8. S. P. Worden, talk at the Harvard College Observatory, Cambridge, MA, 2 April 1984.
9. H. A. Bethe *et al.*, *Sci. Am.* **251**, 39 (October 1984).
10. *Aviat. Week Space Technol.*, 2 July 1984, p. 54.
11. C. R. O'Dell, in *The Space Telescope Observatory*, Special Session of Commission 44, International Astronomical Union 18th General Assembly, Patras, Greece, August 1982 (NASA CP-2244, Space Telescope Science Institute, Baltimore, MD, 1982), p. 7; R. Giacconi, *ibid.*, p. 23.
12. A. B. Carter, "Directed energy missile defense in space," background paper for the Office of Technology Assessment of the U.S. Congress, Washington, DC, April 1984.
13. Martin Marietta Denver Aerospace, "Short burn-time ICBM characteristics and considerations" (Denver, CO, 20 July 1983).
14. C. A. Robinson, Jr., *Aviat. Week Space Technol.*, 24 October 1983, p. 50.
15. *Aviat. Week Space Technol.*, 12 March 1984, p. 31.
16. G. B. Lubkin, *Phys. Today* **37**, 17 (January 1984).
17. J. K. Beatty, *Sky Telesc.* **66**, 189 (1983).
18. J. J. McRoberts, *Space Telescope* (NASA EP-166, Government Printing Office, Washington, DC, 1982), pp. 15 and 18.
19. R. J. Weymann and N. P. Carleton, *Sky Telesc.* **44**, 159 (1972).
20. J. E. Nelson, in *International Conference on Advanced Technology Optical Telescopes*, G. Burbidge and L. D. Barr, Eds. (Society of Photo-Optical Instrumentation Engineers, Bellingham, WA, 1982), p. 109.
21. A. Meinel and M. Meinel, in *Optical and Infrared Telescopes for the 1990's*, A. Hewitt, Ed. (Kitt Peak National Observatory, Tucson, AZ, 1980), vol. 1, p. 95.
22. L. D. Barr *et al.*, in *Advanced Technology Telescopes II*, L. D. Barr and B. Mack, Eds. (Society of Photo-Optical Instrumentation Engineers, Bellingham, WA, 1983), p. 37.
23. R. Teeter and M. Kuhner, *Final Report on the OSS Explorer Program, I: Study Results* (Battelle Columbus Laboratories, Columbus, OH, 1981).
24. J. D. Kiesling *et al.*, *Comsat Tech. Rev.* **2**, 73 (1972).
25. W. L. Pritchard, *Acta Astron.* **7**, 373 (1980).
26. R. H. Hayes and S. C. Wheelwright, *Restoring Our Competitive Edge: Competing Through Manufacturing* (Wiley, New York, 1984), chap. 8.
27. P. Ghemawat, *Harvard Bus. Rev.*, March–April 1985, p. 143.
28. "Department of Defense Appropriations for 1982: Hearings before a subcommittee of the House Committee on Appropriations," 97th Congress, first session, part 9 (Government Printing Office, Washington, DC, 1981), p. 264.

# Rates of DNA Sequence Evolution Differ Between Taxonomic Groups

ROY J. BRITTEN

**The mutation rates of DNA sequences during evolution can be estimated from interspecies DNA sequence differences by assaying changes that have little or no effect on the phenotype (neutral mutations). Examination of available measurements shows that rates of DNA change of different phylogenetic groups differ by a factor of 5. The slowest rates are observed for higher primates and some bird lineages, while faster rates are seen in rodents, sea urchins, and drosophila. The rate of DNA sequence change has decreased markedly during primate evolution. The contrast in rates of DNA sequence change is probably due to evolutionary variation and selection of biochemical mechanisms such as DNA replication or repair.**

THE EVENTS OF SPECIATION AND THE TIMES AT WHICH THEY have occurred are of central interest in the study of evolution. Clear molecular evidence of systematic relationship is valuable both for the identification of these events and for interpolation of dates where the fossil record is incomplete. For example, the determination of DNA sequences of homologous regions for a series of species should disclose many nucleotide substitutions and rearrangements, and the pattern of occurrences can be used to establish the relatedness of the species. Even closely related species, such as man and chimpanzee, differ by almost 2 percent in their nuclear DNA sequences (*1–3*), and thus there are about 60 million sequence differences, most of which have little or no effect on the phenotypes. Human individuals probably differ from each other at as many as 5 million sites (*4*), and new genomic differences appear by the hundreds with every birth (*5*). The rate of occurrence, fate, and significance of these DNA mutations are of interest. As more sequences are measured and compared the differences should resolve questions regarding speciation and the process of evolution.

The constancy of the rate of DNA sequence change requires examination in order to make full use of the measurements and determine how many time calibrations are needed. In this article, many measurements of DNA sequence differences spanning the period since the mammalian radiation are examined. Although good time calibrations are difficult to find and the individual dates are relatively imprecise, clear conclusions can be drawn.

