DNA-DNA Hybridization Evidence of the Rapid Rate of Muroid Rodent DNA Evolution[1]

François M. Catzeflis, Frederick H. Sheldon,† Jon E. Ahlquist,‡ and Charles G. Sibley†*

*Institut des Sciences de L'Évolution, Université Montpellier II (U.S.T.L.); †Tiburon Center, San Francisco State University; and ‡Department of Zoological and Biomedical Sciences, Ohio University

Single-copy nuclear DNAs (scnDNAs) of eight species of arvicoline and six species of murine rodents were compared using DNA-DNA hybridization. The branching pattern derived from the DNA comparisons is congruent with the fossil evidence and supported by comparative biochemical, chromosomal, and morphological studies. The recently improved fossil record for these lineages provides seven approximate divergence dates, which were used to calibrate the DNA-hybridization data. The average rate of scnDNA divergence was estimated as 2.5%/Myr. This is ~10 times the rate in the hominoid primates. These results agree with previous reports of accelerated DNA evolution in muroid rodents and extend the DNA-DNA hybridization data set of Brownell.

Introduction

The reconstruction of phylogenies and the determination of the rate—or rates—of genomic evolution are among the most controversial facets of molecular evolution. Herein, we present DNA-DNA hybridization evidence of the branching pattern of the lineages of seven genera and 14 species in the rodent sister taxa Microtinae and Murinae (superfamily Muroidea), as defined by Carleton and Musser (1984). Thanks to an improved fossil record, we also present evidence that the average rate of genomic evolution in the muroid rodents is much faster than the average rates in hominoids and birds.

Our DNA-hybridization data, as well as those of paleontological (Repenning 1968; Chaline 1974, 1980), morphological (Hooper and Musser 1964; Carleton 1981), chromosomal (Gamperl 1982; Koop et al. 1984; Modi 1987), and other biochemical (Graf 1982; Bonhomme et al. 1985) studies, suggest that, within the Microtinae (=Arvicolidae of Chaline [1974, 1980]), (1) Palearctic and Nearctic species of voles (*Microtus*) are more closely related to one another than to species of water voles (*Arvicola*) or red-backed voles (*Clethrionomys*), (2) *Microtus* and *Arvicola* (tribe Microtinini) are more closely related to one another than either is to *Clethrionomys* (tribe Clethrionomyini), (3) the tribe Lemmini (the lemmings, *Lemmus*, and relatives) is the sister group of the Microtini-Clethrionomyini clade, and (4) that, within the Murinae (=Muridae of Petter [1966] and Eisenberg [1981]), the rats (*Rattus*) are the sister taxon of the clade comprising mice (*Mus*) and wood mice (*Apodemus*), the biochem-

1. Key words: rodent phylogeny, rate of DNA evolution, DNA-DNA hybridization.

Address for correspondence and reprints: Dr. François M. Catzeflis, Institut des Sciences de L'Évolution, Université Montpellier II (U.S.T.L.), Place Eugène Bataillon, F-34060 Montpellier, France; or Dr. Charles G. Sibley, Tiburon Center, San Francisco State University, Box 855, Tiburon, California 94920.

Mol. Biol. Evol. 4(3):242–253. 1987.

0737-4038/87/0403-0004$02.00

ical, chromosomal, and morphological differences among these latter three genera being much greater than those between the voles and lemmings.

In addition, the common ancestor of the Microtinae (hereafter referred to by the common name arvicolines) and Murinae is unknown (Petter 1966; Chaline et al. 1977; Carleton and Musser 1984).

Several molecular studies have indicated that rodent DNAs evolve much faster than those of large mammals. The first DNA-DNA hybridization study suggesting this rate difference was that of Laird et al. (1969). They observed a greater genetic distance between mouse and rat than between cow and pig, although available fossil evidence suggested that cow and pig are genealogically more distinct than mouse and rat. Others to observe an apparent rapid rate of rodent DNA evolution include Rice (1971), Benveniste et al. (1977), Brownell (1983), and Wu and Li (1985). Britten (1986) reviewed the evidence for differences in rates in different taxonomic groups and came to the conclusion that rodent DNA has evolved approximately five times as fast as that of hominoids and birds.

