Molecules vs. Morphology: Of Mice and Men

For those who would reconstruct evolutionary family trees there is now a choice of data: molecules and morphology; do they give the same answers?

Every organism carries the history of its ancestry encrypted within its anatomy and its genes. Theoretically, therefore, it is possible to reconstruct a species' evolutionary history, its phylogenetic tree, which reveals its relatedness with other species.

Phylogenetic reconstruction has traditionally been the province of morphologists, who judge relatedness on the basis of certain shared anatomical characters, principally homologies. But in recent years these anatomically based systematists have witnessed the intrusion into their territory of molecular biologists, who employ genetic information of various types—from proteins and DNA—to build family trees. Inevitably, there was a degree of conflict generated, not least because the new boys seemed to be claiming that their techniques were superior to the established ones.

A recent symposium on this issue* was entitled Molecules versus Morphology, thus apparently emphasizing the methodological divide. In fact, as was noted by the symposium's organizer, Colin Patterson of the British Museum (Natural History), "molecules aren't very different from morphology in this business, because they share similar problems." It is true that most phylogenetic histories derived independently from the two are closely congruent, which is encouraging. But there are important exceptions too, and this is where the interest lies.

Most notable among this latter group, and certainly the most visible, is the continuing dispute over the configuration of the human family tree, which was given close scrutiny at the symposium by Peter Andrews, also of the British Museum. However, some new data on molecules and morphology of inbred mice, presented by Walter Fitch of the University of Wisconsin, promises to stir the debate over the relative merits of the different categories of information: as Fitch and his colleague William Atchley read their data, the morphology appears to be curiously unreliable.

Phylogenetic reconstruction 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. Traditionally this search has been for morphological structures but now, of course, includes molecular sequences, of both proteins and DNA.

For anatomists the identification of homologies is a potential trap, because similarity of function can sometimes fashion similar morphologies in unrelated species: the marsupial radiations of Australia and South America provide many striking examples. Convergence is the systematist's enemy.

In spite of these difficulties, it is usually possible to determine shared ancestry, particularly if more than one homology can be identified. Greater confidence is ensured by the amassing of more and more characters.

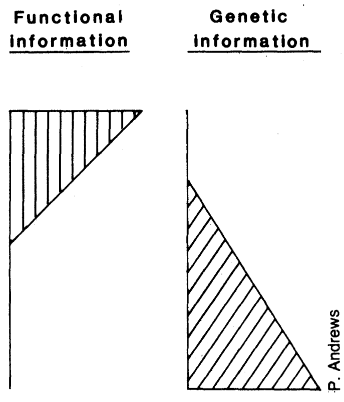
Many molecular biologists have the unfortunate tendency to refer to two similar sequences of proteins or DNA as being homologous, irrespective of questions of ancestry. Molecular phylogeneticists, who do concern themselves with ancestry, nevertheless face serious problems in deciding, say, whether or not a shared amino acid at a particular locus is the result of true homology or convergence. In this respect molecular and morphological data are rather similar. They differ, however, in that the molecular data are usually not clouded by notions of function, a frequent smokescreen surrounding morphological data. The resolution of homology versus convergence in molecular characters is typically a matter of statistical analysis.

One aspect in which molecular and morphology data do differ markedly and potentially very significantly is in the rate of change. Because anatomical structures are expected to alter during evolution at least in part in response to pressures of natural selection, which may vary in time and magnitude, there is no theoretical argument for regularity of change. Morphological modification may occur by turns in fits and starts, and in slow, gradual trajectories. By contrast there is considerable theoretical—and empirical—foundation for expecting molecular change to be on average close to constancy. In other words, that there is a molecular clock. With this temporal dimension available to it, molecular phylogeny does indeed assume a somewhat superior aspect in relation to morphological phylogeny.

Given such a claim, and given also the apparently natural arrogance of molecular biologists, it is perhaps not surprising that the morphologically based systematists were less than enthusiastic in welcoming these new techniques as the solution to their problems: if the molecular biologists were correct in their claims, they, the morphologists, would be out of business.

