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Determinants of Rate Variation in Mammalian DNA Sequence Evolution

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Abstract. Attempts to analyze variation in the rates of molecular evolution among mammalian lineages have been hampered by paucity of data and by nonindependent comparisons. Using phylogenetically independent comparisons, we test three explanations for rate variation which predict correlations between rate variation and generation time, metabolic rate, and body size. Mitochondrial and nuclear genes, protein coding, rRNA, and nontranslated sequences from 61 mammal species representing 14 orders are used to compare the relative rates of sequence evolution. Correlation analyses performed on differences in genetic distance since common origin of each pair against differences in body mass, generation time, and metabolic rate reveal that substitution rate at fourfold degenerate sites in two out of three protein sequences is negatively correlated with generation time. In addition, there is a relationship between the rate of molecular evolution and body size for two nuclear-encoded sequences. No evidence is found for an effect of metabolic rate on rate of sequence evolution. Possible causes of variation in substitution rate between species are discussed.

Key words: Molecular evolution — Molecular clock — Phylogeny — Metabolic rate — Generation time — Body size — Allometry

Introduction

A growing body of evidence suggests that vertebrate lineages differ in their rate of evolution of DNA sequences. Perhaps the best-documented case of lineage-specific rates is the "hominid slowdown" (Li et al. 1987; Hasegawa et al. 1989; Bailey et al. 1991; Seino et al. 1992): Apes have a slower rate of molecular evolution than monkeys, and the lineage leading to humans has evolved more slowly than other ape lineages. Other examples of lineage-specific rates of evolution include fast rats (Li et al. 1990), slow sharks (Martin et al. 1992), and slow turtles (Avise et al. 1992). The general picture that emerges is that the rate of sequence evolution decreases from mammals to birds to amphibians to fish (Adachi et al. 1993). Within mammals, the emergent pattern is that rodents are faster than artiodactyls, and artiodactyls are faster than primates (Li et al. 1987, 1990). Revealing the reasons for rate variation is fundamental to understanding the dynamics of molecular evolution. This paper provides a general method for testing different explanations of rate variation and uses that method on sequence data from a wide range of mammal species.

Identification of rate differences is important for molecular phylogeny reconstruction, as it excludes the use of methods that assume a molecular clock. For example, false assumption of a global molecular clock can result in the placement of the most divergent branches at the root of the tree (Holmes 1991) or inappropriate use of cross-taxon calibration of divergence times (Avise et al. 1992). However, if causes of rate variation can be established, then the possibility of a corrected clock arises, which would provide a means of dating divergences using genetic distances.

Several theories have been put forward to explain observed differences in rates of molecular evolution. Britten (1986) suggested that differences in DNA repair efficiency could be driving rate differences and that a more efficient repair mechanism in primates could account for their slow molecular evolution. A generation

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time effect has been proposed (Li et al. 1987, 1996; Ohta 1993; Mooers and Harvey 1994) based on the assumption that the germ-lines of species with shorter generation times have a greater number of DNA replications per year, and thus a greater chance of replication errors per unit time. The metabolic rate hypothesis (Martin et al. 1992; Martin and Palumbi 1993; Martin 1995) proposes that increased rate of metabolism results in a higher concentration of DNA-damaging metabolites in the cell (particularly free oxygen radicals), which elevates the mutation rate.

However, approaches to testing these hypotheses have been somewhat piecemeal. Limits to the amount of sequence data available have resulted in studies that maximize the number of sequences used by comparing only a few, highly divergent taxa—in particular, relying heavily on rodent/primate comparisons (e.g., Li et al. 1987, 1996; Holmes 1991; Bulmer et al. 1991; Ohta 1993; Easteal and Collett 1994). This approach is problematic for two reasons: (1) Analysis of a small number of taxa cannot reveal statistically sound patterns, because of both a paucity of data and, in many cases, failure to control for phylogenetic bias; (2) such divergent taxa differ in many respects, and it is usually not possible to isolate particular hypothesized causes of rate variation. A more statistically sound approach is to maximize the number of taxa compared and control for phylogenetic bias by including only phylogenetically independent comparisons. While the study reported here is limited by the amount of sequence data currently available for mammals as well as by the absence of a reliable phylogeny for many mammal clades, it illustrates the application of an approach that should become more useful as the amount of sequence data increases.