DNA sequence changes (substitutions, insertions, deletions, and rearrangements) are the likely source of phenotypic variation in evolution since they can affect genes or their regulation and influence biochemistry, development, morphology, and behavior. However, the majority of changes appear to be neutral; that is, they have little or no effect on the phenotype. The mutation rate (underlying or basal rate of DNA sequence change) may be estimated from the interspecies DNA sequence differences that result from the fixation of neutral changes in the genomes of different species.

## Interspecies DNA Divergence

The number of interspecies comparisons of primary DNA sequences is rapidly growing but is still severely limited. Most of the comparisons are for gene regions in which only a small number of neutral substitutions can be identified, and the statistical uncertainty is large. However, there is a fair number of interspecies DNA hybridization measurements, and (as shown below) the two methods give closely similar results. The combination of the results of both methods is required for a full view of the pattern of interspecies

Roy J. Britten is Distinguished Carnegie Senior Research Associate in Biology, Division of Biology, California Institute of Technology, 101 Dahlia, Corona Del Mar, CA 92625.

differences. In the hybridization method, average or median DNA sequence differences can be estimated from the thermal stability of interspecies DNA-DNA duplexes formed in vitro between radioactively labeled single-copy DNA and an excess of unlabeled DNA from different species (*1, 2, 6*). Various calibrations show that a 1 percent sequence difference reduces the melting temperature by about 1 Celsius degree (*7, 8*).

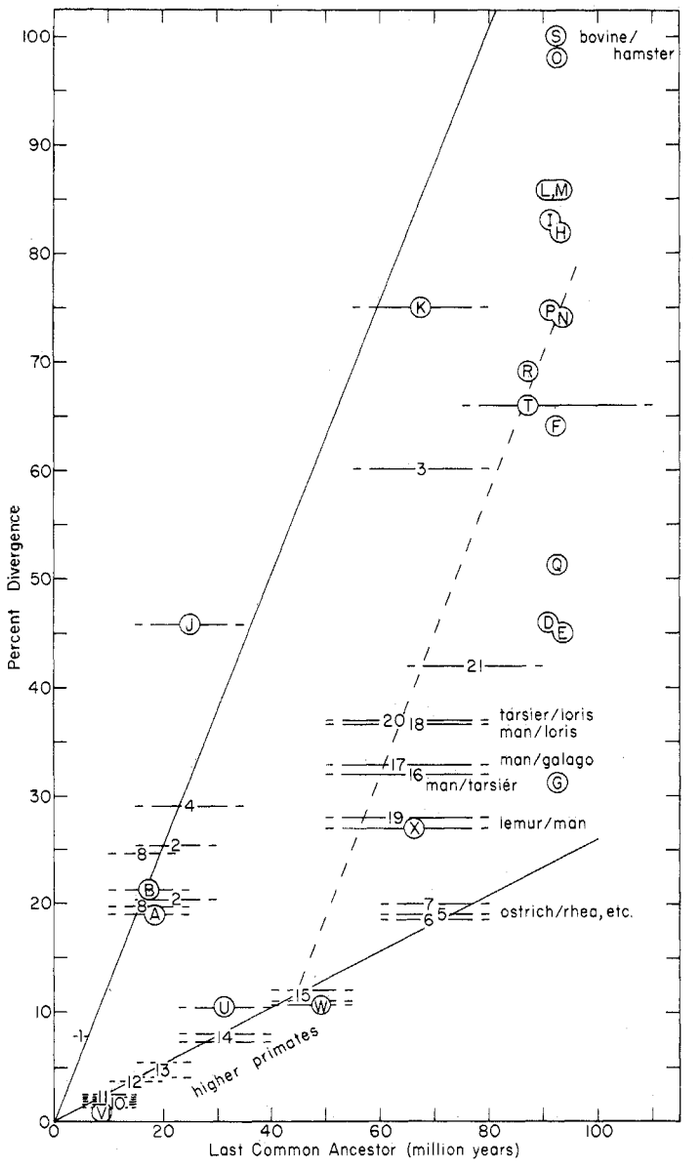


Fig. 1. Fraction of nucleotides substituted as a function of time since last common ancestor, from Tables 1 and 2, corrected for the probability of multiple changes at the same nucleotide. Circled letters are averaged divergences based on primary sequence comparisons for silent substitutions in coding regions. Numbers are thermal stability measurements of interspecies hybrids of single copy DNA, expressed as median divergence [T5OR of (*1, 2*)], where median has its customary meaning, that is, 50 percent of the single-copy DNA has less than the median divergence in an interspecies comparison. The median divergence allows for the reduced formation of interspecies DNA hybrids as well as the reduction in thermal stability of the hybrids that do form, and is calculated from the temperature at which 50 percent of the labeled DNA remains in duplex. Where both measurements have been made, the average divergence for silent substitutions in coding regions is very similar to the median divergence based on thermal stability, probably because both measure neutral drift of DNA sequences. The upper line represents the average of the divergences observed for sequence and thermal stability measurements for sea urchin, drosophila, and rodent comparisons. The lower line is the average of the higher primate comparisons and bird comparisons (*1*).

The disadvantage of the hybridization data compared to primary sequence information is that specific substitutions and rearrangement events cannot be identified. While rearrangements have an uncertain effect on thermal stability, they are not so frequent as to be decisive (*9*). The advantage of the hybridization method is that the median divergence of all of the single-copy DNA can be estimated at once. In contrast, DNA sequences of many genes must be compared in order to estimate the average DNA divergence (for a pair of species) since the number of silent substitutions appears to differ between individual genes (Fig. 1).

