

The Molecular Basis of Evolution

The discovery that mutations accumulate at steady rates over time in the genes of all lineages of plants and animals has led to new insights into evolution at the molecular and the organismal levels

by Allan C. Wilson

The molecules of life are now the chief source of new insights into the nature of the evolutionary process. For a century the main contributors to knowledge of evolution were biologists working at the level of the whole organism. Together with geologists they established that the millions of kinds of creatures living today descended from a few species that lived more than a billion years ago. They also recognized that biological evolution results from heritable change made possible by mutation and natural selection. Until recently, however, investigators could not probe evolution at its most basic level. They could not directly explore changes occurring in genes.

New techniques in biochemistry have made such investigation possible. In recent decades molecular biologists have been able to compare the genes of thousands of living species and a few extinct species. They have measured the extent of the differences among the genes and studied the nature of the differences. One major result of the analysis is the concept of the molecular clock. Because mutations change the DNA in all lineages of organisms at fairly steady rates over long periods of time, one can establish a clocklike relation between mutation and elapsed time. Investigators have calibrated the clock on the basis of a few precisely dated fossils that yield estimates of the elapsed times since particular groups of living species diverged from common ancestors. Molecular differences can then be used to estimate the dates of divergence for multitudes of other species. Evolutionary biology has begun to acquire a quantitative molecular foundation.

My discussion of the molecular basis of evolution rests on two assumptions: (1) that the heritable differences among organisms result from differences in their DNA's, and (2) that molecular evolutionists must not only

measure differences in DNA but also explain the origin of the differences and their relation to organismal differences. In this article I shall describe some of the discoveries and concepts of molecular evolution, attempt to relate it to organismal evolution and then argue that molecular biology has introduced a new way of analyzing organismal evolution. In particular I maintain that pressure to evolve arises not only from external factors such as environmental change but also from the brain of mammals and birds: from the power to innovate.

Two critical elements of molecular evolution are point mutations (specifically, those occurring in the genes coding for proteins) and regulatory mutations. A point mutation is a single replacement of a DNA base. Such a mutation can affect the amino acid sequence of a protein. A regulatory mutation, on the other hand, is any change in a gene or in the vicinity of a gene that determines whether the gene is active or inactive. The investigation of point mutations has resulted in the conceptualization of the molecular clock and in the discovery of a kind of genetic change known as a neutral mutation: a mutation that is neither advantageous nor disadvantageous for

an organism. Work with point mutations has also yielded many important insights into the branching of lineages of species. The inclusion of regulatory mutations has led to an even more thorough understanding of the link between molecular evolution and organismal evolution.

In examining point mutations molecular biologists ideally would like to compare DNA structures directly. Before such comparisons became possible, however, chemists had discovered how to compare the structure of proteins [see illustration on pages 166 and 167]. There is a simple relation between the sequence of amino acids in a protein and the sequence of bases in the gene that codes for the protein. Specifically, each replacement of an amino acid in a protein can be ascribed to a point mutation in a gene. Investigators have therefore gained insight into molecular evolution by comparing amino acid sequences.

During the course of comparative studies of protein structure, several workers began considering how the number of amino acid replacements might be related to the time that had elapsed since any two species of organisms had a common ancestor. By simply counting the replacements (and thus ignoring their nature and their lo-

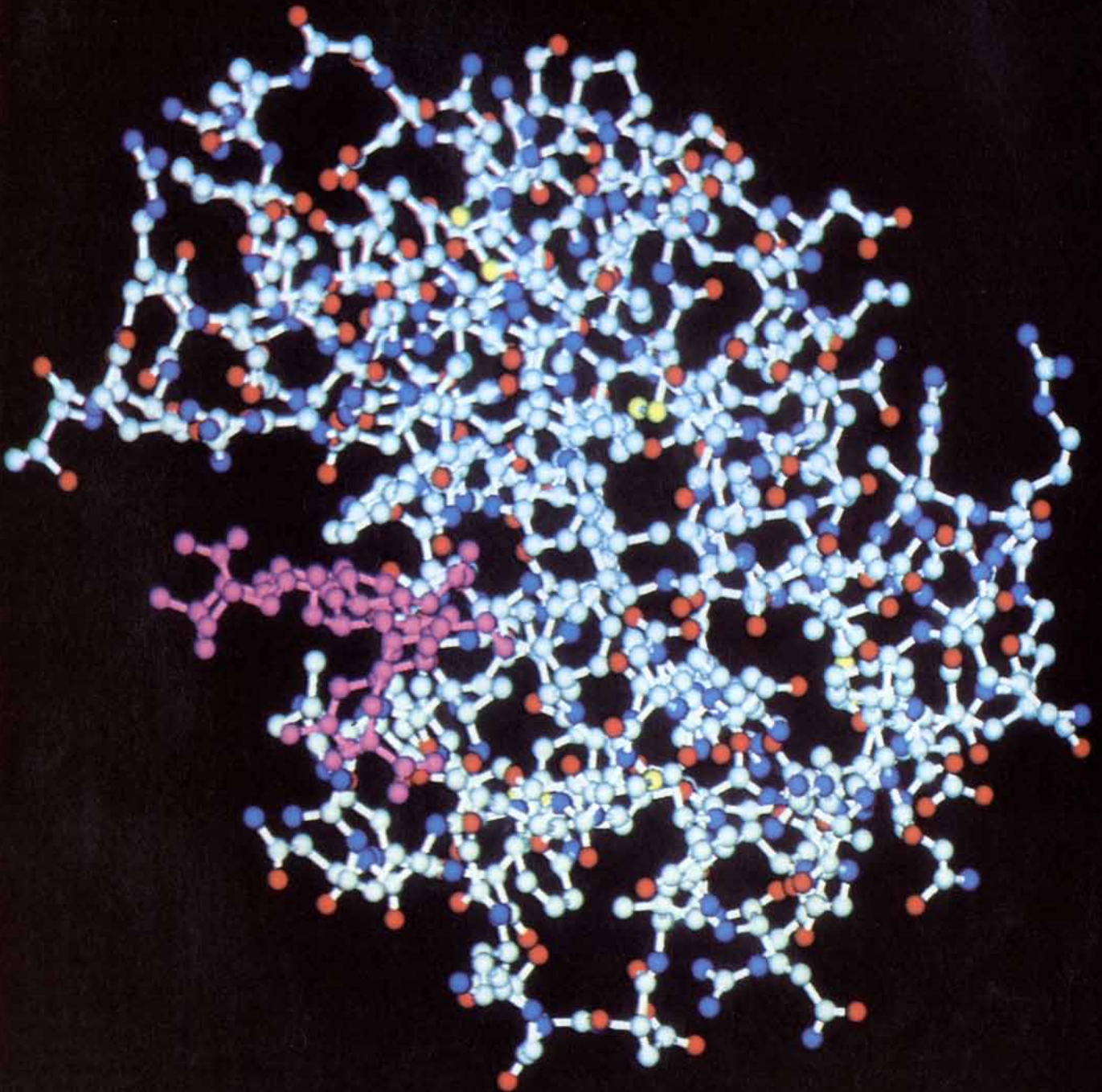
LYSOZYME, the enzyme modeled in the computer image on the opposite page, serves as a measure of regulatory mutations: changes in DNA that determine whether genes are active or inactive. The image depicts a molecule of lysozyme with part of its substrate (purple), the substance on which it acts. The substrate is a sugar polymer found in the cell wall of bacteria. Lysozyme cleaves the polymer and so breaks down the bacterial cell wall. It has therefore been recruited as a major digestive enzyme in ruminant animals (such as cows and sheep) to retrieve nitrogen and phosphorus present in the bacteria in their stomach. (The bacteria function in the digestion of cellulose.) The stomach of most other mammals, on the other hand, contains only a low concentration of lysozyme. The difference in the concentration of the enzyme contrasts with its functional uniformity: lysozymes from all mammals function nearly identically. The difference in concentration is largely attributable to regulatory mutations, which are thought to play an important role in organismal evolution. The computer image was made by the Graphics Systems Research Group of IBM U.K. Limited. The white balls correspond to carbon atoms, the red balls to oxygen, the blue balls to nitrogen and the yellow balls to sulfur; the white sticks represent interatomic bonds.