In her study, which is the most complete set of rodent DNA-hybridization comparisons to date, Brownell (1983) emphasized that the poor fossil record introduces substantial error into the calibration of the amount of genomic change that occurs per unit of time. Sarich (1972), Sarich and Cronin (1977), and Wilson et al. (1977), arguing for overall constancy in DNA evolutionary rates among groups, also made this point and reasoned that apparent discrepancies in rates of DNA evolution are primarily artifacts of a poor fossil record.

However, recent work on the fossils of arvicolines (Chaline 1974, 1980, 1986) and murines (Flynn et al. 1985; Jaeger et al. 1985, 1986) has increased our knowledge of the history of these groups. Together, the molecular data on degrees of genetic divergence and this improved evidence of divergence dates permit a more accurate estimate of the rate of DNA evolution—and hence a determination of the accuracy of the molecular clock.

Material and Methods

Descriptions of our methods have been published by Sibley and Ahlquist (1981, 1983, 1984).

In brief, DNA extracts were obtained from the nuclei of ethanol-preserved tissue cells, purified, and sheared by sonication into fragments with an average length of 500 bases. Single-stranded fragments of the species to be used as radiolabeled tracers were reassociated to Cot 1,000 at 50 C in 0.48 M sodium phosphate buffer, and repeated sequences were removed by hydroxyapatite (HAP) chromatography. The single-copy nuclear DNA (scnDNA), representing 50%–60% of the total genome by volume and ≥99% by complexity, was labeled with ^{125}I.

DNA-DNA hybrids were formed from a mixture composed of one or two parts (=200 or 400 ng) of tracer DNA and 1,000 parts of sheared, whole DNA of the driver species. These proportions ensured that only ~1%–2% of the duplexes formed would be between tracer fragments. The incipient hybrids were denatured at 100 C, then incubated to Cot 16,000 at 60 C in 0.48 M phosphate buffer to permit the single strands to form hybrid duplexes.

After incubation, the buffer was diluted to 0.12 M and the hybrids were bound to HAP columns immersed in a temperature-controlled water bath at 55 C. The temperature was then raised in 2.5-degree C increments from 55 to 95 C. At each of 17

temperatures, the single-stranded fragments produced by the melting of duplexes were eluted with 0.12 M sodium phosphate buffer. The radioactivity of each sample was counted, and the data were used to calculate $T_{50}H$ values.

$T_{50}H$ is the temperature at which 50% of all potential hybrid DNA sequences retain their duplex form and 50% have dissociated into single-strands. Delta $T_{50}H$ is the difference between the $T_{50}H$ value of a homoduplex control (formed by tracer and driver DNAs derived from the same individual) and any heteroduplex hybrid (formed by tracer and driver DNAs of different individuals) measured in the same experiment. Delta $T_{50}H$ measures the median sequence divergence between the genomes of two taxa. It is an estimate of their average percent nucleotide difference, based on the finding that an ~1.0–degree C reduction in melting temperature corresponds to a 1% difference in nucleotide sequence of the DNAs being compared (Britten et al. 1974). As do T_mR (Benveniste et al. 1977; O'Brien et al. 1985) and TMH (Koop et al. 1986), $T_{50}H$ takes into account the final percent hybridization.

The scientific and common names of the species compared in this study are listed in table 1. Of these species, four were radiolabeled: bank vole (*Clethrionomys glareolus*), sibling vole (*Microtus epiroticus*), western Mediterranean short-tailed mouse (*Mus spretus*), and common rat (*Rattus norvegicus*).