Primary sequence comparisons for silent substitutions in coding sequences are identified by letters and thermal stability data by numbers (Fig. 1 and Tables 1 and 2). It appears obvious that very different rates of DNA divergence have occurred in different systematic groups. The upper and lower straight lines (Fig. 1) indicate the fastest and slowest rates of DNA sequence change that can be identified in this set of measurements. Their slopes differ by a factor of 5. The lower line shows the rate of change previously identified for primate and bird DNA (*1*), increased slightly since all the divergence data in the table and figure have been corrected for estimated multiple substitutions at the same site. The upper line shows the average of the rates for the drosophila, rodent, and sea urchin measurements, including both sequence and hybridization data. The abscissa of Fig. 1 is the time since the presence of the last common ancestor of the species being compared (rather than the total evolutionary time which is twice as large). Thus calculated rates are halved to obtain the percentage substitution per million years of evolution. The two slopes are 0.66 percent per million years for the upper line and 0.13 percent for the lower ($6.6$ and $1.3 \times 10^{-9}$ per year, respectively). These two lines were drawn to show that large differences exist and are averages among different phylogenetic groups. They do not imply that the rates have been constant or that the rates are the same among the groups that have been averaged.

## Certainty of the Rate Differences

First the accuracy of the sequence comparison and divergence estimates is considered, and then the interpretation of the dates is discussed. In several cases, totally independent hybridization measurements from several different laboratories are included, and good consistency is shown in each set. For example, all of the thermal stability measurements of the divergence between man and the Old World monkeys are within 0.5 degree of 7.4 degrees. The effect of differences in conditions of hybridization and assay are essentially eliminated by the use of the median divergence (*1, 2, 9*) described (legend to Fig. 1). The median divergence is an approximately linear measure of typical DNA sequence change out to fairly large DNA sequence differences. Even with this method the typical divergence since the time of the mammalian radiation cannot be accurately estimated, since the degree of hybridization is too small (for example, the unplotted comparison of man and rat: item 9, Table 1). This problem is examined below.

A series of comparisons (Fig. 1) shows that the sequence data and the hybridization data are in good agreement. Therefore it is appropriate to combine the results as has been done in this analysis. For example, the silent substitution differences between rat and mouse for two genes (points A and B, Fig. 1) are in good agreement with DNA hybridization measurements (point 8, Fig. 1) and the pseudo-eta globin gene sequence divergences are in excellent agreement with hybridization measurements for the primate divergences

(apes, points V, 10, 11; man and New World monkey, points W and 15; and man and lemur, points X and 19). In fact, sequence data and single-copy DNA measurements agree throughout (Fig. 1). These agreements indicate not only the accuracy of both methods, but together they reinforce the conclusion that there are major systematic differences in the rate of DNA sequence change.

Several methods for time estimation have been used (Tables 1 and 2). The time of the mammalian radiation is plotted at 85 million years (my), with an uncertainty stretching from 75 to 110 my. The three points at the lower right (points 5 to 7, Fig. 1) show the relatively low rates of change of single-copy DNA among the birds (*1*). These time estimates are based on continental drift and represent the opening of the Atlantic Ocean and the Tasman Sea. Because full isolation might be more recent, the error bar extends from 60 to 80 my. In general very conservative error bars have been chosen for the time estimates while their precise termini cannot be defended since the uncertainties are usually unknown.

The divergence for Hawaiian drosophila (point 1, Fig. 1) is based on volcanic events that created the islands of Kauai and Hawaii, and these times are well known. Detailed studies of these species (*10*) leave little doubt that they have remained isolated after rare events in which they colonized islands. The other time values depend on branching in mouse, rat, sea urchin, and primate evolution, and the original reports contain references for the time estimates. Their quality is a matter of judgment, but they appear adequate to identify the large rate contrasts.

It has recently been proposed (*1*) that there is "uniform average rate of DNA evolution" across wide ranges of phylogenetic groups. The data of Fig. 1 are inconsistent with this concept. To fit the data to an intermediate rate it would be necessary to (i) set the Hawaiian drosophila, sea urchin, and the rodent times later by a factor of 2; (ii) set the higher primate and bird dates earlier by a factor of 2; (iii) set the mammalian radiation date at about 150 my; and (iv) make some difficult adjustments of the dates for the lemur and other lower primates. Such a set of changes appears to be inconsistent with modern paleontological knowledge. Thus, there is no generally applicable rate of neutral DNA sequence change (*11*).

