex formation is never 100% complete, even with DNAs from the same species. This unhybridized fraction—which increases as the genetic distance between species increases—is the focus of sharp disagreement between Sarich and his colleagues on one hand and Sibley and Ahlquist on the other. Does it contain valuable phylogenetic information or potentially misleading misinformation? Therefore, should it be encompassed in or excluded from the statistical measure—a single number—that

is derived from each species comparison?

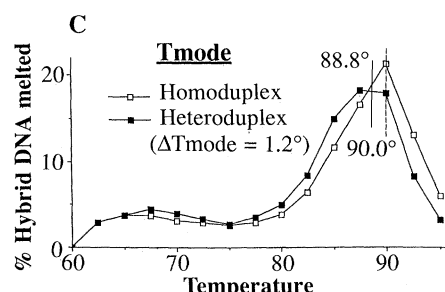
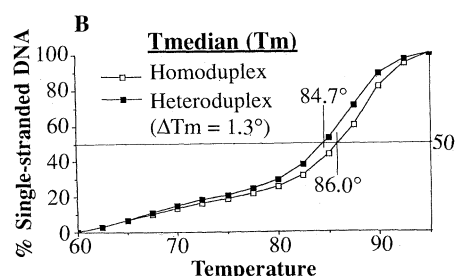
Not only does Schmid believe that this unhybridized DNA fraction is potentially misleading, but he also contends that the kinetics of the initial duplex formation and its subsequent melting are more complex than most practitioners appreciate. This complexity results from the nature of the DNA fragments themselves and from the conditions under which the experiment is usually done, and can be an additional source of uncertainty in the results, he says.

So, when, in early 1986, Marks approached Schmid to discuss potential problems of DNA hybridization in connection with Sibley and Ahlquist's conclusions about the human/African ape relationship, he was more than willing to offer his advice.

Marks was interested in the issue not least because a couple of years previously he had completed a doctoral thesis on chromosome banding in humans and apes, from which he concluded that chimpanzees and gorillas were each other's closest relative. "These data [are] more consistent with an orthodox hominoid phylogeny than with heterodox phylogenies," he wrote in a 1983 review. Shortly afterward the first of the Sibley/Ahlquist papers was published, presenting a distinctly "heterodox" phylogeny.

Marks and Schmid prepared a manuscript for the *Journal of Human Evolution*, submitted midway through 1987, which Marks describes as "a cautionary tale for anthropologists, warning them that DNA hybridization might not be all it seems." Britten, who was a pioneer in developing the DNA hybridization technique, reviewed the manuscript and declared it unpublishable. "They made one melting curve, and discussed every conceivable thing that might be wrong with it, without regard for their significance or relative importance," says Britten. "They proceeded to make this thing into an attempt to trash DNA hybridization in order to keep anthropologists from believing Sibley and Ahlquist's data."

The editors of the *Journal of Human Evolution* put Marks in touch with Britten, and the two discussed the perceived problems with the manuscript. During the conversation Marks explained that he had twice asked Sibley for a sample of his raw data, but had received none. Unbeknownst to Marks, Britten had a sample of the Sibley/Ahlquist data in his desk at Caltech. Ahlquist had given Britten the data in February 1986, when Britten had taken part in a debate at the American Museum of Natural History. The debate had been between cladists and supporters of molecular phylogenetics and had been organized after Stephen Jay Gould, of Harvard University, had declared in the April 1985 issue of *Natural History*



magazine: "We should all rejoice in the success of molecular phylogeny because its techniques have probably solved the biological problem of the ages: namely how, and why, are organisms interrelated?"

This small piece of history was to prove crucial, because Britten, with Sibley's approval, subsequently sent that sample of data to Marks, adding: "[Sibley] will send you more if you need it." The current clash was thus initiated.

Marks received the package from Britten in December, and immediately wrote to Sibley thanking him for this sample of data, and saying, yes, he would indeed like to see more. Marks shared them with Schmid, and sent a copy to Sarich. Most of the data were on humans and apes, with just a few on birds. In fact, the vast majority of Sibley and Ahlquist's work has been on birds: 25,000 individual experiments on birds, 514 on hominoids.

Examining their sample of data, Sarich and his colleagues quickly convinced themselves that, although its quality seemed good, Sibley and Ahlquist had applied an inappropriate statistical analysis.

For the reasons mentioned earlier, Sarich and his colleagues consider that the most accurate interpretation of DNA hybridization melts is one that excludes the unhybridized fraction; in addition, they argue that the DNA that comes out of duplex at low temperatures should also be eschewed. As a result, they concentrate on what is known as Tmode, which is the temperature at which most tracer is melting out of the duplex (see diagram).