This study tests the generation time and metabolic rate hypotheses for mammals and also examines the idea (given below) that body size itself may influence rate of substitution. We circumvent many of the problems of previous studies by using sequence data from a wide range of mammal species, arranged as statistically independent comparisons. The number of genes we could utilize was necessarily small in order to increase sample size of taxa to enable identification of broad-scale patterns among mammals. A range of sequence types (mitochondrial and nuclear, protein coding, RNA, and noncoding) was used because each may evolve in a different way. For example, the rate of evolution of mitochondrial genes may reflect differences in metabolic rate because they suffer greater exposure to oxidative damage in the cytoplasm (Brown et al. 1982; Martin and Palumbi 1993; Rand 1994). In contrast, nuclear genes might show a more pronounced generation time effect, because nuclear DNA replication is linked to cell division, whereas mitochondria can divide many times during the lifetime of a cell. Using a range of sequence types also allows comparison of synonymous, nonsynonymous, and nontranslated substitutions.

Body mass is included as a variable in this analysis to allow testing of a body-size effect on substitution rate and to identify trends due to covariation of life-history parameters. Body size may exert selective pressure on the level of DNA replication fidelity and repair efficiency. Because repair is expensive (Kunkel 1992), an organism might be expected to evolve the minimum level of repair needed for maintenance of life. Increased size demands more error-free replication because large animals must maintain more cells over a longer life span. DNA replication must be reliable enough to produce enough cell generations without lethal mutation; as the majority of mutations can be considered deleterious, in effect the maximum allowable mutation rate will be related to the reciprocal of the number of cell generations. Large mammals not only require more cell generations to grow their bodies but also have a greater number of somatic cells, and so they must have higher DNA repair rates in order to have the same probability of maintaining all their cells (Promislow 1994).

Metabolic rate and generation time are correlated with each other and also with life-history variables such as body size, and thus a causal relationship between one of these variables and genetic distance is likely to result in a significant correlation with the others. Partial correlation coefficients measure the association between two variables when another variable is held constant, thereby allowing the relationship over and above the effects of other life-history variables to be assessed. Use of phylogenetically independent comparisons (Felsenstein 1985; Burt 1989; Harvey and Pagel 1991) reduces the number of possible comparisons that can be made between taxa (particularly if the phylogenetic tree is poorly specified with many polytomies rather than being fully bifurcating) but must be used in order to satisfy the assumptions of independence of data inherent in statistical tests. Two methods for measuring rate of molecular evolution are used here: maximum-likelihood (ML) trees and relative-rates tests.

Methods

We chose the most widely sequenced mitochondrial and nuclear mammalian genes available from the GenBank database: cytochrome *b* (cyt*b*), 12s ribosomal RNA (12s), beta-globin, and epsilon-globin (Table 1). For each of these genes, phylogenetically independent pairs of taxa were identified for which complete or nearly complete sequences and data on body mass, generation time, and metabolic rate were available.

Independent comparisons are required because the statistical tests used in this analysis assume the independence of data points. Species cannot be treated as independent units because they form part of a hierarchical phylogeny, so the set of traits for each species does not represent an assortment chosen randomly from a distribution of possible traits, independently of other taxa (Harvey and Pagel 1991). In-

Table 1. DNA sequences used in this study

Gene	Gene product	Genome	Length of alignment (bp)	Number of species	Number of comparisons
Cytochrome *b*	Protein	Mitochondrial	1,140	33	16
12s	rRNA	Mitochondrial	904	22	10
Beta-globin:					
Exons	Protein	Nuclear	454	15	7
Introns	Untranslated	Nuclear	404	15	7
Epsilon-globin					
Exons	Protein	Nuclear	462	20	8
Introns	Untranslated	Nuclear	1,489	20	8

stead, similarity between taxa will vary with their level of relatedness. Using only independent comparisons circumvents this problem, as the data are divided into statistically independent units, each contributing one degree of freedom to the statistical test (Burt 1989).

Many aspects of the phylogenetic relationships between mammals are uncertain, so we used the following safeguards to help ensure independence of pairs. We assumed monophyly of families but took no more than one pair per family. Where between-family or between-order comparisons were made, published phylogenies were used to identify phylogenetically independent comparisons (Purvis 1995 for primates; Eisenberg 1981 for rodents; Garland and Janis 1993 for artiodactyls; Novacek 1992 for cross-order comparisons). Polytomies among taxa (Novacek 1992; Eisenberg 1981; Purvis 1995) are best interpreted as representing uncertainty of the true relationships rather than simultaneous divergence of multiple lineages, so only one pair arising from each polytomy was used. This severely limited the number of between-family and between-order comparisons that could be included in the analysis.