Results and Discussion

Reciprocity

A matrix of the delta $T_{50}H$ distances derived for pairs of rodents in this study is presented in table 2. From these data, the degree of reciprocity—i.e., the degree to which the distance from labeled taxon A to driver taxon B agrees with the distance from labeled B to driver A—was calculated. The average delta $T_{50}H$ value for comparisons between labeled *Microtus* DNA and driver *Clethrionomys* DNA equals 10.02 ± 0.71 (n = 10). The reciprocal distance is 11.02 ± 0.85 (n = 5). *Mus* to *Rattus* and

Table 1
Names and Localities of Specimens Used in Present Study

Family and Common Name (Genus Species)	Locality
Microtinae:	
Sibling vole (*Microtus epiroticus*)[a]	Bulgaria
Meadow vole (*Microtus pennsylvanicus*)	Connecticut
Prairie vole (*Microtus ochrogaster*)	Kansas
Water vole (*Arvicola terrestris*)	Switzerland
Bank vole (*Clethrionomys glareolus*)[a]	Switzerland
Northern red-backed vole (*Clethrionomys rutilus*)	Alaska
Boreal red-backed vole (*Clethrionomys gapperi*)	Connecticut
Siberian lemming (*Lemmus sibiricus*)	Alaska
Murinae:	
House mouse (*Mus musculus*)	Laboratory strain
Western Mediterranean short-tailed mouse (*Mus spretus*)[a]	France
Common rat (*Rattus norvegicus*)[a]	Laboratory strain
Wood mouse (*Apodemus sylvaticus*)	Switzerland
Striped field mouse (*Apodemus agrarius*)	Italy
Yellow-necked mouse (*Apodemus flavicollis*)	Switzerland

[a] Radio-labeled taxon.

Table 2
Mean ± SD Delta $T_{50}H$ Distances between Pairs of Muroid Rodents

	Microtus epiroticus	*Clethrionomys glareolus*	*Mus spretus*	*Rattus norvegicus*
M. epiroticus		10.4 ± 0.4 (3)	32.4 ± 0.5 (4)	ND
M. pennsylvanicus	4.3 ± 0.1 (4)	ND	ND	ND
M. ochrogaster	4.5 ± 0.2 (3)	ND	ND	ND
Arvicola terrestris	8.7 ± 0.3 (5)	10.8 ± 0.5 (4)	30.8 ± 1.0 (4)	31.4 ± 0.9 (6)
C. glareolus	10.2 ± 0.5 (2)		ND	31.3 ± 1.3 (6)
C. gapperi	10.1 ± 1.0 (5)	1.8 ± 0.3 (4)	ND	ND
C. rutilus	9.8 ± 0.2 (3)	2.9 ± 0.7 (4)	31.7 ± 2.1 (2)	ND
Lemmus sibiricus	11.3 ± 0.3 (6)	12.6 ± 0.5 (4)	ND	ND
M. spretus	30.0 ± 0.8 (2)	ND		ND
M. musculus	31.4 ± 1.0 (4)	32.6 (1)	3.6 ± 0.4 (6)	20.1 ± 0.8 (6)
Apodemus (three species[a])	31.6 ± 0.4 (3)	30.2 (1)	18.3 ± 0.8 (9)	21.0 ± 0.6 (6)
R. norvegicus	ND	31.2 (1)	19.9 ± 0.8 (23)	

NOTE.—Labeled species are given on the horizontal axis. Numbers in parentheses are number of comparisons. ND = not determined.

[a] *A. sylvaticus, A. agrarius,* and *A. flavicollis.*

vice versa are 19.94 ± 0.76 (n = 23) and 20.15 ± 0.79 (n = 6), respectively, and *Microtus* to *Mus* and vice versa are 30.93 ± 1.08 (n = 6) and 32.40 ± 0.49 (n = 4), respectively. When t-tested, these reciprocal values indicate no significant difference at the P = 0.05 level.

Brownell (1983) reported a significant degree of nonreciprocity between delta mode values of *Mus* and *Rattus.* However, we found that when delta $T_{50}H$ values were calculated from data provided by Dr. Brownell, these values exhibited excellent reciprocity: *Mus* to *Rattus,* 20.7 (n = 2); *Rattus* to *Mus,* 20.0 ± 1.7 (n = 3).