cation in the protein structure) they discovered that proteins behave like approximate evolutionary clocks. A great deal of evidence points to the fact that amino acid replacements accumulate at fairly steady rates over long periods of evolutionary time. Techniques that allow direct comparison of genes confirm the hypothesis that the steady evolution of proteins is rooted in the steady evolution of DNA. In nucle-

ar DNA and in the DNA of other cellular components (such as mitochondria and chloroplasts), for example, the average accumulation of base replacements is nearly as clocklike as the process of radioactive decay.

The molecular clock, however, does not tick at the same rate at every position along the DNA molecule. The rate of evolution at a site in DNA that

directly affects the function of a protein is slow; it is faster at a position that does not affect such a function. In other words, evolutionary change at the molecular level is slow where there are strong functional constraints and faster where they are weak. The active sites of most enzymes, for instance, evolve slowly compared with many other parts of the enzyme structure. The structures of other proteins also



illustrate the concept of functional constraint. The hemoglobin of horses and that of human beings differ from each other by amino acid replacements at 43 out of 287 positions. In spite of these many differences, the chains of amino acids in these two hemoglobins are shown by crystallographic studies to fold in identical ways. Moreover, the two proteins behave nearly identically in functional tests: the point mutations ascribable to the 43 replacements are subject to weak functional constraints.

Comparisons of codons (triplets of DNA bases, each of which specifies a particular amino acid) provide a third example of functional constraint. The rate of change at the third position of codons is greater than the rate of change at the second position. This observation corresponds to the fact that whereas any base change at the sec-

ond position results in an amino acid substitution, about half of the base changes at the third position do **not** result in a substitution. The functional constraint on evolutionary change at the second position is strong because change occurring there is more likely to affect protein function; the constraint on change at the third position is weak because change can occur there without disrupting the function of proteins.

Observations of the high rate of evolutionary change at weakly constrained DNA positions have encouraged biologists to regard molecular evolution as an accumulation of neutral mutations that do not interfere with protein function. This way of looking at molecular evolution has been uncomfortable for Darwinists accustomed to thinking of evolution as resulting from the accumulation of ad-

vantageous mutations. The reconciliation of the two points of view lies in the fact that even though neutral mutations may dominate molecular evolution, the abundance of genetic variation allows for the accumulation of enough advantageous mutations to enable natural selection to have its effect at the organismal level.

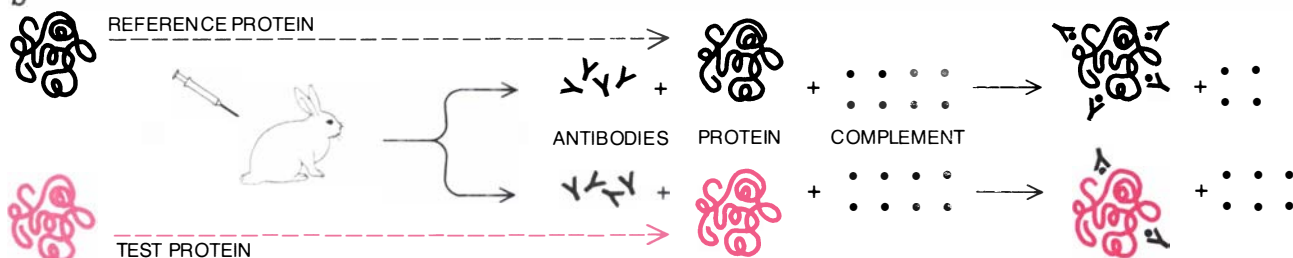
The revolutionary idea that genetic change is dominated by neutral mutations has helped to explain the finding that molecular evolution depends more on years than on generations. If positive selection were driving molecular evolution, one would expect to find higher rates of evolution in short-lived species such as flies or mice than in long-lived species such as the higher primates. Instead base replacements accumulate at about the same rate in coding sequences along both kinds of lineages.

PROTEINS

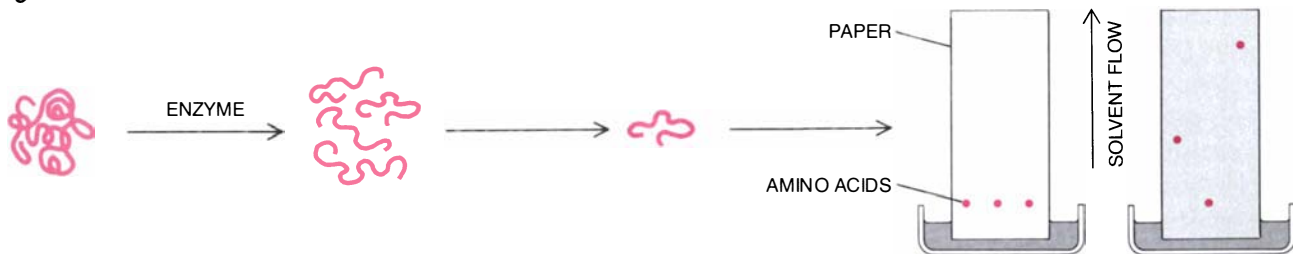
a



b



c



MOLECULAR EVOLUTION is measured by comparing proteins (a-c) or DNA's (d-f). Gel electrophoresis (a) can separate proteins on the basis of charge. Since the charge varies with the amino acid composition of a protein, the technique serves as a measure of the extent to which that composition varies in different versions of a protein. The method is most valuable when the electrophoretic mobilities of a set of 30 or more kinds of proteins from one individual are compared with the mobilities of the corresponding proteins from another individual. Microcomplement fixation (b) relies on the ability of antibodies to detect small differences between pro-

teins. Antibodies made by immunizing rabbits against a pure protein are tested in the presence of complement (a mixture of substances in blood) for their ability to bind with the immunizing protein and with related proteins. Complement interacts only with antibody bound to a protein antigen; the disappearance of complement measures the amount of antibody-antigen complex formed and therefore indicates differences in the proteins. In chemical sequencing (c) a purified protein is fragmented by an enzyme. The amino acids of each fragment are cleaved sequentially, beginning at one end of the fragment, and are identified by chromatography, a process in which

Nevertheless, many biologists who make mathematical models of the evolutionary process are coming to believe many of the mutations accumulated during molecular evolution are not neutral. They argue that instead of proceeding smoothly, molecular evolution might be characterized by long periods of inactivity punctuated by bursts of change. If they are right, the challenge of finding an explanation for the molecular-clock phenomenon grows. The explanation of the phenomenon may entail a deeper grasp of the nature of the evolutionary process.