## Retardation of the Primate Rate of DNA Divergence

The rate of DNA sequence change has itself apparently changed during primate evolution. There seems no doubt that the DNA divergence among the apes and monkeys (higher primates) has been slow compared with that of most other groups shown in Fig. 1. It is also likely that the early primate drift rate was higher in the period after the mammalian radiation and before the lower and higher

Table 1. Interspecies DNA sequence divergences based on thermal stability of DNA hybrids. For the thermal stability measurements the column headings represent: No., identification for Fig. 1; MOD, method of estimating time (see below); MY, branching time, million years; NR%, percent hybridization for interspecies comparison, normalized to self-hybridization; DT, reduction in melting temperature (50 percent of hybridized DNA); DM%, percent median sequence divergence (*9*) equal to T5OR (*1, 2*) (see legend to Fig. 1); C%, data of previous column corrected for expected multiple substitutions at the same site. Some species have been abbreviated as follows: Sea urchins: Pm, *Psammechinus miliaris*; Pl, *Paracentrotus lividus*; Sp, *Strongylocentrotus purpuratus*; Sf, *Strongylocentrotus franciscanus*. For column 2 (MOD), the symbols and methods are: KH, the time difference between the creation of Kauai and Hawaii (4.8 my); FR, the date estimated from the fossil record; NA, the opening of the North Atlantic; SA, the opening of the South Atlantic; TA, the opening of the Tasman Sea; MR, the time of the mammalian radiation (now thought to be 75 my, but drawn to 110 my as a conservative estimate of the accuracy); V, dates from a variety of sources, with error bars covering the full range of time estimates summarized by Sibley and Ahlquist (*1*).

| No. | MOD | MY | NR% | DT | DM% | C% | Species compared | Ref. |
|---|---|---|---|---|---|---|---|---|
| 1 | KH | 5 | 59 | 2.1 | 8 | 8 | *Drosophila picticornis/ D. heteroneura* | (*10*) |
| 2 | FR | 15–30 | 90 | 15 | 18 | 20 | Sp/Sf (HAP method) | (*9*) |
| 2 | FR | 15–30 | 64 | 13 | 21 | 25 | (HAP S1 method) | (*9*) |
| 3 | FR | 65 | 30 | 17 | | (60) | Pm/Sp | (*24*) |
| 4 | FR | 25 | 54 | 15 | (24) | 29 | Pm/Pl | (*24*) |
| 5 | SA | 60–80 | | | 17 | 19 | Ostrich/rhea | (*1*) |
| 6 | NA | 60–80 | | | 17 | 19 | New/Old World passerines | (*1*) |
| 7 | TA | 60–80 | | | 18 | 20 | New Zealand wrens/ Australian passerines | (*1*) |
| 8 | FR | 10–25 | 62 | 14 | 18 | 20 | Rat/mouse | (*6*) |
| 8 | FR | 10–25 | 76 | 18 | 21 | 25 | Rat/mouse | (*25*) |
| 9 | MR | 75–110 | 13 | 25 | | | Human/rat | (*2*) |
| 10 | V | 5–15 | 95 | 1.1 | 1.7 | 1.7 | Man/chimpanzee | (*2*) |
| 10 | V | 5–15 | | | 1.9 | 1.9 | Man/chimpanzee | (*1*) |
| 10 | V | 5–15 | | 1.4 | 2.4 | 2.4 | Man/chimpanzee | (*26*) |
| 11 | V | 5–15 | | | 2.4 | 2.4 | Man/gorilla | (*1*) |
| 11 | V | 5–15 | | | 2.5 | 2.5 | Man/gorilla | (*26*) |
| 12 | V | 5–15 | | | 3.7 | 3.8 | Man/orang | (*1*) |
| 13 | V | 15–25 | 79 | 3.5 | 4.1 | 4.2 | Man/gibbon | (*2*) |
| 13 | V | 15–25 | | | 5.2 | 5.4 | Man/gibbon | (*1*) |
| 14 | V | 23–40 | 76 | 5.5 | 7 | 7 | Man/Old World monkey | (*2*) |
| 14 | V | 23–40 | | | 7 | 7 | Man/Old World monkey | (*12*) |
| 14 | V | 23–40 | | | 7.7 | 8 | Man/Old World monkey | (*1*) |
| 15 | FR | 40–55 | 71 | 10 | 12 | 13 | Man/New World monkey | (*2*) |
| 15 | FR | 40–55 | | | 11 | 12 | Man/New World monkey | (*12*) |
| 15 | FR | 40–55 | 71 | | 12 | 13 | Man/New World monkey | (*26*) |
| 16 | FR | 50–75 | | | 27 | 32 | Man/galago | (*12*) |
| 17 | FR | 50–75 | | | 26 | 33 | Man/tarsier | (*12*) |
| 18 | FR | 50–75 | | | 28 | 37 | Man/loris | (*12*) |
| 19 | FR | 50–75 | (70) | 15 | 22 | 28 | Man/lemur | (*12*) |
| 20 | FR | 50–75 | 48 | 21 | 29 | 37 | Tarsier/loris | (*12*) |
| 21 | FR | 65–85 | | | 32 | 42 | Man/tree shrew | (*12*) |

Table 2. Interspecies DNA sequence divergences based on silent substitutions in coding regions from DNA sequences. The column headings have the same meaning as in Table 1, except for columns 4, 5, and 6 which signify: No., the actual number of silent substitutions scored, to estimate the statistical uncertainty; DIV%, the divergence as percent of possible silent substitutions; C%, the percent divergence corrected for expected multiple substitutions.