The mean percent reciprocal deviation was also calculated, using the formula of Champion et al. (1974). For *Microtus* to *Clethrionomys,* the mean deviation from the average reciprocal distance for 30 tests was 3.6%. In *Mus*-to-*Rattus* comparisons, 120 tests yielded a 2.5% mean deviation. Even in tests between arvicolines and murines, the mean deviation is small: 2.4% for 24 *Mus*-to-*Microtus* tests. These deviations are much lower than the values commonly observed for immunological distances derived on the basis of data for serum albumins and transferrins (e.g., such values are 6%–8% in Champion et al. [1974]).

Complete reciprocity, demonstrated even for taxa as distant as *Mus* and *Microtus,* suggests that all reciprocal values are likely to be equivalent, including those not actually measured. Making this assumption, we averaged all reciprocal distances (i.e., folded the distance matrix) before clustering our data into phylograms (see below).

Relative-Rate Tests

At least four sets of comparisons qualify as relative-rate tests (i.e., those tests in which two of three taxa are more closely related to one another than either is to the third [Sarich and Wilson 1973]). These sets are outgroup *Mus* to ingroups *Arvicola* and *Microtus, Rattus* to *Arvicola* and *Clethrionomys, Microtus* to *Mus* and *Apodemus,* and *Rattus* to *Apodemus* and *Mus.* From the data in table 2, it is apparent that the distances from the outgroup to each of the two ingroups are essentially equal (t-test,

$P = 0.05$). Thus, in all cases in which the outgroup is unambiguous, our data indicate that the scnDNAs of arvicolines and murines are evolving at approximately the same average rate. Similarly, Brownell (1983) found that nucleotide substitution was occurring at a uniform average rate in several arvicoline and sigmodontine rodent lineages.

Comparisons with Previously Published Data

To compare our delta $T_{50}H$ values with the distances derived in other DNA-hybridization studies, we calculated the relationships among mode, T_m, and $T_{50}H$, using distances ranging from delta 4 to delta 34 (data to be published elsewhere). Delta mode and delta $T_{50}H$ are related by the following power regression:

$$T_{50}H = 0.8\ \text{Mode}^{1.17} \quad (n = 84; r = 0.996). \tag{1}$$

This equation was used to estimate delta $T_{50}H$ from modal values published by Brownell (1983). The relationship between delta T_m and delta $T_{50}H$ is described by the following function:

$$T_{50}H = 0.63\ T_m^{1.32} \quad (n = 43; r = 0.993). \tag{2}$$

This regression was used to estimate $T_{50}H$ from the T_m values of Laird et al. (1969), Rice and Straus (1973), and Rice (1974).

Our estimates of the mean number of differences per site conform well to some of the previously published distances between the same taxa. Brownell's (1983) delta mode of 8.6 ± 0.6 between *Microtus* and *Clethrionomys* corresponds to a delta $T_{50}H$ of 9.9, a value nearly identical to our 10.1 ± 0.7 (n = 13). For *Mus* to *Rattus,* Laird et al. (1969) and Rice and Straus (1973) derived delta T_m values of 14 and 14.9, respectively. These distances correspond to delta $T_{50}H$'s of 20.4 and 22.1 and are therefore close to our average distance 19.9 ± 0.8 (n = 23) and to that which we calculated from Brownell's raw data (20.2). The distances between the Microtinae and Murinae have been estimated from Rice (1974) and Brownell (1983) to range from delta $T_{50}H$ 28.2 to $T_{50}H$ 37.2, thus agreeing with our values of 29.4–33.2 (table 2).

The smaller delta $T_{50}H$ distances measured between *Mus* and *Rattus* by Benveniste et al. (1977) are possibly the result of differences in DNA-hybridizing techniques. These authors treated their samples with S1 nuclease before thermal fractionation, thus eliminating single-stranded tails, which we include in our measurements.

Phylogeny

The existence of a single average rate of scnDNA evolution among the arvicolines and murines permits the use of phenetic methods for clustering taxa into phylograms. We chose the *u*nweighted *p*air *g*roup *m*ethod of *a*nalysis (UPGMA; Sneath and Sokal 1973), rather than least squares or other fitting algorithms, because average linkage allows the combining of matrix cells when pairwise comparisons are missing, as was the case in our data set.