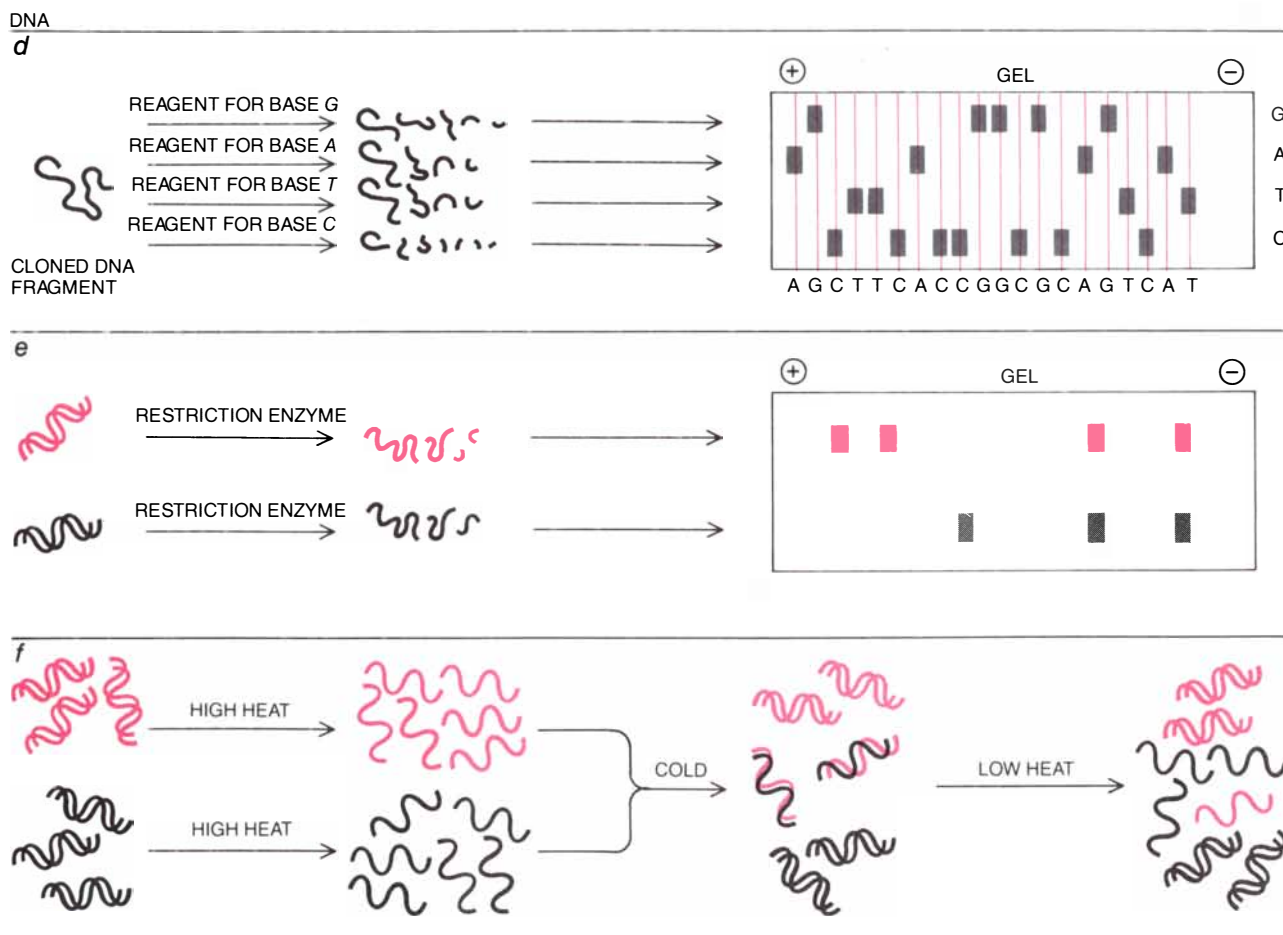
On one point all molecular biologists agree: changes in the sequence of DNA's and the proteins they encode are mainly divergent. Investigators can therefore construct molecular trees, or branching diagrams, showing the genealogical relations among

these sequences. Such diagrams help one to think clearly and quantitatively about how present-day sequences evolved from a common ancestral sequence. Molecular trees also illuminate the genealogical pathway by which the species containing the sequences evolved from a common ancestral species. The order of lineage branching leading to modern species provides a valuable framework in which to organize knowledge of the differences among species.

To choose among alternative genealogical hypotheses molecular biologists follow the principle of Occam's razor: the simplest of competing theories is selected over more complex ones. The tree is chosen that requires the fewest mutations to explain the evolution of particular sequences from a common ancestral sequence. This approach allows molecular evolutionists

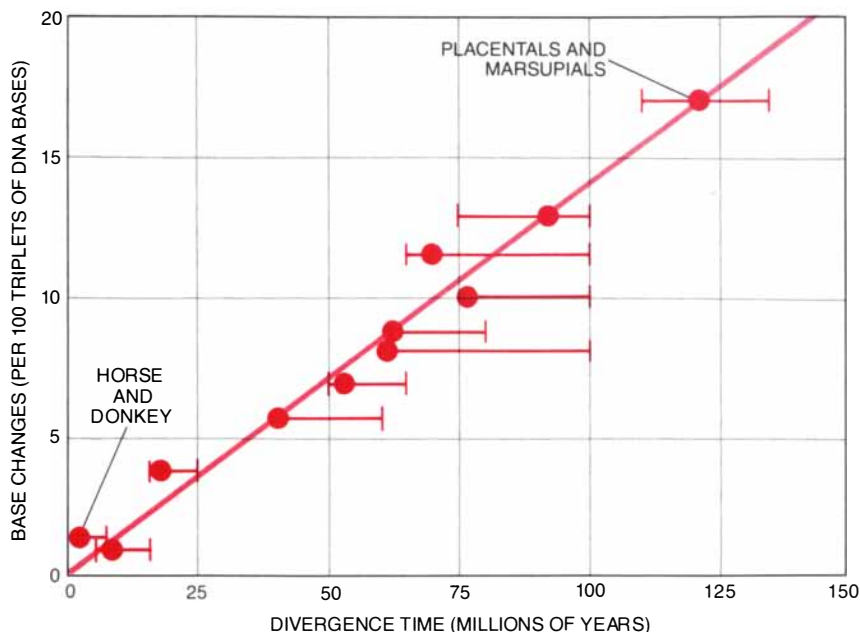
to choose objectively and quantitatively among alternative trees. How, for example, are human beings related to orangutans and African apes (chimpanzees and gorillas)? A branching diagram linking human beings most closely to African apes explains the molecular data by postulating fewer mutations than are required for diagrams linking humans most closely to orangutans. In other words, one diagram explains the observed sequence diversity so much more simply than others do that the complicated ones can be ruled out statistically.