| | MOD | MY | No. | DIV% | C% | Gene | Species compared | Ref. |
|---|---|---|---|---|---|---|---|---|
| A | FR | 10–25 | 60 | 17 | 19 | Amylase | Mouse/rat | (*27*) |
| B | FR | 10–25 | 13 | 19 | 21 | IGC kappa | Mouse/rat | (*28*) |
| C | FR | 40–55 | 22 | 26 | 32 | Beta globin | Man/cebus | (*29*) |
| D | MR | 75–110 | | | 51 | Beta globin | Man/rabbit | (*30*) |
| E | MR | 75–110 | | | 45 | Beta globin | Man/mouse | (*31*) |
| F | MR | | | | 64 | Beta globin | Rabbit/mouse | (*31*) |
| G | MR | | | | 32 | Alpha globin | Man/rabbit | (*31*) |
| H | MR | | | | 82 | Alpha globin | Rabbit/mouse | (*31*) |
| I | MR | 75–110 | 44 | 51 | 83 | Alpha globin | Man/mouse | (*31*) |
| J | FR | 25 | 23 | 29 | 46 | Histone III | Pm/Pl | (*24*) |
| K | FR | 65 | 138 | 46 | 75 | Four histones | Sp/Pm | (*24*) |
| L | MR | 75–110 | | 51 | 86 | IGC lambda | Man/mouse | (*32*) |
| M | MR | | | 51 | 86 | IGC kappa | Man/mouse | (*28*) |
| N | MR | | | 47 | 74 | Growth hormone | Man/rat | (*33*) |
| O | MR | | | 55 | 98 | Prolactin | Man/rat | (*34*) |
| P | MR | | | 47 | 75 | Insulin | Man/rat | (*35*) |
| Q | MR | | | | 51 | Glucagon region | Man/bovine | (*36*) |
| R | MR | | | | 68 | Glucagon region | Man/hamster | (*36*) |
| S | MR | | | | 102 | Glucagon region | Hamster/bovine | (*36*) |
| T | MR | 75–110 | | | 66 | Eight-gene average | Man/rat, mouse, rabbit, hamster, bovine | |
| U | V | 23–40 | 29 | | 11 | Antitrypsin | Man/baboon | (*37*) |
| V | V | 5–15 | 36 | | 1.6 | Eta globin | Man/gorilla, chimp | (*3*) |
| W | FR | 40–55 | | | 11 | Eta globin | Man/owl monkey | (*16*) |
| X | FR | 50–75 | 74 | | 27 | Eta globin | Man/lemur | (*16*) |

primate lineages split, indicating retardation in the rate of DNA change during primate evolution.

Differences in primate rates of DNA divergence have been proposed. Bonner *et al.* (*12*) have made a set of reciprocal thermal stability measurements among the lower primates including comparisons to human DNA (points 16 to 20, Fig. 1) and concluded that the lemur and higher primate lineages showed a slower rate of DNA change than the lorises and tarsiers. Some time ago Goodman (*13*) argued from globin protein sequence comparisons that globin evolution had decelerated, and DNA sequence data appear to confirm this conclusion (*3, 14, 15*). More recently an analysis of the DNA sequences of several genes (*16*) indicates that the average rate of DNA drift over the whole primate lineage since the mammalian radiation was slower than for the rodents over this same period.

Recently, there have been three reports on primate sequences of the eta globin pseudogene (formerly human pseudo-beta) and its evolution. Since this gene was silenced early in primate evolution and has apparently not been subject to conversion, it is particularly useful. The evidence is convincing that there has been slow sequence change for higher primate DNA (*3*) and indicates that much of the deceleration occurred after the branch between the lower and higher primate lineages (*14*). Goodman *et al.* (*15*) have suggested that the slow rate could be correlated with an improved DNA repair mechanism, but they also raise the possibility that DNA sequence dependent selection may have been responsible for the reduced rate of change in higher primate DNA. While it is likely that a deceleration of DNA change occurred about 30 to 50 million years ago in the lineage of the higher primates, reconstruction of the history of the rate of DNA change is difficult with the available data. Two questions arise. What have been the rates of change in the lineages of the lower primates since they branched from the higher primates, and what were the rates of change in the period between the mammalian divergence and the branches to the lower primates? These questions are taken up in succession in the next paragraphs.

If the rate of DNA divergence of the lineages leading to the modern lower primates had been constant and slow during the whole period since the branch between lower and higher primate lineages, the pattern would be different from that in Fig. 1. In such a case, the measured divergences between human and lower primate (tarsier, loris, galago, and lemur) DNA would fall on the lower line of Fig. 1. However, these divergences have intermediate values, implying that the rate of change of the DNA in the lower primate lineages has had intermediate values. This conclusion is primarily dependent on measurements of the divergence between different lower primates (*12*) of which an example is given (point 20, Fig. 1). The fact that all points in Fig. 1 are averages for two lineages must be considered in judging the intermediate values since the two lineages may have very different rates (*17*). A reasonable view is that the lower primate lineages split off from the higher primate lineage before the retardation was complete and thus do not share with the higher primates all of the genomic, behavioral, and biochemical features that may be related to the slow rate.