To produce more accurate and additive distances, we followed the example of Koop et al. (1986) and converted the delta $T_{50}H$ values of table 2, which are the mean number of base-pair differences per 100 sites between the DNA sequences of two taxa, into TMH-C values (table 3). TMH-C is the mean number of substitutions per 100

Table 3
Mean ± SD Delta $T_{50}H$ Values, Mean Delta $T_{50}H$ Values Transformed into TMH-C Distances by Correcting for Multiple Substitutions, and Estimated Times since Divergence of Various Arvicoline and Murine Clades

Clade Pairs	Delta $T_{50}H$	TMH-C[a]	Divergence Date[b] (MYBP)
Palearctic/Nearctic *Microtus*	4.4 ± 0.2 (7)	4.5	1.2–1.8
Microtus/Arvicola	8.7 ± 0.3 (5)	9.2	3.5
Microtini/Clethrionomyini	10.3 ± 0.7 (17)	11.1	3.7
Microtini-Clethrionomyini/Lemmini	11.8 ± 0.8 (10)	12.9	4.8
Mus/Apodemus	18.3 ± 0.8 (9)	21.1	7–10
Mus-Apodemus/Rattus	20.5 ± 0.7 (29)	24.1	8–11
Murinae/Arvicolinae	31.4 ± 1.0 (34)	40.7	20

NOTE.—Numbers in parentheses are number of comparisons.

[a] Calculated using the Jukes and Cantor (1969) correction factor for multiple hits at single sites. The mean number of substitutions for the Arvicolinae/Murinae comparison is probably larger than indicated, since the Jukes and Cantor formula tends to underestimate the number of multiple substitutions as delta $T_{50}H$ becomes large (Tajima 1985).

[b] Based on the fossil record (see text).

sites that have occurred since two taxa diverged from a common ancestor. It is calculated with Jukes and Cantor's (1969) conversion factor for multiple substitutions (hits) per site. The phylogram of figure 1 was drawn using the TMH-C values from table 3.

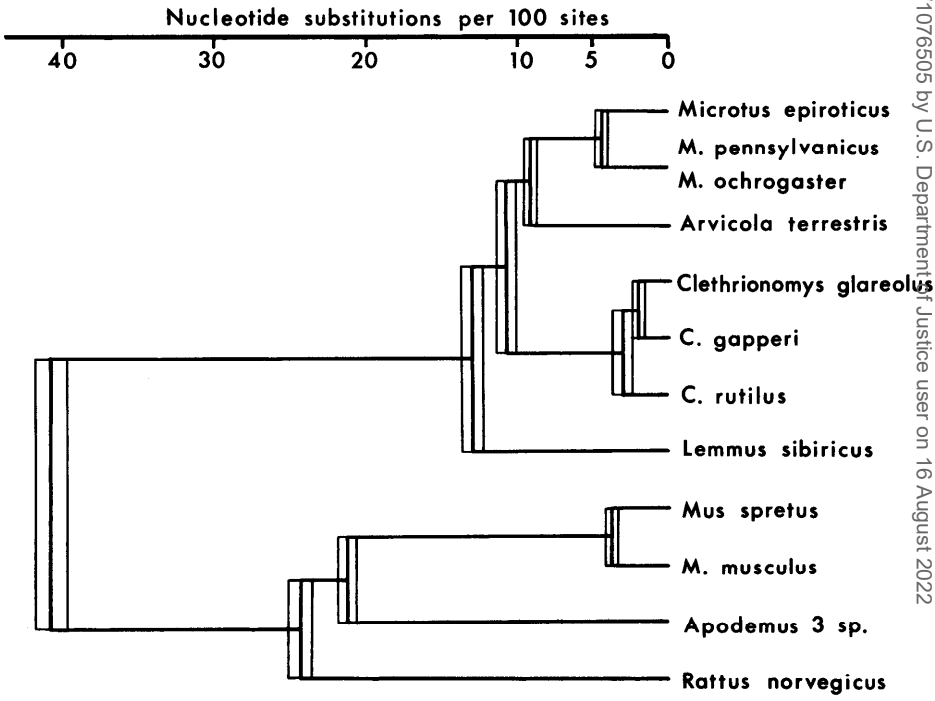

FIG. 1.—Phylogram of arvicoline and murine relationships. The branching pattern and distances were computed by average linkage using the TMH-C values in table 3.

