says. "The costs of cloning will make the base pair costs look like chicken feed." Hood, whose group is one of several now tackling the "front end," expects to see automated cloning devices within a year or so. The eventual goal is to automate all the sequencing steps and tie them together.

At DOE, however, "we are not interested in small incremental improvements in existing technology, but in new methods that offer great possibilities," according to Gerald Goldstein, who is overseeing the technology development for the genome project. "We must have vastly improved technologies," he says. And vast improvement, according to Charles DeLisi, who headed the genome effort at DOE before moving to Mount Sinai School of Medicine this fall, means thousands of bases per second.

For fiscal year 1988, DOE has set aside several million dollars for such technology development. Proposals are due 2 November, but some work is already under way. For the past few years a group at Los Alamos National Laboratory has been trying to apply flow cytometry to DNA sequencing. The idea is to tag the bases with a fluorescent label, then cleave the bases off one at a time and flow them by a detector. Currently, flow cytometers detect single cells, but the Los Alamos group is working on an "extra-sensitive" optical system capable of detecting a single molecule, Goldstein says.

DOE is also funding work at Brookhaven National Laboratory on scanning transmission electron microscopy. So far the effort has been focused mainly on mapping techniques, but it could conceivably be used for sequencing, Goldstein says. This might involve labeling the bases with clusters of gold or tungsten atoms. "It's not exactly a crazy idea, but it is a long way from being proved."

Other possibilities include mass spectrometry—DOE is evaluating several proposals—and scanning tunneling microscopy, which creates a sort of contour map with atomic resolution. Both are "highly speculative," Goldstein admits. DOE may fund a feasibility study of another approach that involves immobilizing DNA in a solid matrix and then knocking off the bases one by one, perhaps with an ion beam, and then detecting them in some as yet unidentified way.

Few expect these innovative approaches to figure in the genome project any time soon. Hood, for one, does not anticipate any "earth-shattering new approaches that will change things fundamentally. Maybe in 5 years someone will have a new idea and there will be a big jump." But for now, most of the gains will come from tinkering with the current generation of DNA sequencers. ■ **Leslie Roberts**

My Close Cousin the Chimpanzee

Recent evidence of molecular biology indicates that humans and chimpanzees are each others' closest relative, a conclusion that remains at odds with most anatomical inferences

"If Morris Goodman is correct in his conclusion, we will just have to go back to the anatomical evidence and find out what we've been missing," says Lawrence Martin, an anthropologist at the State University of New York at Stony Brook. The conclusion to which Martin refers is that, contrary to most expectations, humans are genetically closer to chimpanzees than either is to the gorilla. On the basis of both superficial physical similarity and more formal anatomical analysis, chimpanzees and gorillas certainly appear to be each others' closest relative. "It would be remarkable if this proved not to be the case," says Martin.

And yet, if a score had been kept during the past few years of the various lines of molecular evidence that have emerged on the human-chimp-gorilla relationship, the unexpected would be seen to be gaining majority support, by more than two to one. The latest offering, by Goodman and his colleagues at Wayne State University and the University of Florida, is published on page 369 of this issue, and is described by Martin as "by far the best molecular dataset to date." Goodman's data, which he collected with Michael Miyamoto and Jerry Slightom, are in the form of a 7100–base pair sequence of a locus in the beta-globin region in humans, chimpanzees, gorillas, and the orangutan.

"If we had only our dataset, the question of a human-chimpanzee association wouldn't be decisive," acknowledges Goodman. "And maybe putting all the datasets together still would leave room for some doubt. But I think it is getting pretty close to being decisive." Backing up this conclusion are two additional papers about to be published.

The first is by Nobuyo Maeda and her colleagues at the University of Wisconsin and presents a further 3000–base pair sequence from the same genetic region that Goodman analyzed. The second is a new batch of DNA-DNA hybridization data by Charles Sibley of San Francisco State University and Jon Ahlquist of Ohio University. Although there had been earlier indications scattered in the literature, it was Sibley and Ahlquist's publication 3 years ago of their first set of DNA-DNA hybridization data that forced molecular biologists and anthropologists alike to take seriously the possibility that the chimpanzee's closest genetic relative might be *Homo sapiens*, not the gorilla.