The reconstruction of the early rates of DNA change is difficult primarily because the neutral DNA drift cannot be accurately estimated over the long period since the mammalian radiation. The interpretation is difficult for both the single-copy divergence measurements and the sequence data, for different reasons in each case. When human and rat single-copy DNA are hybridized (Table 1, line 9) the reaction is only 13 percent; thus it is impossible to calculate the median divergence, and the measurement only implies that it is very large. The sequence data (Table 1 and Fig. 1) are restricted to silent substitutions in coding regions and, although these changes do not affect the amino acids, choices among synonymous codons are not always free of selection. There is probably a small selected residue of unchanged but possible synonymous substitutions at this great evolutionary distance. This may account for the wide range of the points (Fig. 1, D through I and L through R) and suggests that these sequence comparisons may be underestimates of the neutral drift for the period since the mammalian divergence. Thus the data merely indicate that the neutral drift rate was very large in the period

before the retardation of the higher primate rate of DNA change and do not give a precise estimate of the rate in this early period.

The dashed line in Fig. 1 indicates a possible history of primate divergence that is consistent with all of the measurements. It is constructed on the basis that the rate of very early primate neutral DNA sequence drift was about the same as the typical rate for other groups (upper straight line), and that the recent rate is established by the comparisons between higher primate DNA's. The uncertainty in the divergence after the mammalian radiation and the uncertainty of the dates allow many alternative curves, but they should all pass through the New World monkey values (Fig. 1, points 15 and W) and through the lower primate points. Future measurements will be required to establish the true time course of the retardation.

## Neutrality and Drift

The DNA sequence data (Table 2) have been purposefully restricted to silent substitutions in coding regions since they form a consistent base of neutral changes. While all synonymous substitutions may not occur at the neutral rate of drift, many do. If the great majority are free of selection then a reasonable estimate of the rate of neutral drift can be made from this set of data. The agreement between hybridization data and DNA sequence measurements shows that there are no large systematic differences between the two methods indicating that the majority of changes in the total single copy DNA are also neutral.

The implication that DNA sequence changes in most of the DNA have little or no effect is consistent with the fact that coding sequences make up only a small part of the total DNA and few other regions have been identified where changes might be significant. Obviously we do not know the function of most of the DNA or even if it has any function that depends on the nucleotide sequence. If function is difficult to demonstrate it is reasonable that the bulk of the DNA has a neutral drift rate. A part of the single-copy DNA sequences, including gene coding regions, are under selection; but, since they amount only to 5 or 10 percent of the DNA, they do not seriously affect the calculation of the neutral drift rate; but it is probable that the estimates made from Fig. 1 are low by at least 5 or 10 percent. There is no doubt that substitutions occur much more rapidly in the total single-copy DNA than do those in coding regions that lead to amino acid replacement. It has long been recognized that if most changes in the genome were eliminated by selection the genetic load would be excessive (*18*).

In conclusion, both interspecies comparisons of total single-copy DNA and silent substitutions can probably be used to measure the rate of change of unselected DNA sequence and we can assume that most DNA sequence changes enter populations by the process of neutral drift. In other words, chance and random fluctuation primarily determine whether the great majority of sequence changes are fixed in the genome of species or ultimately lost.

## Lack of Effect of Population Size on the Drift Rate

Population models show that the rate of drift of neutral substitutions is independent of the population size for a fixed rate of mutation per individual per generation and for a steady population size (*19*). Thus primary effects of population history on the rate of change of DNA sequences are not expected. However, if there were an effect of population on the rate it would weaken the conclusion that the underlying rate of mutation has changed and is different in different lineages. Therefore a search has been made by computer modeling for population conditions that might affect the neutral drift rate.

Two types of model population history have been examined. The first includes small and large populations coupled to each other by gene flow. In these cases the rate of drift is just the mutation rate per individual per generation even for extreme values of the parameters. In the second type of model dynamic effects on the neutral drift rate were tested with an extreme "boom-and-bust" population model. The conclusion is that neither such dynamic population histories nor the presence of coupled small and large populations have a significant effect on the average rate of neutral drift.

## Replication and Repair Mechanism Differences

The identification of the processes that cause the differences in the rates of DNA change could be fundamentally important to our understanding of the evolution of the genome. The sections above indicate that neither selection nor differences in population history could be the cause of the observed rate contrasts. The alternative causes are differences in the number of germline DNA replications per year or in the mutation rate itself. The data of Fig. 1 suggest that differences in generation time are not the primary cause of differences in rates of DNA change. Sea urchins, rodents, and drosophila show fast rates of change; and, while rodents and drosophila do have short generation times, sea urchins do not. It takes nearly a year for sea urchins to become sexually mature (*20*); they are still very small at that time and achieve the maximum rate of production of gametes only several years later (*20*). Thus sea urchins may have longer generation times than do many birds, and the large differences in DNA sequence change rate cannot be attributed to generation time for these groups (*2, 16*). However, the number of DNA replications per year is probably large for sea urchins since they produce very large quantities of gametes (up to $10^7$ per year). Thus, a part of the reason for rapid DNA drift among sea urchins, rodents, and drosophila could be a larger number of germline DNA replications per year than for the other species shown in Fig. 1.