The capacity to make such determinations is one of the notable achievements of molecular evolutionary biology. Previously investigators had based trees exclusively on differences in anatomical traits. The comparison of such traits is highly subjective. In addition the workers had no way of

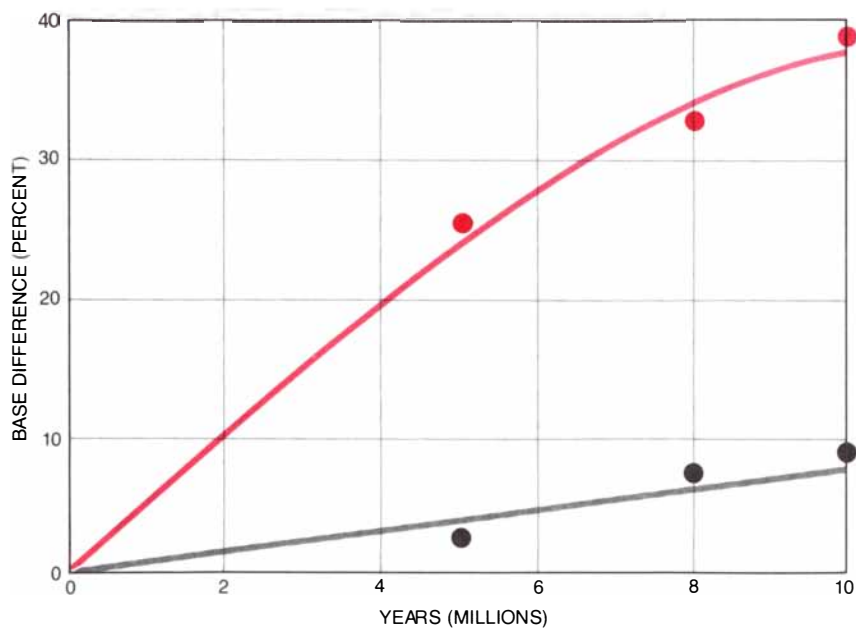


the migration of amino acids depends on size and charge. Two cloned DNA's can be compared in detail by sequencing (d). A piece of DNA, to which a radioactive label has been attached at one end, is cleaved by a reagent specific for one of the four DNA bases (G, A, T, C) under conditions such that each molecule is on the average cleaved at only one of the susceptible sites. The DNA sequence (AGCTTCAACGGCGCAGTCAT in this case) is inferred by reading the distances the cleaved fragments move through a gel under the influence of an electric field. A faster but less accurate method for comparing DNA's is restriction analysis (e). A piece of DNA

is fragmented by a set of restriction enzymes, each of which recognizes and cleaves a specific sequence of from four to six bases. Differences in the sequences affect the size of the fragments, so that the pattern of fragments of two DNA's subject to cleavage and electrophoresis reflects their degree of similarity. In DNA hybridization (f) the double helix of analogous DNA's from two sources is disrupted by heating. When the two sets of single strands are cooled together, hybrid duplexes consisting of a strand from each of the DNA's can form. The stability of the hybrids to heat is a measure of the degree of sequence similarity between the DNA's.



CLOCKLIKE EVOLUTION is shown for the genes of mammals whose times of divergence are known approximately from fossil evidence. The amino acid sequence for each of seven proteins was determined for 11 pairs of mammals or mammalian groups and the number of amino acid differences between the two members of each pair was calculated. The number of point mutations, or replacements of individual DNA bases, required to account for those differences was estimated, and it is indicated on the vertical axis of the graph. The horizontal axis indicates how long ago the particular lineages of each member of a pair diverged from each other. The most distantly related mammalian groups compared are placentals and marsupials, whose common ancestor lived about 120 million years ago. The most closely related pair are the horse and the donkey. The bars indicate the uncertainty in the estimates of divergence time. The curve shows that DNA-base replacements accumulate at fairly steady, or clocklike, rates over long periods of evolutionary time.



FUNCTIONAL CONSTRAINT is illustrated by comparing the rate of change at the second position of codons (black curve) to the rate of change at the third position (colored curve). A codon is a triplet of DNA bases that encodes a particular amino acid. Change takes place more rapidly at the third position than at the second one. The reason is that whereas any base change at the second position results in an amino acid substitution, about half of the base changes at the third position do not result in a substitution. The data are from comparisons made of the DNA of mitochondrial (cellular organelles) of apes and humans. In broader terms, the rate of evolution at a site in a gene that directly affects the function of a protein is slow; it is faster at a position that does not affect any such function.

knowing the number of mutations necessary to produce an observable difference in a trait. They also could not know whether a mutation giving rise to a difference in one anatomical trait also contributes to differences in other anatomical traits. Molecular trees built from sequence data require no subjective decisions about traits. Moreover, biologists know the minimum number of base replacements needed to account for the sequence differences. Finally, the number of countable genetic traits revealed by comparison of DNA and protein sequences has begun to exceed the number of anatomical traits available for tree analysis.

In addition to disclosing the order of lineage branching, molecular trees contain information about times of divergence among lineages. The first application of this approach to evolutionary dating involved estimating when hominoids such as human beings and African apes diverged from a common ancestor. Working in my laboratory at the University of California at Berkeley, Vincent M. Sarich measured the structural differences of serum albumin, a protein found in both humans and African apes. He determined the mean rate of evolutionary change by comparing the albumins of species whose divergence times were known from fossil evidence. He was thus able to calculate that humans and African apes diverged five million years ago. This was only a fraction of the time postulated by anthropologists: from 20 to 30 million years. Subsequent DNA studies have confirmed Sarich's work, leading to a reinterpretation of the fossil record and a revision in thinking about the pathway of evolution from ape to man.

Molecular trees have yielded many other insights into the genealogical links between species. Trees based on fast-evolving DNA positions link species that diverged rather recently (such as the hominoids). Whereas these positions facilitate the exploration of the twigs of the evolutionary tree, highly conserved positions allow the probing of the deepest branches. Genes containing many highly conserved positions reveal four primary branches of descent. The branches diverged from one another nearly three billion years ago, when all cells were at the bacterial level of organization. The pattern of branching offers new insights into the sequence of steps characterizing the evolution of metabolism in early cells.