The morphologists' understandably critical reception of molecular phylogeny was, however, fueled by the considerable disagreement among the molecular biologists themselves about just how clock-like the molecular clock is. By now the debate has evolved into part

Information source	Information status [character (ch.) or distance]
*Morphology:	single/multiple ch.
*Chromosomes:	single ch.
*Blood groups:	polymorphic ch.
*Proteins:	distance statistic
*Amino acid sequence:	single ch.
*DNA hybridization:	distance statistic
*DNA sequence:	single ch.



Raw material for reconstructing phylogenetic trees

From morphology to DNA, the nature and status of information changes.

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*Third International Congress of Systematic and Evolutionary Biology, 4 to 10 July, Brighton, England.

substantive issues and part a tangle of sociological and philosophical posturing.

One somewhat tangible aspect of the foment is the acceptability of so-called distance techniques, such as DNA hybridization. This technique, which has been promoted in recent years by Charles Sibley and his colleagues at Yale as a powerful way of establishing phylogenies, is based on the physical strength with which DNA strands from different species will bind to each other in solution: the greater the identity, the more tenacious the binding. What is measured is a "melting temperature," from which the degree of sequence similarity is inferred.

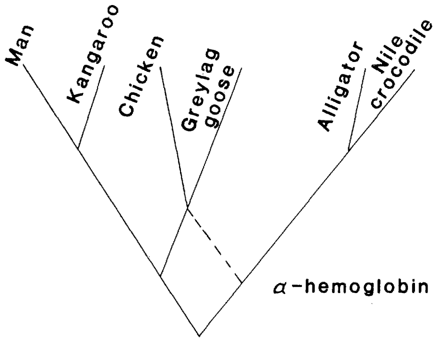
Although this is simply a distance statistic, and not a determination of a character state, Sibley and his colleagues claim that an estimate of phylogenetic divergence between species is possible, because the sequence similarity is a true reflection of homology. Now, although this cannot be true absolutely, because some convergence and back mutation is inevitable, the fact that the method typically involves 1 billion nucleotides makes the assumption of homology statistically acceptable, as Andrews noted at the symposium. Patterson, a morphologist, also considers Sibley to be on relatively safe ground.

It is clear, however, that when one is dealing with characters, such as a long DNA or protein sequences, there is potentially a great deal of detailed information to be gleaned, most of which is lost in the albeit powerful statistics of DNA hybridization. Joseph Felsenstein of the University of Washington considers that, given a free choice, one would opt for character information for phylogenetic reconstruction, but matters of practicality make DNA hybridization a very acceptable alternative for the comparison of multiple taxa, as the Yale group has demonstrated in their revision of much of the world's bird phylogeny.

When Fitch and his colleagues began pioneering serious molecular phylogeny in the late 1960's they were faced with the task of interpreting their data. Because the data always had some uncertainties and internal inconsistencies within them, the question was one of finding the most likely tree among the many possible. There began the development of several lines of numerical analysis of competing phylogenies, an approach that was adopted independently by the morphologists. Although this was not part of the recent symposium, the issue of how different types of data are most appropriately analyzed is becoming a very important aspect of phylogenetics. Until now there has been a strong tendency for practitioners to stick with one type of data and one type of analytical methodology, no matter what the problem was that they faced. This is beginning to break down.

Together with these developments is the fast-growing practice of casting answers in a statistical framework, which assigns relative probabilities to favored trees. This is a major departure from the typical single-tree answer of the traditional approach, which inevitably assumed a somewhat dogmatic air.

Molecular phylogeny has often been applied to large-scale questions, which is what Adrian Friday, of the University of Cambridge, England, addressed. Following a recent suggestion based on anatomical analysis that birds may be more closely related to mammals than is usually allowed, Friday turned to the molecules for an answer. He would have preferred to use DNA sequences, but there simply are insufficient data yet available for this wide analytical sweep. So, instead, he employed sequence data for myoglobin and alpha-hemoglobin.



