surface temperature did show a significant result, but only by dint of opposing 15-year extremes at either end of the record.

Barnett and Schlesinger do suggest that some parts of the greenhouse fingerprint will be more useful than others. In some cases, they say, natural variability could easily mimic the greenhouse signal, especially if the signal is a weak one. The pattern of atmospheric temperature variability, for example, resembles that of the temperature greenhouse signal. But sea surface temperature has a patchy pattern of natural variability and is expected to increase rather uniformly under the greenhouse.

Barnett and Schlesinger's results do not deny that there has been a global warming this century. They simply show that the variation in warming from place to place does not match the pattern predicted by the models very well. Unfortunately, the fault may lie in the warming or in the models. As Hugh Ellsaesser and his colleagues at Lawrence Livermore Laboratory have pointed out, surface temperature changes on time scales of decades to many millennia have always had a patchy pattern. But the models call for a steady pattern of warming symmetric about the equator. That suggests to the Livermore group "that our climate models are inadequate or that there is a fundamental difference in character between climate changes of the past" and those of the future.

Alternatively, present models may be adequate for what has been asked of them so far—the calculation of conditions once the climate system has adjusted throughout to a doubling of carbon dioxide. But early detection requires knowledge of conditions as different parts of the system respond at different rates to increasing carbon dioxide. As Stephen Schneider and Starley Thompson of the National Center for Atmospheric Research in Boulder have suggested, the climate halfway to a carbon dioxide doubling may not look much like the final, equilibrium climate, as assumed by Barnett and Schlesinger. So far, climatologists do not have the months and years of supercomputer time they need to model accurately the current transition to equilibrium.

Fingerprinting is only just getting under way, but the chase is on. Both the subtle clues and the visage of the culprit itself await refinement. ■ **RICHARD A. KERR**

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Molecular Clocks Turn a Quarter Century

It is now 25 years since Linus Pauling and Emile Zuckerkandl proposed the molecular evolutionary clock hypothesis, and a controversial quarter century it has been. Counterintuitive in the sense that anything in evolution might "tick" in a regular manner, the notion of and evidence for a molecular clock nevertheless persists and has become even more pervasive than originally conceived.

Some of the controversy derives from theoretical and experimental considerations of the fixation of DNA mutations. And some derives from clashes over the implication of specific molecular clock results when compared with classical morphological interpretations—the arena of human/ape evolutionary patterns is most notorious here. Nevertheless, molecular clock information is emerging as a potentially powerful tool in reconstructing evolutionary trees (phylogenies) among a wide range of organisms.

To mark the molecular evolutionary clock's quarter century the Journal of Molecular Evolution *recently devoted an entire issue to the subject. Articles touch on clock theory, molecular and whole genome phenomena that could cause deviations from metronomic ticking, and applications of the approach to specific cases. A selection is presented here.*

Origins and Evolution of the Molecular Clock

Emile Zuckerkandl describes how he joined Linus Pauling at the California Institute of Technology in 1959 and began working on the evolution of primate hemoglobin. The notion of a degree of regularity in protein evolution that might provide the basis of a molecular clock developed during the next half dozen years.

"In June 1960," writes Zuckerkandl, "I assumed that numbers of differences in sequence between homologous polypeptide chains could be expressed as the approximate evolutionary time since divergence." This, he says, was "a conceptual jump," the birth of the clock hypothesis.

However, that conceptual jump faced a formidable conceptual barrier—and still does in many ways—namely an antithesis of associating the idea of any kind of regularity with the process of evolution, and for good reason. "Twenty-five years ago," remembers Zuckerkandl, "the diversity of rates in morphological evolution was the strongest argument, in the minds of biologists, against seriously considering the possibility that graded differences between informational macromolecules might be proportional to evolutionary time."

In other words, biologists knew that morphology changed in fits and starts through evolutionary time, so why should macromolecules be any different? It was soon demonstrated, of course, that molecular and morphological changes were not linked in lockstep: a single base substitution in DNA might produce small morphological change, large morphological change, or no change at all, depending on where the substitution occurred. And the notion of neutral evolution began to emerge too, a useful, but not essential, support for the clock notion.

"It became clear early on that the rate at which the clock ticked differed not only between different informational macromolecules, notably between different sectors of DNA, but also between different parts of such molecules, in fact from molecular site to molecular site," says Zuckerkandl. This variability was evident first in sequence comparisons carried out by Zuckerkandl among hemoglobin molecules and by Emmanuel Margoliash on cytochrome c's. Later Margoliash and Walter Fitch constructed extensive evolutionary trees using cytochrome c sequence information.

A complex set of factors combines to make the chance and consequence of change at one nucleotide position different from that at another. As a result macromolecules offer a quasi-clock based on stochastic changes, not a perfect metronomic clock.

Three major issues must be addressed, says Zuckerkandl: "1) Evolutionary clocks have been explored for different compartments of genomes. Where should one look for the best clock? 2) It is now widely considered that most substitutions, even in coding nucleotides, are functionally neutral. Is this legitimate? 3) Are there other biological evolutionary clocks and, if so, is there a common foundation for all biological evolutionary clocks?