Impressive though the recent accumulation of genetic results in favor of the human-chimp association is, resolution of the issue will not be settled by majority vote, not least because the data are not equivalent. In addition to the basic divide between anatomical and molecular information, there are different types of genetic data: some are more direct than others.

For instance, DNA sequence data offer direct information about the species being compared, and the sequences themselves can be thought of as being analagous to series of anatomical characters, such as the shape of a bone or the pattern of muscle attachment. By contrast, DNA-DNA hybridization data—which match the overall fit of two separate genomes—are an indirect reflection of two species' relatedness, and simply offer a measure of the genetic distance between them: the poorer the fit, the greater the distance.

In general, biologists with an interest in reconstructing phylogenies—or family trees—prefer to use characters rather than distance data because, in principle, characters allow unique links between species to be identified. For this reason Goodman's latest DNA sequence results are seen as being of special importance in resolving what has clearly become a hot issue in anthropology.

The issue is hot for several reasons. First, if Goodman and others are correct, ideas about the beginnings of the human lineage would be significantly altered. Specifically, because both chimpanzees and gorillas move about by means of a mode of locomotion known as knuckle-walking, it becomes more likely than not that the common ancestor of these two African apes and humans was also a knuckle-walker. Sherwood Washburn, of the University of California, Berkeley, has advocated just this scenario for many years, but with virtually no support from any quarter. "If the common ancestor were a knuckle-walker, then this must affect the way you think about the biology and behavior of the first hominids," he says.

The second reason that the notion of a human-chimp association is being hotly debated has to do with the reliability of using anatomical characters to infer relationships. Reconstructing phylogenetic trees requires the search for signs of shared ancestry, specifically the identification of homologous characters that uniquely link two or more species as an evolutionarily derived group. And for chimpanzees and gorillas, the entire anatomical complex that underlies knuckle-walking looks like a very good bet to fulfill this requirement. If it turns out that, in spite of sharing the knuckle-walking adaptation, chimpanzees and gorillas are not each others' closest relative, and instead chimps are more closely allied to humans, "it could strike the death knell of comparative anatomy," as one observer pessimistically put it.

It was the limitations of inferring phylogeny from anatomy—particularly from fossil anatomy—that led Harvard anthropologist David Pilbeam recently to conclude that "branching patterns are best determined using mainly genetic data." Most practitioners in his profession are, however, not as enthusiastic about yielding the high ground of phylogenetics to molecular biologists. In short, the newest results and conclusions of Goodman, Sibley, Maeda, and others will bring into even sharper focus the current debate over how data from anatomy and various forms of molecular biology can be utilized in building family trees.

The introduction of modern molecular biology into anthropology began in the early 1960s, and it immediately set a tone of conflict that echoes to this day. Morris Goodman used immunological tests to compare serum proteins from humans and the three great apes, and in 1963 concluded that chimpanzees and gorillas formed a natural group with humans, leaving orangutans more distant. This pattern was at odds with the then popular notion that the three great apes—under the family name, Pongidae—were the natural group, with humans separate, the single occupant of the family Hominidae.

Goodman even went so far as to suggest that, on the basis of the molecular data, gorillas, chimpanzees, and humans should be placed in the same family, hominids all. The proposal was not well received. Nevertheless, Goodman's branching pattern became the norm. Later, in 1967, Allan Wilson and Vincent Sarich, of the University of California, Berkeley, using the immunological approach, produced some dates and sparked a controversy. The human line diverged from the African apes about 5 million years ago, they concluded. Not so, responded the anthropologists, who argued from fossil evidence that the first hominid lived at least 15 million years ago. Like Goodman, Wilson and Sarich had been unable to resolve the details of the African ape-human branching pattern: it was often shown as a three-way split.

> ***"If Morris Goodman is correct in his conclusion, we will just have to go back to the anatomical evidence and find out what we've been missing."***

During the next decade and a half more and more molecular evidence flowed from more and more laboratories. The original immunological data were joined by protein sequencing, DNA-DNA hybridization, restriction enzyme mapping and sequencing of mitochondrial DNA, gene sequencing, and chromosome anatomy. The outcome was that date of the African ape-human divergence was confirmed as more like 5 million than 15 million years, but the precise topology of the divergence remained unresolved. True, there were from time to time some indications of the shape of the tree—sometimes grouping chimps with gorillas, sometimes chimps with humans, and sometimes gorillas with humans—but nothing was persuasive.