By contrast Sibley and Ahlquist plot their data cumulatively, producing an S-shaped curve rather than a bell-shaped distribution. Making an allowance for the fraction of DNA that did not hybridize initially, they then calculate the temperature at which 50% of the DNA that theoretically could have hybridized has melted out of duplex: this measure is known as T50H. A third measure, Tm, gives the temperature at which 50% of the DNA that actually did hybridize has melted out of duplex.

Using the hominoid data at their disposal, Sarich and his colleagues recalculated the genetic distance between the three species, using Tmode and Tm rather than the T50H measure. In one of their manuscripts, Sarich and his colleagues recall the tremendous debate that followed the publication of Sibley and Ahlquist's 1984 paper, and then say the following: "Unfortunately, as we shall show, there should have been no debate in the first place, for the actual data do not discriminate between the [human/chimpanzee/gorilla genetic distances]." In other words, according to Sarich and his col-

leagues, using the Tmode measure on Sibley and Ahlquist's own data, the hominoid trichotomy cannot be broken.

Ahlquist told *Science* that he and Sibley had used the Tmode statistic in their early work on birds, but had shifted to the T50H measure, "precisely because sometimes it isn't sensitive enough to make discriminations between species." Oftentimes they had found themselves with bell-shaped curves for two species, both of which having exactly the same mode, but with the height of one lower than the other. "We considered that the difference must be telling us something," says Ahlquist, "and eventually found that T50H could discriminate between such species." Since 1980 the T50H statistic has been their measure of choice. (The issue of data analysis will be addressed further in next week's article.)

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At about the same time that Marks received the sample of Sibley and Ahlquist's data from Britten—December 1987—a special issue of the *Journal of Molecular Evolution* was published on molecular clocks. This included an article by Sibley and Ahlquist reporting about three times as much DNA hybridization data on the hominoid relationship as they had available in their previous, 1984, publication. Sibley and Ahlquist took the opportunity to respond at length to some of the negative commentary they had received following their 1984 publication, and again concluded that the DNA hybridization data split the human/chimpanzee/gorilla trichotomy, making chimpanzees closer to humans than to gorillas.

The new paper identified each experiment by number, making it possible for Sarich and his colleagues to check the published T50H comparisons with the raw data they had in their hands. Before they did that, however, Marks wrote again to Sibley, on 11 January, asking again for more data and requesting permission to use the data they had in publications of their own. By this time Marks and Schmid's original paper for the *Journal of Human Evolution* had been scrapped and two new drafts were in prepa-

ration, one again to *JHE* and the other to the *Journal of Molecular Evolution*.

Both papers would question the reliability of DNA hybridization as commonly used, and both would attempt to show that Sibley and Ahlquist's own data do not support their widely publicized conclusions. Each listed Sarich, Schmid, and Marks as authors, and were tailored for the different audiences of the two journals. That was the plan. It was soon to change.

Meanwhile, Sibley had not replied to Marks' two letters, but instead had passed them on to Ahlquist. Both men had recently left Yale and were preoccupied with setting up new laboratories and courses, Sibley at San Francisco State University, Tiburon, and Ahlquist at Ohio University, Athens. Ahlquist eventually replied to Marks, on 15 January, saying "It will take me some time to assemble the data, since they are not 'computerized' and must be copied. . . . I do not have any assistance with this sort of thing." He also asked Marks in what form he would like the data.

Marks replied a week later, saying that the data should be in the same form as those that Britten had obtained earlier. He added: "While I shall look forward to receiving the rest, at the moment it will be adequate simply to make sure that you have no objections to our citing and discussing the data we already have."

Shortly after that Sarich and his colleagues made the crucial comparisons: they calculated T50H measures from the raw data they had, and checked them with what should have been the same measures in Sibley and Ahlquist's recent *Journal of Molecular Evolution* paper. Forty percent of them were significantly different (that is, by more than 0.5°C in melting temperature differences in the range 1°C to 3°C). "We were stunned," recalls Sarich. "I was sure we were face to face with a disaster for the field of molecular phylogenetics."

Marks elected to write again to Ahlquist, beginning with an apparently rather low-key request: "While analyzing the data sent to me by Dr. Britten, I have come up with a few questions, and I hope you can assist me in answering them. . . ." Sibley and Ahlquist are now embroiled in answering these questions: their reputations and the credibility of molecular phylogenetics may be resting on the answers. ■ ROGER LEWIN

Next week's article will address the impact of Sarich and his colleagues' critique of Sibley and Ahlquist's work; it will examine the nature and extent of Sibley and Ahlquist's data manipulation; and will reveal how other evidence influences judgment on the episode.