Of Apes, Men, and Statistical Analysis

No roundup of current ideas about molecular clocks would be complete without a

nod in the direction of the debate over the human/ape family tree. Three papers address the issue, one on DNA-DNA hybridization, one on sequence data from the beta-globin locus, and one on the statistical analysis of it all.

In 1984 Charles Sibley and Jon Ahlquist, then at Yale University, presented DNA-DNA hybridization data—183 comparisons in all. Contrary to most molecular work of the time, these new data indicated that humans and chimpanzees were genetically closer to each other than either was to the gorilla. Since that time more and more molecular data have pointed in the same direction. In their latest offering Sibley and Ahlquist report on a total of 514 DNA-DNA comparisons, which give the same branching order as previously, but with more precision. Their divergence dates are as follows: Old World monkeys, 25 to 34 million years ago; gibbons, 16.4 to 23 million; orangutan, 12.2 to 17 million; gorilla, 7.7 to 11 million; and chimpanzee-human, 5.5 to 7.7 million.

In their analysis of 2040 sites in the beta globin locus, Masami Hasegawa and his colleagues from the Institute of Statistical Mathematics, Minato-ku, Japan, obtain the same branching order as Sibley and Ahlquist, but with slightly younger divergence dates. The American and Japanese workers also agree that a slowing down of mutation rate has occurred within the primates, being most significant within the ape and human lineages. The notion of a slowdown has long been a matter of disagreement among proponents of the molecular clock and promises to continue being so for some time to come.

Sibley and Ahlquist's 1984 paper provoked a series of statistical criticisms from several workers, many of whom disagreed with the interpretation of the shape of the tree. Joseph Felsenstein of the University of Washington has analyzed the new data set and concludes that "there is significant evidence for resolving the [human-chimpanzee-gorilla] trifurcation in favor of a [human-chimpanzee] clade."

Felsenstein notes that, although the DNA-DNA hybridization technique is attractive because it is effectively comparing very large numbers of nuculeotide sites, experimental error in carrying out the method makes it equivalent to the resolving power obtained from direct sequencing of 4472 bases. "There is therefore the prospect that, if hybridization methods do not increase in power, sequence data could overtake hybridization data in the near future," he says. There is no doubt too that many biologists prefer sequence data for family tree reconstruction.

How to Perturb a Regular Clock

There are widely varying probabilities that mutations at different nucleotide sites will become fixed, depending on precisely what the mutation is, whether the site is in a coding region, and, if so, which of the three positions in the codon it occupies. Moreover, some codons are more resistant than others to change, depending on the role of the encoded amino acid in the protein that is produced. Such nuances of codon chemistry and biology influence the rate at which mutations will accumulate in a genome, inevitably making the clock less than the "ideal" metronome.

Further potential perturbations come in at a grander scale, some of which relate to internal genome dynamics (known collectively by some people as molecular drive) while others are essentially external intrusions (cross-species gene exchange).

Although even closely related species, by definition, are reproductively isolated from each other, a potential channel for the transfer of genetic material from one to another is on the backs of viruses that can infect them. Enough examples of putative cross-species gene transfer have been documented by now to make it an interesting, albeit rare, phenomenon of molecular natural history. Michael Syvanen of the University of California at Davis argues, however, that the frequency of such transfer is much higher than is generally suspected.

If Syvanen is correct, then some—perhaps many—comparisons of genes between organisms will be distorted: gene transfer would make the species concerned appear to be more closely related than they really are.

"The argument has been made that transfer of DNA sequences cannot be important because of the data base upon which molecular systematics and the molecular clock are based," says Syvanen. "That is, if different macromolecules yield the same phylogeny, then the occurrence of cross-species gene transfer must be unlikely. . . . However, this objection should not be a serious problem if cross-species gene conversion events occur uniformly throughout an entire genome and if, furthermore, parts of genes, not entire genes, are the usual unit of conversion."

Syvanen cites two cases where apparent conflicts between molecular and morphological data might be the result of species sharing genes through common viral infections: the first is the relationship between humans and the African apes, and the timing of their split from the Asian ape, the orangutan; and the second concerns the correct relatedness of the song birds of Australia.

The gorilla, chimpanzee, and human lineages all apparently evolved in Africa, where they will have provided a common pool for a range of viral infections. They might therefore now appear to be more closely related to each other than they really are, and more distant from the orangutan, as a result of cross-species gene transfer, reasons Syvanen.

Similarly, although several Australian song birds seem to be closely related to certain European species on the basis of anatomy, molecular information tells a different story. The molecular data—principally DNA-DNA hybridization—imply that all the Australian song birds are more closely related to each other than any is to the European species: anatomical similarities between particular Australian and European species are explained as being the result of convergent evolution.

Syvanen offers the explanation that close phylogenetic relationship between certain European and Australian song birds has been "washed out" at the molecular level, simply because the Australian species have recently exchanged genes with other Australian birds as a consequence of common viral infections.

Dynamic processes of the genome itself produce a second potential perturbing influence on the steady accumulation of mutations. Such processes—including unequal crossing-over, gene conversion, slippage, transposition, and RNA-mediated changes—are known collectively as molecular drive, a term coined by Gabriel Dover of the University of Cambridge.