The subdivision of the microtines into three tribes—Microtini, Clethrionomyini, and Lemmini—has been proposed by Gromov and Poliakov (1977) on morphological grounds. Our data support such an arrangement and show that Lemmini is the sister group of the clade comprising Microtini and Clethrionomyini. Evidence from dental morphology (Chaline 1974, 1980), electrophoretic comparisons of proteins encoded by 19–22 loci (Graf 1982), and chromosomal analyses (Modi 1987) also indicate this sister-group relationship. However, comparative anatomy of the glans penis (Hooper and Musser 1964) does not.

Within the Clethrionomyini, our data indicate a slightly closer affinity between *C. glareolus* and *C. gapperi* than between either of them and *C. rutilis.* However, the G- and C-banded karyotypes of *C. glareolus* and *C. rutilus* are more similar between these two species than any of them are to those in *C. gapperi* (Modi and Gamperl 1986).

Our comparisons also suggest that *Mus* and *Apodemus* form the sister group of *Rattus.* Jacobs (1978) came to the same conclusion on the basis of fossil evidence, as did Sarich (1985) on the basis of albumin immunological data. However, on the basis of a comparative study of molar teeth, Misonne (1969) proposed a closer relationship between *Mus* and *Rattus* than between *Mus* and *Apodemus.*

Mus spretus and *M. musculus* are as genetically distant from one another as are species of *Microtus* or *Clethrionomys;* all of these congeneric distances are between delta $T_{50}H$ 2 and delta $T_{50}H$ 5. Our findings also agree with the electrophoretic data (based on 24 structural loci), which show that *M. musculus* and *M. spretus* are more similar to each other than either is to several species of *Apodemus* (Bonhomme et al. 1985).

Dates, Rates, and Possible Causes

Zakrzewski (1985) has estimated that *Microtus* arrived in the Nearctic ~1.8 million years before the present (MYBP), and Chaline (1974) and Repenning (1980) dated the divergence of Nearctic and Palearctic *Microtus* as being 1.2–1.8 MYBP.

The morphological characters differentiating *Microtus* from *Arvicola* appear in fossils dating from ~2.0 MYBP (Gromov and Poliakov 1977), but it is possible to trace the lineage leading to *Arvicola* back to 3.5 MYBP (Chaline 1986, and personal communication).

Gromov and Poliakov (1977) and Chaline (1974, 1977) estimated that the Microtini and Clethrionomyini diverged ~3.7 MYBP.

Lemmus was distinct from *Synaptomys,* another member of the Lemmini, at 2.5–3.0 MYBP (Gromov and Poliakov 1977; J. Chaline, personal communication), and the ancestors of the Lemmini are represented by fossils that are distinct from the Microtini and Clethrionomyini at ~4.5–5.0 MYBP (Chaline 1974, 1977; Gromov and Poliakov 1977).

Recently discovered murid fossils from Pakistan and a reexamination of the oldest known murids (Jacobs 1978; Flynn et al. 1985; Jaeger et al. 1986) suggest that the *Mus-Rattus* split occurred ~8–11 MYBP. L. J. Flynn (personal communication) believes that the divergence date is probably close to 11 MYBP. The *Mus-Apodemus* divergence is dated by Flynn (personal communication) at 7–10 MYBP—i.e., within 1–2 Myr after the *Rattus* lineage branched. A succession of closely spaced divergence dates is also indicated by our DNA data (fig. 1).

The murine lineage split from the microtine lineage ≥20 MYBP (Jaeger et al.

1985; L. J. Flynn, personal communication) and more likely 25–30 MYBP (Lindsay 1978).