The first strong statement of topology came in 1984, when Sibley and Ahlquist described the results of their DNA-DNA hybridization work on the great apes, gibbons, and humans in comparisons with Old World monkeys. Calibrating their molecular clock against fossil evidence, Sibley and Ahlquist concluded that gibbons split off between 18 million and 22 million years ago, orangutans 13 million to 16 million years ago, and gorillas 8 million to 10 million years ago, leaving humans and chimps briefly united, finally to diverge between 6.3 million and 7.7 million years ago. The recalcitrant three-way split had finally been broken.

The new break was not, however, to everyone's satisfaction. The DNA-DNA hybridization technique has been—and in some quarters continues to be—criticized as inherently inelegant. It involves measuring the binding strength between the genomes of two species, after the removal of repeated sequences which can distort the picture. Critics argue that the removal of repeated sequences is often incomplete, that the physical chemistry of the binding between the genomes under comparison is more complex than imagined by proponents, and that the measure of similarity—in effect, a point on a temperature scale—is too imprecise.

After Sibley and Ahlquist's data became public, there soon developed a small industry obsessed with their statistical reevaluation. Criticism of the conclusions from one quarter would be followed by support for them from another. Indeed, the scrutiny of other people's data, giving rise to published counterconclusions, has become something of a hallmark of molecular anthropology in recent years, and includes data from DNA mapping and sequencing in addition to hybridization.

Among other things, this lack of immediate consensus over the implications of the molecular data indicates how very close-knit the chimp-gorilla-human relationship really is. During this same period the anthropologists were apparently experiencing the same problem. One researcher concluded, for instance, that, on the basis of anatomical comparison, chimpanzees and gorillas formed a natural group with orangutans, and excluded humans altogether. Another said that humans and orangutans were most closely related.

Recently there have been efforts to reduce the confusion by bringing together both the molecular and anatomical results and scrutinizing them by using the same systematic approach: that of cladistic analysis. The latest such assessment is soon to be published by Martin and Peter Andrews, of the British Museum (Natural History).

The cladistic technique depends on the use of evolutionarily shared characters—either anatomical or molecular—which are then identified as being either primitive, that is, present in the ancestral population of the species under study, or derived, that is, novel with them. Finger nails are primitive characters for the African apes, for example, because all primates have them. But the knuckle-walking adaptation is found only among the African apes and therefore gives the appearance of being a derived character. Only derived characters can be invoked in inferring unique links between species.

In searching through anatomical features that might uniquely link humans and chimpanzees as a group, or clade as it is known with this technique, Martin and Andrews came up with extremely thin pickings. "The morphological data are much clearer as concerns the recognition of an African ape clade," they note. "In particular, two character complexes, forelimb features associated

Rival trees

The most recent analysis of the anatomical evidence suggests that chimpanzees and gorillas are each other's closest relative (right), whereas the molecular evidence makes humans and chimps the closest.

Les Owen/FPG

with knuckle-walking, and enamel formation and structure associated with enamel thickness on the molar teeth, seem to provide strong evidence for a period of common ancestry for the chimpanzees and gorillas which did not include ancestors of either humans or orangutans."

The molecular evidence yields a different picture. Based on amino acid and DNA sequences only, Martin and Andrews count some 17 shared derived characters that appear uniquely to unite humans and chimpanzees as against 11 that support a chimpanzee-gorilla clade. "If each genetic event is counted equally, then a chimpanzee-human clade appears the most likely," they note, "although . . . this conclusion can hardly be said to be strongly indicated." Their reservation is due in part at least to the fact that, rather than having arisen within their immediate ancestral stock, some of these apparent shared derived characters are certain to have developed independently in parallel.