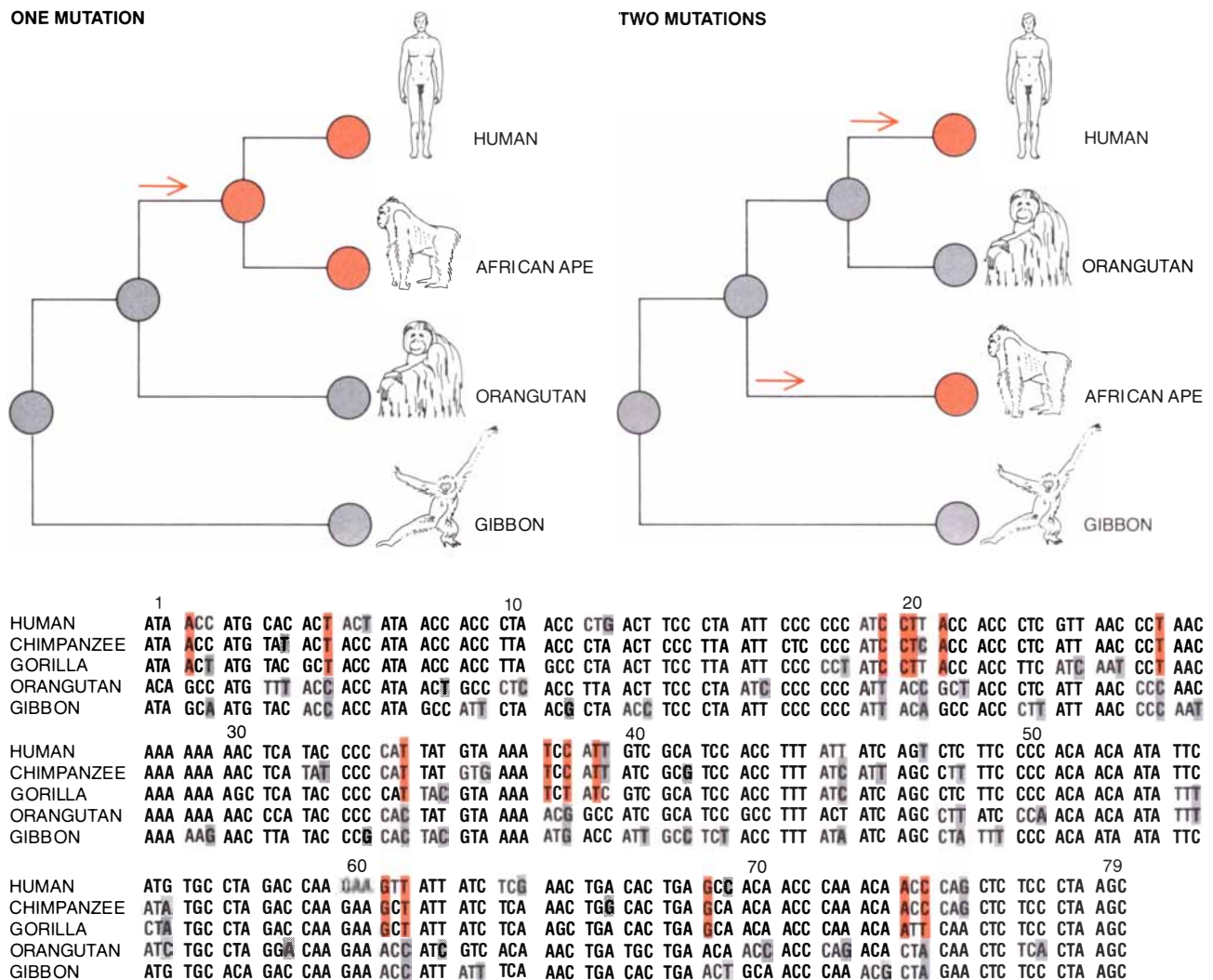
Tree analysis has also supported the theory that eukaryotic cells (the nucleated cells of organisms higher than

bacteria) arose by the fusion of two or more types of bacterial cells about a billion years ago. Eukaryotic cells contain DNA in distinct compartments: the nucleus, the mitochondrion and, in the case of photosynthetic cells, the chloroplast. The genome of each compartment includes a set of very conservative genes specifying the structure of RNA molecules in the ribosomes (the organelles on which proteins are assembled) of that compartment. Sequence comparisons show that whereas the ribosomal RNA genes in the nucleus stem from one of the four primary branches in the bacterial tree, those in the chloroplast and the mitochondrion stem from another.

Tree analysis has also helped to explain how the nuclear genome of eukaryotic cells has grown hundreds of times bigger than the bacterial genome. The pattern of genealogical relations among genes and other repetitive sequences within the nucleus offers clues about the steps involved in the process. These steps include the duplication of entire genes and their movement to new locations in the genome. The duplicate genes usually diverge independently, either acquiring new functions or becoming inactive pseudogenes: duplicate stretches of DNA that contain mutations preventing them from encoding a functional polypeptide, or short protein

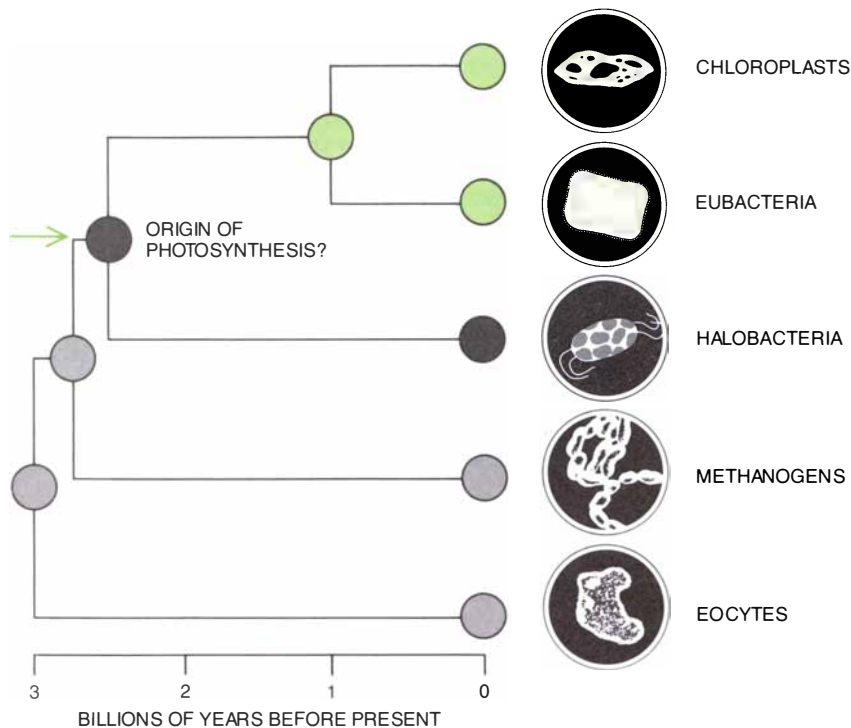
chain. In other cases the duplicated DNA's communicate (exchange genetic information) with one another at varying rates as they evolve.