From these dates (summarized in table 3 and illustrated in fig. 2) and the TMH-C distances, we calculated the number of nucleotide changes that have occurred per unit of time for the muroid lineages. The average rate of DNA divergence is delta TMH-C 1.0 = 0.38 ± 0.07 Myr (n = 10). Assuming that delta 1.0 corresponds to a 1%-bp mismatch (Britten et al. 1974), 1 Myr of divergence between two species results in an ~2.5% difference in their DNAs. A rate of 2.5% nucleotide change/Myr is ~10 times as fast as the rate calculated for the hominoid primates (Sibley and Ahlquist 1984). This difference is illustrated in figure 2.

To determine the rate for hominoids, Sibley and Ahlquist (1984) used the divergence date of the Orangutan clade, set at ~13–16 MYBP by Pilbeam (1983). This gave an average rate of 0.23%/Myr. To calculate the rate for ratite birds, Sibley and

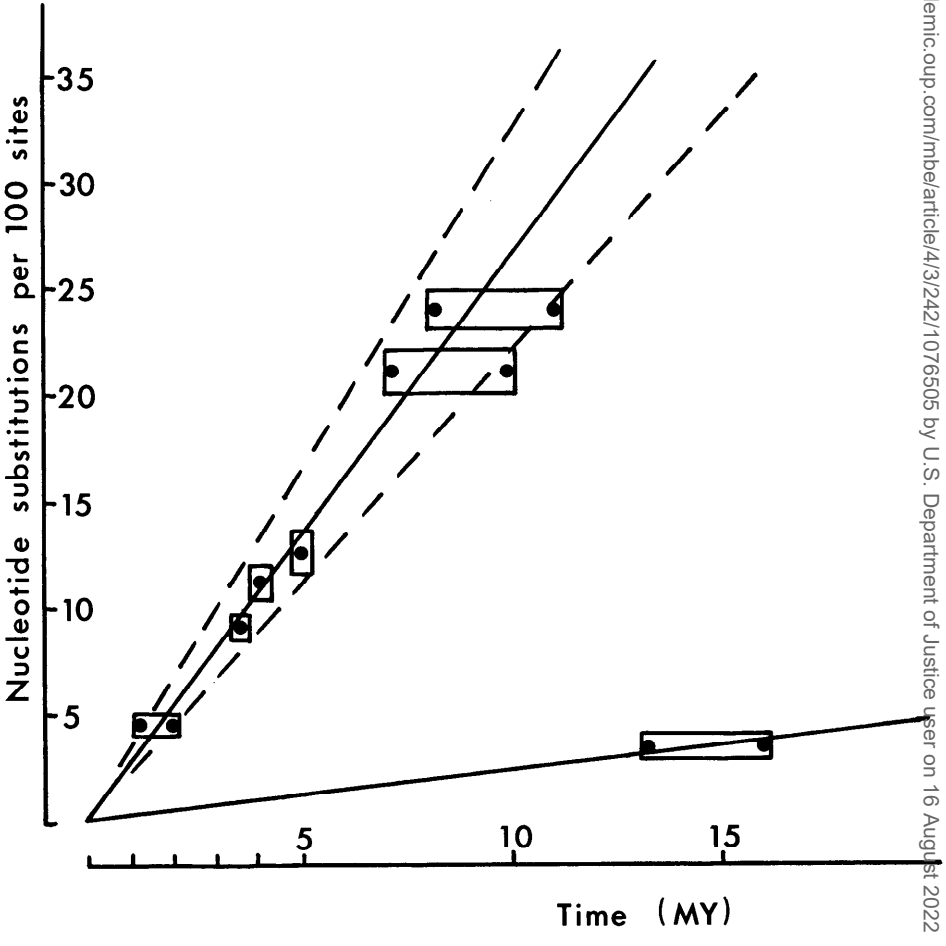

FIG. 2.—Graphic representation of single-copy genomic-rate differences between rodents (upper solid line) and hominoids (lower solid line). The slope of the rodent line is 2.5% nucleotide substitutions/Myr. The dotted lines on either side of the rodent line represent ±1 SD borders to the rodent line. The slope of the hominoid line is 0.23% nucleotide differences/Myr (from Sibley and Ahlquist 1984). The dots date events given in table 3, and the frames around the dots represent ±1 SD in two dimensions.