Evolutionary change in the biochemistry of DNA replication or repair and change of other mechanisms including transposons are also possible causes of changes in mutation rate. Comparative measurements have been made of the DNA repair systems, and the effectiveness of repair appears to have increased during the evolution of the higher primates (*21, 22*). Therefore changes in repair mechanisms are a likely source of the differences in mutation rates and of the retardation in the DNA drift rate that occurred during primate evolution (*23*).

## Evolutionary Significance

The mutation rate is apparently different in different lineages and perhaps at different locations within a single genome (*23*). A reduction of the mutation rate occurred during primate evolution. Thus variation and selection have probably influenced one or all of the mechanisms affecting the mutation rate, such as DNA replication, DNA repair, or transposable elements. During primate evolutionary history there has been a trend toward increased care of individual offspring and reduced birth rate, as well as increased generation time and longevity. A selective advantage would have resulted if the mutation rate decreased during the same period of evolution since the reproductive strategy depends heavily on the survival of individual offspring. Thus this reproductive strategy might have favored the reduction in mutation rate and vice versa, for an extended period of time. On the other hand, a relatively sudden

change in the biochemistry of replication or repair could have been the cause. More precise examination of DNA divergences between existing species may distinguish between these alternatives.

Variation and selection have probably affected the history of the mutation rate—itself a primary mechanism of evolution. The resulting feedback reduces the simplicity of scenarios of natural selection, but the theoretical issues probably do not differ in kind from, for example, those deriving from major induced changes in the environment. Thus, in general, effects of a species' evolutionary history can be stored either in the genome or the environment and then continue to affect the evolution of the species itself for long periods of time. However, for the mutation rates a quantitative record exists in the genomes of living species and measurement may permit analysis of the underlying mechanisms.