In addition tree analysis has contributed to knowledge of the evolutionary role of gene transfer between species that do not interbreed. Some viruses and plasmids (small circles of bacterial DNA) can transfer cellular genes from one species to another, but the stable integration of such genes from one species into the genome of another species is rare in nature. If it were common, the genome of each species would be a mosaic made up chiefly of horizontally transferred contributions from diverse species. In that case at-

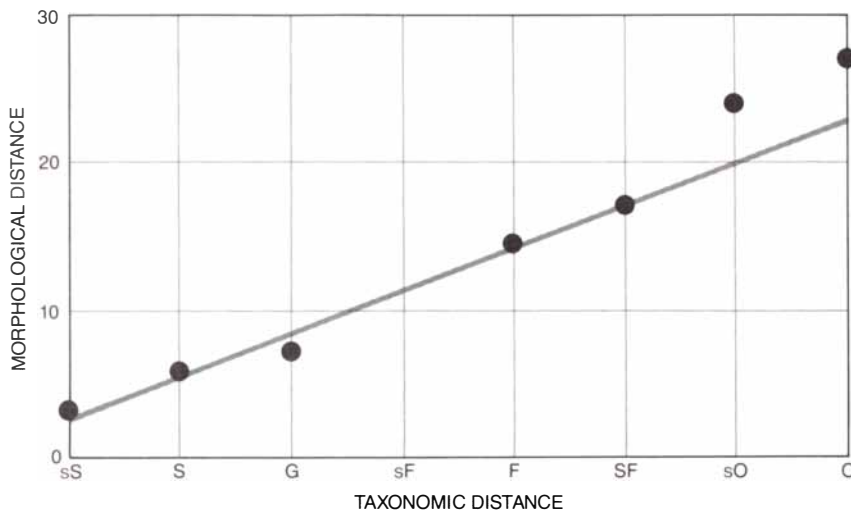


BRANCHING DIAGRAMS, or phylogenetic trees (*top*), showing the descent of the hominoids can be constructed on the basis of DNA sequences such as those at the bottom. Colored disks in the diagrams show the presence of a particular base (or amino acid) at a given position in a DNA (or protein) sequence for both humans and African apes (chimpanzees and gorillas). Gray disks show that a different base (or amino acid) is present at that position in orangutans and gibbons (Asian apes). The diagram at the upper left accounts for the sequence differences among the hominoid lineages with one mutation on the lineage leading to the common ancestor

of humans and African apes (*arrow*). The diagram at the upper right, in contrast, requires two mutations (*arrows*) to account for the data; it is less likely to be correct. The order in which humans diverged from chimpanzees and gorillas is still in dispute. The 79 codons shown in the bottom section of the illustration code, in the various hominoids, for part of a protein (NAD dehydrogenase 5) that functions in energy production within the mitochondrion. The sequences differ mostly by base replacements at third positions of codons (*gray panels*). The 16 colored panels indicate positions at which the African ape sequence resembles the human sequence.



EVOLUTION OF EARLY CELLS began nearly three billion years ago and led to the emergence of chloroplasts (organelles in which photosynthesis takes place) and four main groups of bacteria: eubacteria (the major current form) and halobacteria, methanogens and eocytes (sulfur bacteria). Chloroplasts share with many eubacteria the capacity for photosynthesis based on chlorophyll and carotenoids. Halobacteria also exhibit a type of photosynthesis based on a carotenoid. Photosynthesis probably originated in the common ancestor (arrow) of chloroplasts, eubacteria and halobacteria. The phylogenetic tree was inferred from comparing the base sequence of ribosomal RNA found in the various organisms.



MORPHOLOGICAL DISTANCE, a measure of the extent to which animals differ in body plan, correlates with distance in taxonomic classification. The graph summarizes the results of more than 20,000 anatomical measurements on nearly 400 species and suggests that the accumulation of point mutations cannot explain the accelerated rate of organismal evolution in mammals. To estimate the morphological distance between two animals a standard set of bones from the head, forelimb, trunk and hindlimb of each animal is measured. The relative length of each trait is then calculated by dividing the length of that trait by the sum of the lengths of all traits measured in that animal. The morphological distance between two animals is the sum of the absolute values of the differences in the relative lengths for all traits. The smallest taxonomic distances in the classification are those between subspecies (sS), followed by those between species (S), genera (G), subfamilies (sF), families (F), superfamilies (SF) and suborders (sO). The largest taxonomic distances shown are for different orders (O). The points represent the mean morphological distances among birds. The line was fitted to analogous morphological-distance values for frogs, lizards and mammals.

tempts to build a tree for a set of species would prove futile; a tree based on one particular gene would probably disagree with a tree based on another gene. In practice, however, trees based on several different genes usually agree with one another. Most purported cases of horizontal transfer do not receive support from tree analysis. In both the bacterial and the eukaryotic worlds the predominant mode of evolution has been vertical: from parent to offspring.

Although the investigation of point mutations has increased understanding of evolutionary processes, it has failed to describe completely the link between molecular and organismal evolution. The sharp difference in the rates of organismal evolution for two groups of species, frogs and mammals (such as cats, bats, whales and humans), for instance, does not reflect the similarity in the rates at which point mutations accumulate for both groups. Frogs are an ancient group of animals consisting of thousands of species. Yet they share so many anatomical similarities that zoologists classify all frogs in one order. Indeed, during the period that saw the rise of cats, bats, whales and humans from a common ancestor, one type of frog evolved so slowly that both fossils 90 million years old and the present-day representatives of its lineage are classified in the same genus, *Xenopus*. Placental mammals, on the other hand, even though they represent a younger group, differ so much from one another that zoologists classify them in 16 orders.

Facts such as these indicate that the pace of organismal change in mammals has been much faster than it has been in frogs. Yet point mutations accumulate in the DNA of mammals at the same rate as they do in frogs. Similar contrasts between the rate at which point mutations accumulate and the rate of organismal evolution characterize many other groups.

The argument that there is a contrast between the rate of accumulation of point mutations and the rate of organismal evolution rests on the supposition that taxonomic classifications summarize, without bias, information about the degrees of anatomical similarity among species. To assess the validity of this assumption, Lorraine M. Cherry, then at the University of California at Berkeley, and Susan M. Case of Harvard University collaborated with me in developing a quantitative and objective way of estimating the degree to which species differ in body plan [see illustration at bottom left]. The results from our method agree

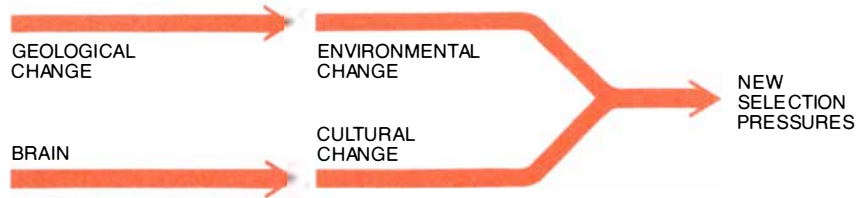
with those obtained from traditional taxonomic methods.