Ahlquist (1981) used the opening of the South Atlantic at ~80 MYBP, setting the rate at ~0.22%/Myr. Even if these fossil and vicariance dates are off by a large amount (e.g., by a factor of two in birds, as suggested by Helm-Bychowski and Wilson [1986]), which we do not believe them to be, the average genomic rate of change in rodents must have been much faster than those of hominoids and some birds.

Laird et al. (1969), Kohne (1970*a*, 1970*b*), and Kohne et al. (1970), using a *Mus-Rattus* divergence date of 10 Myr, found that rodents have diverged at a rate of ~2%/Myr. Benveniste et al. (1977, p. 859) analyzed thermal stability profiles of DNA duplexes of several taxa of primates and rodents and observed a "6- to 10-fold increase in the accumulation of base pair mutations in rodent cellular DNA as a function of time." Wu and Li (1985) have discovered that rates of evolution in 11 rodent genes were faster than those of their homologues in hominoids. However, some questions have been raised concerning their use of the relative-rate test to determine these rate differences (Easteal 1985); Wu and Li used the bovine lineage as outgroup, and the ungulate-primate-rodent branching pattern is disputed. However, even if the precise branching pattern is not known, Wu and Li's conclusions are still valid, because ungulates, primates, and rodents diverged in a short span of time, too short for the large number of nucleotide changes in the rodents to have been caused by genealogical separation rather than by differences in rates (Li and Wu 1987). Finally, as noted above, Britten (1986) concluded that the rate of DNA evolution in rodents is at least five times that in birds and hominoids.

Arguments against variable rates have usually been proposed on the basis of protein evidence (e.g., Wilson et al. 1977), but the effects of variable rates among proteins have made conclusions based on them questionable. Sibley and Ahlquist's (see, e.g., 1983) mistaken belief in uniform rates of scnDNA evolution was based partly on the remarkable similarity of hominoid and ratite bird rates (0.23% vs. 0.22% change/Myr), partly on the constancy of rates among most passerine birds, and partly on the use of faulty relative-rate tests. The outgroup taxa employed in these rate tests (heron and plover) had slow rates of evolution, and, when employed as references to various nonpasserine ingroups, they tended to equalize discrepancies in branch lengths.

Now that the consensus of information has turned in favor of variable rates of molecular evolution, the next frontier is to discover the cause(s) of rate changes. Evidence for the rapid evolution of rodent DNA certainly points toward a generation-time effect and related phenomena (see, e.g., Laird et al. 1969; Kohne et al. 1970; Goodman 1985; Wu and Li 1985). However, Britten (1986) has suggested that generation time, population history, and selection are not likely to be the primary causes. Instead, he has proposed that the effectiveness of DNA repair mechanisms in various groups of organisms may be involved.

We conclude that there is not a single, global DNA clock ticking at the same average rate in all mammals; rather, the rate of genomic evolution in each group must be determined separately by calibrating numbers of nucleotide changes with absolute divergence dates that are derived from fossil or vicariant events. Whether the differences in average genomic rates among groups of organisms are due to differences in generation times, repair mechanisms, or other causes is not yet clear.

Acknowledgments

We thank Roy Britten, Jean Chaline, William S. Modi, and Jean-Jacques Jaeger for providing preprints of manuscripts. Marie-Claude Durette-Desset, Robert Hoff-

mann, and Fred Sibley provided tissue samples. Linda Wallace assisted in the laboratory. Funds for laboratory work were provided by Yale University and the U.S. National Science Foundation (grant BSR 83-15155 to C.G.S.). F.M.C. was supported by a postdoctoral fellowship from the Swiss National Science Foundation.

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WALTER M. FITCH, reviewing editor

Received March 31, 1986; revision received November 7, 1986.