## REFERENCES AND NOTES


1. C. G. Sibley and J. E. Ahlquist, *J. Mol. Evol.* **20**, 2 (1984).
2. D. E. Kohne, J. A. Chiscon, B. H. Hoyer, *J. Hum. Evol.* **1**, 627 (1972).
3. L.-Y. E. Chang and J. L. Slightom, *J. Mol. Biol.* **180**, 767 (1984).
4. R. J. Britten, *Stadler Symp.*, in press.
5. With the primate neutral drift rate of 0.13 percent per million years, the expectation is about 160 mutations per individual diploid genome for a 20-year generation time. Probably 95 percent of these are neutral changes.
6. R. J. Britten, *Carnegie Inst. Washington Yearb.* **65**, 102 (1966); the first of the eukaryotic single-copy DNA hybridization measurements.
7. ———, D. E. Graham, B. R. Neufeld, *Methods Enzymol.* **29E**, 363 (1974).
8. J. R. Hutton and J. G. Wetmur, *Biochemistry* **12**, 558 (1973).
9. T. J. Hall, J. W. Grula, E. H. Davidson, R. J. Britten, *J. Mol. Evol.* **16**, 95 (1980).
10. J. A. Hunt, T. J. Hall, R. J. Britten, *ibid.* **17**, 361 (1981).
11. In any case, there was no defensible theoretical basis for this concept. The argument (*1*) reduces to the statement that since very many individual changes are being averaged the "law of large numbers" requires that all of the observed rates be the same. This could only be true if the mechanisms controlling the occurrence of sequence changes did not differ from species to species, or between higher taxonomic groups.
12. T. I. Bonner, R. Heinemann, G. J. Todaro, *Nature (London)* **286**, 420 (1980).
13. M. Goodman, in *Molecular Anthropology*, M. Goodman, R. E. Tashian, J. H. Tashian, Eds. (Plenum, New York, 1976), pp. 321–353; M. Goodman, J. Barnabas, G. Matsuda, G. W. Moore, *Nature (London)* **233**, 604 (1971). Also see (*3, 14, 15*).
14. S. Harris, P. A. Barrie, M. L. Weiss, A. J. Jeffreys, *J. Mol. Biol.* **180**, 785 (1984).
15. M. Goodman, B. F. Koop, J. Czelusniak, M. L. Weiss, J. L. Slightom, *ibid.*, p. 803.
16. C.-I. Wu and W.-H. Li [*Proc. Natl. Acad. Sci. U.S.A.* **82**, 1741 (1985)] compare DNA sequence divergences for several genes between three species (rodent, human, and another mammal for reference), and the results indicate that since the mammalian radiation the average rate of neutral drift of primate DNA has been about half of that for the rodent lineage.
17. This problem might be avoided by a complete set of comparisons including a reference species representing the common stem lineage, but the data are now too limited.
18. M. Kimura, *Nature (London)* **217**, 624 (1968).
19. J. F. Crow and M. Kimura, *An Introduction to Population Genetics Theory* (Harper and Row, New York, 1970).
20. A. P. McMahon *et al.*, *Dev. Biol.* **108**, 420 (1985).
21. A. Ganesan, G. Spivak, P. Hanawalt, in *Manipulation and Expression of Genes in Eukaryotes*, P. Nagley, A. W. Linnane, J. A. Peacock, J. A. Pateman, Eds. (Academic Press, Sidney, 1983), pp. 45–54; A. A. van Zeeland, C. A. Smith, P. C. Hanawalt, *Mutat. Res.* **82**, 173 (1981); B. Zelle, R. J. Reynolds, M. J. Kottenhagen, A. Schuite, P. H. M. Lohman, *ibid.* **72**, 491 (1980).
22. R. R. Tice and R. B. Setlow, in *Handbook of the Biology of Aging*, E. L. Schneider Ed. (Van Nostrand Rheinhold, New York, 1985), p. 202.
23. V. A. Bohr, C. A. Smith, D. S. Okumoto, P. C. Hanawalt [*Cell* **40**, 359 (1985)] show differing repair effectiveness in different genomic regions. Studies of sea urchin DNA variation and divergence [J. W. Grula *et al.*, *Evolution* **36**, 665 (1982)] show that some fractions of the total single-copy DNA are more variable than the average and have a faster evolutionary drift rate while other fractions are less variable than the average and show a slower drift rate. It appears that the rate of mutation is different in different regions of the genome of a single species, due perhaps to local differences in repair or replication mechanisms.
24. M. Busslinger, S. Rusconi, M. L. Birnstiel, *EMBO J.* **1**, 27 (1982); M. Busslinger, thesis, University of Zurich (1980).
25. N. Rice and P. Paul, *Carnegie Inst. Washington Yearb.* **72**, 262 (1972); E. Brownell [*Evolution* **37**, 1034 (1983)] has measured single copy DNA divergences of a number of rodents and states "Although the rate of DNA sequence divergence is stochastically constant within the rodents, the rodent clock may be calibrated differently from that of other groups."
26. B. H. Hoyer, N. W. van de Velde, M. Goodman, R. B. Roberts, *J. Hum. Evol.* **1**, 645 (1972); R. E. Benveniste and G. J. Todaro, *Nature (London)* **261**, 101 (1976).
27. H. Hayashida and T. Miyata, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2671 (1983); R. J. MacDonald *et al.*, *Nature (London)* **287**, 117 (1980); O. Hagenbuchle, R. Bovey, R. A. Young, *Cell* **21**, 179 (1980); P. P. Minghetti, S. W. Law, A. Dugaiczyk [*Mol. Biol. Evol.* **2**, 347 (1985)] report albumin and α-fetoprotein silent substitution differences of 37 and 42 percent between mouse and rat.
28. O. Heidmann, C. Auffray, P.-A. Cazenave, F. Rougeon, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5802 (1981); P. A. Hieter, E. E. Max, J. G. Seidman, J. V. Maizel, Jr., P. Leder, *Cell* **22**, 197 (1980).
29. S. L. Martin, E. A. Zimmer, W. S. Davidson, A. C. Wilson, Y. W. Kan, *Cell* **25**, 737 (1981).
30. R. M. Lawn, A. Efstratiadis, C. O'Connell, T. Maniatis, *ibid.* **21**, 647 (1980).
31. A. Efstratiadis *et al.*, *ibid.*, p. 653.
32. P. A. Hieter, G. F. Hollis, S. J. Korsmeyer, T. A. Waldmann, P. Leder, *Nature (London)* **294**, 536 (1981); A. L. M. Bothwell *et al.*, *ibid.* **290**, 65 (1981).
33. F. M. Denoto, D. D. Moore, H. M. Goodman, *Nucleic Acids Res.* **9**, 3719 (1981); G. S. Page, S. Smith, H. M. Goodman, *ibid.*, p. 2087.
34. N. E. Cooke, D. Coit, J. Shine, J. D. Baxter, J. A. Martial, *J. Biol. Chem.* **256**, 4007 (1981); N. E. Cooke, D. Coit, R. I. Weiner, J. D. Baxter, J. A. Martial, *ibid.* **255**, 6502 (1980).
35. G. I. Bell *et al.*, *Nature (London)* **284**, 26 (1980); P. Lomedico *et al.*, *Cell* **18**, 545 (1979).
36. G. I. Bell, R. F. Santerre, G. T. Mullenbach, *Nature (London)* **302**, 716 (1983); G. I. Bell, R. Sanchez-Pescador, P. J. Laybourn, R. C. Najarian, *ibid.* **304**, 368 (1983); L. C. Lopez, M. L. Frazier, D.-J. Su, A. Kumar, G. F. Saunders, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5485 (1983).
37. A. Bollen *et al.*, *DNA* **2**, 255 (1983).
38. More interspecies comparisons might show greater contrasts in rate, and possibly rate variation within lineages. I would much appreciate receiving copies of single copy DNA measurements including full melting curves and extents of reaction needed for a median divergence calculation and references and data regarding dates that are fairly well established and that open opportunities for new sequence comparisons to establish rates. As new interspecies comparisons or sequence alignments appear I would appreciate notification and plan to coordinate a collection of dates and DNA divergence data for future publication.


39. I thank Eric Davidson for advice and useful criticism of this manuscript; John Roberts for discussion and assistance in the sequence comparisons; all those who made the measurements and dating observations; M. Goodman and W.-H. Li for sending manuscripts before publication (*15, 16*); and B. Barth for manuscript assistance. Supported by National Institutes of Health grant GM34031.