Molecular drives can have several different effects on new sequences within a genome, sometimes spreading them rapidly through the genome, and by extension through the local population, and sometimes eliminating them. As a result, says Dover, "turnover mechanisms have the potential to retard, maintain, or accelerate the rate of DNA differentiation between populations." In other words, in those sequences affected by the processes of molecular drive the clock may sometimes appear to run fast, run slow, or stand still.

"DNA clocks are occasionally observable," agrees Dover, "but . . . they are probably a reflection of DNA turnover mechanisms in defined components, as well as reflecting the diffusion dynamics of neutral mutations." In general, however, molecular drive, like natural selection on specific sequences, may perturb an otherwise steady rate of ticking. "To understand the full range of internal and external forces influencing DNA divergence, each sequence needs to be examined case by case," he cautions. "For the moment we can only guess at what lies beneath the tip of the iceberg."

Evidence for a Universal Rate of Mutation

If one concentrates attention on the possible perturbations of an otherwise regular molecular clock it is easy to gain the impression that clock-like activity in DNA is all but impossible. Proponents of the clock are always careful to point out that any species comparisons must be subject to "the rate test," which is designed to reveal any unclock-like behavior. And no one would rely on sequence information from a single gene as the sole basis of a proposed phylogeny: using hybridization of single copy DNA from the entire genome is the ultimate extension of this caveat. And by now there are enough data to show that clocks can and do work, even if there appear to be many cogent reasons why they should not.

Moreover, Allan Wilson of the University of California, Berkeley, and Howard Ochman of Edinburgh University present data on nucleotide substitution rates in organisms as disparate as bacteria, flowering plants, and vertebrates, and find a remarkable degree of constancy.

For instance, the average rate of substitution at "silent" sites—those at which mutation produces no change in amino acid encoded—is about 0.7% per million years for bacteria. This compares with 0.9% for the nuclear genes of mammals and 1% for plants. "The similarity between the evolutionary rates in eubacteria and vertebrates raises doubts about the recent claims that nuclear genomes of multicellular animals vary in rates of substitutions by factors of two to five," note Ochman and Wilson.

There has for a long time been a debate over the potential effect of generation time on nucleotide substitution rate: mice should accumulate mutations faster than men do, it was thought. In fact, although there are some data that indicate a faster substitution rate in rodents compared with large mammals, the difference is much less pronounced than would be predicted by a simple generation-time effect. And, as Ochman and Wilson note above, they, and others, question whether the reported differences are in fact real at all. "In the best studied animals (for example, birds, carnivores, ungulates, primates) average rates of nuclear DNA divergence may be the same."

The debate is certain to continue for a while, which at least has the beneficial effect of stimulating the collection of more and more data from many different laboratories.

■ ROGER LEWIN

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Carl Frank/Photo Researchers, Inc.

Recount on Amazon Trees

As you travel from high latitudes toward the equator you encounter increasingly rich species diversity. Clearly, there is something about tropical climes that favors high levels of speciation, a pattern that is repeated among animals and plants throughout the globe. Until now, however, there has been one striking exception to this rule: the rain forests of Southeast Asia were thought to carry twice as many tree species compared with similar forests in Africa and the New World, a discrepancy that had researchers scratching their heads for a biological explanation.

It turns out, however, that the explanation is methodological, not biological. Alwyn Gentry of the Missouri Botanical Gardens says that "Although the Asian tree plots span the full gamut of habitat types from poor soil, relatively low diversity, heath or kerangas forests to high diversity, continually moist rain forests on relatively fertile soils, all the equivalent neotropical and African data sets have been until recently from sites anticipated to be at the low end of the tropical diversity gradient." This, combined with identification and counting problems that tended to lump different species as one, conspired to give a low diversity count for the neotropics: about 90 species per hectare compared with 160 for Southeast Asia.*

Gentry undertook extensive tree counts at six 1-hectare sites in Peru and one along the Brazil-Venezuela border, and produced figures that match and sometimes exceed those for Southeast Asia. "I conclude that the ever-wet forests of Upper Amazonia may be the world's richest in tree species," says Gentry. "Indeed, it is hard to imagine a more diverse forest than at Yanamono where there are only twice as many individuals . . . as species in a 1-hectare patch of forest, with 63% of species represented by single individuals and only 15% of species represented by more than two species." The Yanamono site had 283 tree species.

"The new data presented here suggest that, instead of being a striking anomaly, patterns of tree species richness parallel those of birds, butterflies, reptiles and amphibians, and mammals," says Gentry, "with the world's greatest local concentrations of species in the relatively moist and fertile forests near the base of the Andes." Some researchers argue that the exceptionally high species diversity in this area is a consequence of the repeated fragmentation and coalition of communities through the fluctuating ice ages of the last million years, a process that might favor high rates of speciation. In any case, the documentation of high species diversity in these areas has implications for conservation. "The emerging generality that diversity may be uniquely concentrated in upper Amazonia suggests that special focus on preserving remnants of these rapidly disappearing ecosystems is of the utmost conservational important. ■ ROGER LEWIN

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