Where are the birds in the tree?

The most favored tree from alpha-hemoglobin sequence data allies the birds with the mammals, but the more traditional grouping (dotted line) is a close second.

Pairwise comparisons of sequences from representative species produced a series of trees, the most favored one of which in the case of myoglobin appeared to support "reasonably clearly" the bird/mammal affinity. The answer from alpha-hemoglobin was more equivocal, with there being very little to choose between a tree linking birds and mammals and another rooting them in their more traditional home, with the reptiles. Michael Miyamoto of Morris Goodman's department at Wayne State University presented an alternative analysis of myoglobin, which, he said, did not support Friday's. What is clear is that with the molecular data available, an unequivocal answer is not yet possible.

Perhaps the most conspicuous test case of molecules versus morphology has been the resolution of the hominoid family tree, the great apes and humans. Sentiment on this issue has swung back and forth through the decades, and virtually all points of opinion are still represented today. For example, Arnold Kluge of the University of Michigan argues from morphological analysis for a grouping of all the great apes together (a great ape clade), leaving humans as a separated branch. He also points out that mitochondrial DNA sequences can be interpreted as supporting a great ape clade, albeit not strongly.

In his analysis of morphological data, Andrews concludes that a good case can be made for an African ape clade (chimpanzees and gorillas, but excluding orangutans) because of the shared knuckle-walking adaptation and dental anatomy. Many of the other anatomical features that appear to link the African apes with the orangutan are simply related to similarity in body size, he says, and are therefore not necessarily diagnostic of shared ancestry.

The molecules, however, tell their own story. Ever since Goodman's initial analysis in the early 1960's placed humans, chimpanzees, and gorillas as very close cousins of each other, there has been a struggle to untangle the detailed configuration: was it a three-way split? do chimpanzees and gorillas share some slightly closer ancestry in relation to humans? or are humans more allied to one of the great apes, to the exclusion of the other, and if so, which one?

In recent months there has been an accumulation of evidence that favors the last of these three, placing humans and chimpanzees with a briefly shared ancestry after splitting from gorillas. This evidence includes that from DNA hybridization and mitochondrial DNA sequencing. For Andrews, however, the strongest support for this relationship comes from the sequences of alpha- and beta-hemoglobin, in which humans and chimpanzees uniquely share two amino acid substitutions. Overall, however, the weight of evidence is as yet far from conclusive in any direction, he cautions.

The mitochondrial DNA sequence support for a human/chimpanzee relationship comes from Masami Hasegawa of the Institute of Statistical Mathematics in Tokyo. In his reanalysis of sequence data produced by Allan Wilson's group in Berkeley, in which he concentrated on certain types of base substitutions (transversions), he not only placed humans and chimpanzees with a shared ancestry but also produced a date for the origin of the human line, 2.5 million years ago, that is far more recent than is generally accepted.

One interpretation is that some of the putative hominid fossils from East Africa have nothing to do with human evolution, because they are older than 2.5 million years. Alternatively, the apparent intimacy and recency of the human/chimpanzee association is the result of an interspecific hybridization 2.5 million years ago between the two lines, which had separated some time earlier. Interspecific transfer of mitochondrial DNA is known to have occurred between different species of mice, which left them with different nuclear genomes but identical mitochondrial genomes.

Fitch switched the attention from men to mice and produced a puzzle. He and Atchley recently discovered that genetic evolution proceeds apace in inbred mice strains, but showed nevertheless that several methods of analysis of the molecular data were able to reconstruct the known history of the different lines. Given that their albeit brief phylogeny—a mere 70 years—is securely known, inbred mice apparently offer a perfect system in which to test methods of phylogentic reconstruction. The molecular data and analysis appear to pass the test, which contrasted markedly with similar attempts based separately on seven life-history variables and 14 measurements on the lower jaw. The best trees from these latter two sets of data were different from each other, from that produced by the molecular analysis, and hence from the known history.