The work of Cherry and Case lends quantitative support to the notion that the accumulation of point mutations cannot explain the accelerated rate of organismal evolution in mammals. The recognition of this discrepancy has led molecular biologists to ask two questions: What relation exists between molecular evolution and evolution at higher levels of organismal organization? What makes mammals evolve so fast at these higher levels?

One possible answer to the first question is that the majority of point mutations accumulating in nucleic acids and in the proteins they encode may be neutral or nearly so from the standpoint of natural selection. Only a minority may underlie adaptive evolution at the organismal level. The fraction of accumulated mutations having adaptive significance could be higher for mammals than for frogs but still too low to contribute significantly to the overall rate of molecular evolution in mammals.

In all likelihood, however, it is the regulatory mutation that establishes the link between molecular evolution and organismal evolution. A regulatory mutation is any mutation that affects the expression of a gene: particularly the turning on or off of specific genes in the course of development. In particular, attention has been paid to the idea that most adaptive evolution at the organismal level is due to mutations affecting the relative concentrations of specific proteins rather than to mutations affecting their structures.

To test these ideas one needs a strategy for picking genes with which to link molecular change to organismal change. Until the molecular basis of embryonic development is better understood, it does not seem profitable to search for those genes whose differences account for the anatomical differences between species of multicellular organisms. The best strategy at present is to work at the chemical interface between organism and environment. That is why investigators in my laboratory such as Deborah E. Dobson, Caro-Beth Stewart, R. Tyler White, Michael S. Hammer and Ellen M. Prager have probed genes coding for enzymes in the mammalian gut. The biochemistry and digestive physiology of mammals are well-developed subjects. Mammalian species diverge quickly from one another with respect to their diets. Biochemists can often guess which enzymes are necessary to cope with a chemical present in one diet but not in another. Genes coding for such enzymes therefore hold a key



PRESSURE TO EVOLVE, the author argues, comes both from geologic forces such as erosion and mountain building and from the brain of mammals and birds. By suddenly exploiting the environment in a new way, a relatively big-brained species quickly subjects itself to new selection pressures that foster the “fixation” of mutations complementary to a new habit. A mutation is said to be fixed in a population when descendants that bear the mutant gene predominate greatly over those individuals that bear the original gene.

to understanding the relation between molecular and organismal evolution.

The investigation of bacteria-digesting enzymes confirms the significance of regulatory mutations. Although most mammals are not enzymically equipped to digest bacteria, on several occasions during mammalian evolution species have acquired the necessary enzymes. Ruminant animals such as cows and sheep, for example, need to digest bacteria in order to retrieve nitrogen and phosphorus that has been captured by the microorganisms. (The bacteria function in the digestion of cellulose.) The enhanced ability to digest bacteria is due to the presence of the enzyme lysozyme, which cuts open the cell wall of bacteria. Ruminant stomachs contain high concentrations of lysozyme, whereas most other mammalian stomachs contain low concentrations of the enzyme. Lysozyme has evidently been recruited as a major digestive enzyme in ruminants.

Although the recruitment of lysozyme depends on both regulatory mutations and structural mutations, regulatory change appears to have had the primary role. A similar picture emerges from studies of evolution in the test tube. The net conclusion from many experimental studies of evolution with both bacterial and animal cells in culture is that regulatory mutations may play a primary role in adaptive evolution.

The specific kind of regulatory mutation, however, remains unknown for many evolutionary processes. Although gene duplications and point mutations in regulatory DNA are responsible for most of the altered rates of protein synthesis observed in laboratory experiments, for instance, they may not account for the changing lysozyme levels in mammalian evolution. Because the lysozyme changes are tissue-specific, enhancers (regulatory DNA sequences recognized by factors specific to a given tissue) may prove to be responsible for controlling the levels of the enzyme. The tissue-specific activation of a gene can be accom-

plished by moving an enhancer into any one of a variety of noncoding positions within or near the gene. It remains to be seen whether the recruitment of lysozyme depends on enhancers and whether the lysozyme case typifies that of other genes taking part in major adaptive shifts.

The final question I address is why mammals evolve so fast at the organismal level. I maintain that the high rate of evolution for mammals with respect to that for frogs may be due to the large brain of mammals. A large brain generates an internal pressure to evolve that frogs lack. In reaching this conclusion I assume that organismal evolution is a Darwinian process driven by selection and therefore has two components: mutation and fixation. In other words, although a newly arisen mutation is initially present in a single individual within a population, the mutation has not been “fixed” until descendants bearing the mutant gene predominate greatly over individuals bearing the original type of gene. Quantitatively, the basic equation of evolution states that the rate of evolution within a population equals the number of mutations arising per unit of time multiplied by the fraction of those mutations destined to be fixed.

The high rate of mammalian evolution might therefore be attributed to either a large number of mutations or a large fraction of fixation, or to both. Even though the mammalian genome may indeed be more prone to mutation, or more unstable, than the genomes of “living fossils” (such as *Xenopus*) are, a large fraction of fixation seems more likely to account for the trend. In particular I consider the following possibility: the number of mutations arising per unit of time is the same for frogs and mammals, but the fraction of those mutations that are fixed is higher for mammals than it is for frogs. This would mean that mammals fix a larger fraction of their morphological mutations than frogs.

The opportunity to fix advantageous

mutations arises whenever the direction of selection changes. There are two basic sources for change in the direction of selection, which is to say there are two basic pressures to evolve adaptively. One comes from outside a species and the other comes from inside. Evolutionary biologists have tended to think only about the external factors, such as environmental change, which is largely driven by such geologic forces as erosion and mountain formation. The second pressure to evolve comes from the brain of mammals and birds. This internal pressure, a conse-

quence of the power of the brain to innovate and imitate, leads to culturally driven evolution.