"If you add Goodman's new data to this, the argument for a chimp-human clade does look a little stronger," says Martin, "but not overwhelmingly so." The numbers would become 23 and 12, with the same caveat about parallelisms. "When the cladistic analyses of morphological and molecular data are combined," conclude Martin and Andrews, "we believe that the most parsimonious interpretation of the data is that the African apes form a clade which is the sister taxon of the human clade."

Martin and Andrews' analysis excludes Sibley and Ahlquist's DNA-DNA hybridization results, because they are distance data, not character data. "This is not to say that distance data have nothing to contribute," says Martin and Andrews, "simply that we remain to be convinced that they can be analyzed in the cladistic fashion by which we are analyzing our other data." Sibley finds the approach too restrictive. "The notion that protein and gene sequences are more informative than the data from DNA hybridization is erroneous," he asserts. "The difference lies not in whether 'exactly what is changing' is known, but in the *complexity* of the data and whether they can distinguish analogy from homology."

Nevertheless, argue Martin and Andrews, when two species' genomes are matched against each other, what is measured is simple similarity. "There is no way of knowing whether the similarity is the result of commmon ancestry—homology—or parallel change—analogy," says Martin." Sibley counters, claiming that "in effect, the two genomes that are hybridized 'sequence' one another."

The technique involves cutting the two genomes into fragments of about 500 bases in length. It is the binding strength of these fragments that the hybridization technique measures. "A DNA sequence of 500 bases can occur in 10^{301} different arrangements," notes Sibley. "It is therefore inconceivable that chance, or convergence, could produce the 75% to 80% of base pairing required for the production of a stable duplex at 60°C." In other words, Sibley is arguing that, in effect, the similarity that DNA-DNA hybridization measures can confidently be assumed to be homology.

The power of the DNA-DNA hybridization technique lies in the averaging of very large numbers: two billion bases of single-copy DNA to be precise. "DNA hybridization data are enormously complex," notes Sibley, "indexing essentially the entire information content of the genome. . . . There is no obvious advantage in knowing 'exactly what is changing' unless one is committed to the [cladistic] procedure." In any case, argues Sibley, the true test of the value of DNA-DNA hybridization as against sequencing is the results it gives. "The meager evidence available suggests that the two methods will give the same answer to any given question."

If the commonly arrived at answer in the case of the human-chimpanzee relationship proves to be correct, then the two anatomical characters—knuckle-walking and molar tooth enamel structure—that are apparently shared uniquely by the African apes have to be explained. One possibility is that chimps and gorillas independently evolved these two characters after their two lineages had diverged. Another is that the characters were present in the common ancestor of the African apes and humans, and were subsequently lost in the human line.

The fact that both characters are extremely complex and made up of many components makes the possibility of independent evolution seem unlikely, particularly for the knuckle-walking. Washburn and Sarich notwithstanding, the alternative—that knuckle-walking was lost in the human line—appears equally unlikely, according to most anthropologists. "Apart from one small character, there's nothing I'd call evidence for knuckle-walking in human anatomy," concludes Randall Susman of the State University of New York at Stony Brook. "Even in the earliest hominid fossils—*Australopithecus afarensis*—I can find not a whit of evidence for knuckle-walking."

Martin would view as "extremely surprising" the prospect of all signs of knuckle-walking having been lost from human anatomy. "Nevertheless, all these developments are stimulating us to go out and try to find evidence that might one way or the other resolve the apparent conflict between the molecular and morphological evidence," he says. "It is a very exciting time."

Meanwhile, Goodman urges that the conclusions from the genetic data should be reflected in the formal classification of apes and humans. "I should like to see chimpanzees, gorillas, and humans placed in the same subfamily, the Homininae," he says. "In 1963, I suggested they should be in the same family, but I now think they should be shown to be even more closely related than that."

Goodman is likely to find some support for this proposal among certain cladistically inclined morphologists—including Martin and Andrews—but not among more conventional anthropologists who continue to insist that humans deserve family status of their own. ■ **Roger Lewin**

ADDITIONAL READING

C. G. Sibley and J. E. Ahlquist, "DNA hybridization evidence of hominoid phylogeny," *J. Mol. Evol.*, in press.

P. Andrews and L. Martin, "Cladistic relationships of extant and fossil hominoid primates," *J. Hum. Evol.*, in press.