Patterson was unsurprised by this apparent failure of morphology, saying that the data are morphometrics, which are distance statistics and therefore noisy; they are not characters, he says, which could be expected to tell the truth. Felsenstein believes it is stretching the point to make a clear distinction between characters and distance statistics in this kind of situation, but is as yet uncertain how the results should be interpreted.

If the use of inbred mice strains is valid, in spite of their somewhat unnatural history, and the results are substantiated with more thorough anatomical analysis, the message will be difficult for morphologists to swallow. But this should not be viewed as molecules versus morphology, but rather an opportunity to use molecular data in order to learn something of the evolution of morphology. Instead of conflict, here is a pathway for gaining keener insights into evolutionary change.—**Roger Lewin**

Atomic Physics Tests Lorentz Invariance

Accurate measurements of the frequencies of atomic transitions lead to tighter limits on the velocity dependence of the laws of physics

One of the fundamental assumptions of modern physics is that physical laws do not change with the velocity of the frame of reference in which experiments are done. Two recent atomic physics measurements by groups at the National Bureau of Standards, Boulder, Colorado, and the University of Washington provide the most stringent limits yet on the magnitude of any violations of this assumption, which physicists call local Lorentz invariance.

A velocity dependence could enter into the laws of physics if there were in the universe a preferred frame of reference. An obvious candidate for such a preferred frame is that in which the mean distribution of matter in the universe is at rest. For example, if one supposed that the distribution of matter in the universe affects the nongravitational laws of physics by means of some as yet undiscovered long-range force (some new aspect of gravity), this would single out the frame of reference at rest with respect to the mean distribution of matter in the universe as a preferred frame.

If such a force existed that influenced the laws of electromagnetism, for example, there could be many observable consequences. In particular, the rate at which an atomic clock ticked could depend on the orientation of the atoms in the clock relative to their velocity through the distribution of matter. The NBS and Washington experiments, as well as in a third project that has started at Princeton University, test just this idea.

As the earth rotates, the orientation of a clock fixed to its surface does in fact change relative to its motion through the distribution of matter in the universe. So, the physicists compared the rates at which an atomic clock ticks at different times throughout the day or, equivalently, looked for changes in the position (frequency) of an atomic spectral line with time of day. However, neither group (John Prestage, John Bollinger, Wayne Itano, and David Wineland at NBS, and Blayne Heckel, Frederick Raab, E. Norval Fortson, and Steve Lamoreaux at Washington) found any measurable change.

What difference would it make if researchers eventually did find that there was a preferred frame of reference? One far-reaching consequence would be the violation of the Einstein equivalence principle, of which local Lorentz invariance is a fundamental component. Simply put, the equivalence principle says that scientists can count on physical laws being the same at all times and everywhere in the universe. More precisely, there exist local Lorentz frames, anywhere and anytime in the universe, in which all the nongravitational laws of physics must take on their familiar special-relativistic form. A Lorentz frame is one in four-dimensional space-time in which bodies are freely falling, so that gravity "disappears," as in an orbiting satellite. It is always possible to establish a Lorentz frame, even near a black hole, by considering sufficiently small volume of space-time that gravity is constant within it.

Violation of the equivalence principle would require changes in the way of thinking about physics. Consider the famous rods and clocks that figure in discussions of special and general relativity. " If the length and time intervals measured with different rods and clocks depended on their location in and motion through the universe, then the ability to define a unique geometric structure of space-time like that in relativity would be lost," notes theorist Mark Haugan of Purdue University. Fortunately, because of the already tight experimental limits, the practical effects of any violation would be not be as catastrophic as the philosophical ones.

Without the equivalence principle, for example, general relativity would no longer suffice as a complete description of gravity. General relativity is the leading member of a broader class of so-called metric theories, in which gravity is ex-