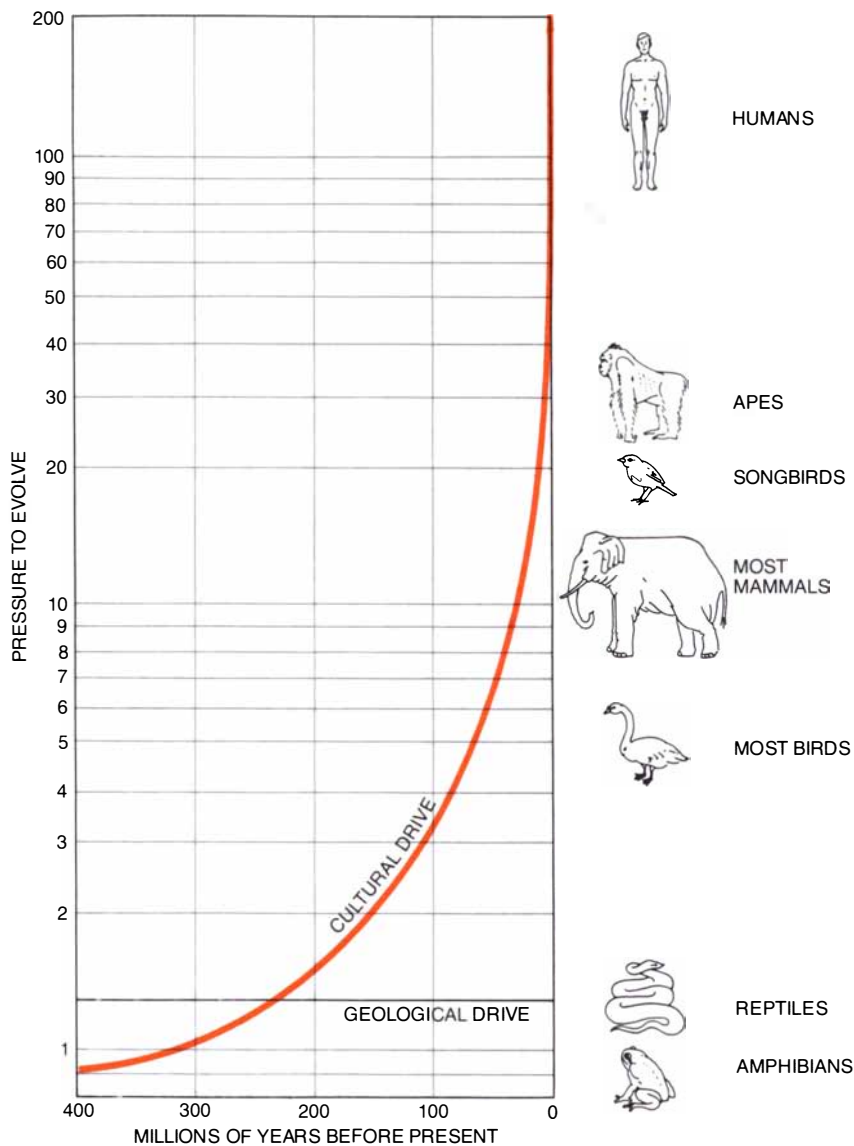
Once a species has a dual capacity to evolve, a new way of exploiting the environment can arise in a single individual and spread rapidly to other individuals by imitative learning. By suddenly exploiting the environment in a new way, a big-brained species quickly subjects itself to new selection pressures that foster the fixation of mutations complementary to the new habit. The larger the population is to which the new habit is communicated,

the more likely it is that such a mutation will already be available, or will arise, so that selection can act on it. The time required for a population to fix a mutation that complements a new behavior is shorter if the new behavior spreads quickly not only to offspring (vertically) but also to other members of the population (horizontally).

The lineage leading to the human species has been under the highest internal pressure to evolve. The rise of agriculture, for instance, imposed new selection pressures that led to swift genetic changes in human populations. Consider the introduction of milk sugar (lactose) into the diet of adults as the result of the invention and social propagation of dairy farming. The genetic capacity of adults to digest this sugar has evolved only within populations dependent on dairy products. In the short period of 5,000 years genes conferring the ability to handle milk sugar as an adult reached a level of 90 percent in populations that depended heavily on dairy farming. In contrast, the level of the genes is virtually zero in human populations that do not drink milk and in all other mammalian species tested.

The potential for culturally driven evolution is by no means confined to humans. Imitative learning occurs in many species having brains that are relatively large in relation to body size, such as primates and songbirds. Imitative learning may also occur in some fishes, squids and insects, although it has not yet been demonstrated in them. The most celebrated case of a rapid shift in nonhuman behavior was provided by songbirds known as British tits. Some of these songbirds, which resemble American chickadees, learned how to open milk bottles. Soon they were imitated by millions of other tits. Within a couple of decades most of these British songbirds were engaging in the practice. Finally human beings stopped the evolutionary experiment: they put the bottles in crates. Biologists did not have the opportunity to ascertain whether or not the songbird population responded genetically to the new selection pressures generated by their new behavior.

My work with Jeff S. Wyles of Berkeley and Joseph G. Kunkel of the University of Massachusetts at Amherst supports the hypothesis that the brain of mammals and birds is the major driving force behind their organismal evolution. The investigators found that the larger the size of the brain in relation to the size of the body, the higher the mean rate of anatomical evolution. During the evolution of vertebrates on land, the relative size of the



INCREASE IN BRAIN SIZE with respect to body size supports the theory that high selection pressure for mammals comes from the brain. A dramatic rise in the pressure to evolve at the organismal level is postulated to have occurred in the lineages leading from early amphibians to present-day species of birds and mammals. The vertical axis represents a measure of pressure to evolve. The curve for cultural drive was determined by dividing the mass of a species' brain (in grams) by the total mass of its body (in kilograms) raised to the two-thirds power. The level of the line plotting geologic drive is based on the assumption that pressure coming from geologic change has not undergone a net increase in the past 400 million years. Because humans, apes and songbirds have relatively large brains, they are under higher pressure to evolve than most mammals, birds, reptiles and amphibians.



BRITISH TIT, a songbird, perches on a milk bottle after pecking open the foil cap. In the 1930's and 1940's the practice of opening milk bottles spread throughout the tit population of Britain, providing the most celebrated case of a cultural shift known in nonhumans. Human beings finally stopped the practice by putting the milk bottles in crates; biologists did not have an opportunity to learn whether or not the songbird population would have responded genetically to the new selection pressures that were generated by their behavior.

brain has increased by a factor of 100 along the lineage leading from the first amphibians to humans. Furthermore, the rate of increase in relative size has accelerated. The lineages leading from those same early amphibians to other mammals and to birds exhibit a similar but less pronounced tendency for the relative size of the brain to increase over time. In contrast, the relative brain size of modern frogs and salamanders does not differ significantly from the relative brain size of the first amphibians.

Since the rate of organismal evolution correlates with relative brain size, its rate may also have risen by a factor of nearly 100 along the lineage leading to humans and by smaller factors along the lineages leading to other big-brained creatures. Organismal evolution in the vertebrates may provide an example of an autocatalytic process mediated by the brain: the bigger the brain, the greater the power of the species to evolve biologically. When cultural evolution becomes extremely

fast, however, such a process presumably does not keep accelerating. In such a case the pressures generated by one cultural shift will sometimes be relieved by the next cultural shift, rather than by a genetic response. This has probably been true of the human species for some 35,000 years, when the human brain reached its present size.

The study of molecular evolution occupies a special position in contemporary biology. In trying to link gene to organism, it touches molecular biology, cell biology, developmental biology, physiology, anatomy and behavioral biology. It also requires an understanding of how genes behave in populations, and the disciplines of taxonomy, paleontology and geology are involved. No other field touches all these aspects of biology and geology. The study of molecular evolution provides an opportunity to build bridges between biological disciplines and by so doing contribute to the unification of the life sciences.

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