Life-History Data. Estimates of body size, generation time, and metabolic rate for each species were gleaned from the literature (Table 2). Body size is represented by mean female adult body weight (average of all available population means). In some cases no estimate was available, so the mean for both sexes was used, or the geometric mean of the maximum and minimum was calculated.

For the purposes of this study, the relevant measure of generation time is the time it takes for the germ-line DNA to reproduce itself. This can be pictured as the amount of time it takes for a gamete to product another gamete (via syngamy, mitotic growth of body and germ cells, and gametogenesis). The easiest way to measure DNA generation time is fertilization-to-fertilization (age at first mating plus gestation period) or birth-to-birth (age at first birth) (Fig. 1). Because not all reproductive data is available for all taxa, we have included either or both of these measures—the mean is given where both are recorded or several estimates were available.

Published values of basal metabolic rate (BMR) were considered acceptable only if they were ''made on adults within the region of thermoneutrality, during the normal period of inactivity, and while the animals are inactive, thermoregulating and post-absorbative'' (McNab 1988). In addition, the weight of the animal from which the measurement of BMR was taken must be available in order to convert the value to mass-specific BMR, expressed in watts per gram ($W.g^{-1}$). Published values expressed in cm^3 O_2 min^{-1} or similar units were converted to $W.g^{-1}$ by assuming 1 l O_2 = 20.09 kJ (McNab 1988).

Molecular Data. Sequences were obtained from GenBank and aligned by eye. Regions of genes that could not be confidently aligned (hypervariable regions of 12s and globin introns) were excluded from the analysis.

To compare the rates of molecular evolution for two taxa, we must estimate the relative amount of evolution that has occurred down the two lineages since their common ancestor. We have done this in two ways: maximum-likelihood ''trees of three'' and relative-rates tests. Trees of three, consisting of two taxa and an outgroup, were constructed using the HKY85 model of nucleotide substitution (using a test version of PAUP* 4.0 provided by D.L. Swofford). For each sequence, we estimated the maximum-likelihood transition/transversion ratio (TS/TV) for the tree of all available taxa. For the protein-coding genes, the relative rates of evolution at each codon position were also estimated using maximum likelihood. These parameters were then used for all the trees of three for that gene. Trees for cyt*b* and 12s were also constructed using an empirically determined TS/TV for comparison (5.5 for cyt*b*, 9.5 for 12s: Purvis and Bromham in press). For the globin genes, ML trees were constructed for introns only, exons only (using separate rate categories for each codon position), and for exons plus introns (four rate categories: codon positions plus introns).

A relative rates test utilizes pairwise distances between pair and outgroup to estimate branch lengths (Sarich and Wilson 1973). Relative rates tests were performed as described in Li and Graur (1991) using pairwise distances which were measured in a number of ways. For protein-coding sequences (cyt*b*, globin exons), Ina's ''New Method 1'' was used to estimate both the number of synonymous and nonsynonymous changes between pairs of sequences (Ina 1995). This is an unweighted pathway method, based on Kimura's two-parameter model, which estimates number of fourfold degenerate substitutions (changes at sites where all possible mutations are synonymous) per site by using a matrix of all possible substitutions based on the TS/TV (estimated from the third codon position). This method is particularly useful for mitochondrial genes, because it allows for high TS/TV (Ina 1995).

The non–protein-coding sequences (12s, globin introns) are characterized by a mosaic of both highly conserved sites and regions of high variability in both nucleotide sequence and length. A gamma distance allows for variability in the rate of change at each site by specifying that the amount of change per site follows a gamma distribution described by the shape parameter α (the inverse of the coefficient of variation of the substitution rate λ) (Kumar et al. 1993). We used MEGA (Kumar et al. 1993) to calculate the gamma distance for the non–protein-coding sequences, using the default value $\alpha = 1$. Gamma distances for 12s was also calculated using $\alpha = 0.3$ (a maximum-likelihood estimate for the global tree of all sequences).

Both the maximum-likelihood trees-of-three and the relative rates tests require an outgroup to the pair. Published phylogenies (Eisenberg 1981; Novacek 1992; Garland and Janis 1993; Purvis 1995) were used to choose the closest definite outgroup. If a number of sequences fitted that requirement (i.e., were equally distant from the pair), one was chosen at random.

Statistical Analysis. Each data point in the analysis represents the difference between a pair of species (Tables 3 and 4). Correlations were

Table 2. List of species with life-history variables taken from the literature[a]

Order	Family	Species	Mass (g)	Generation time (mth)			Metabolic rate (W/g)	Source
				A	B	Avg		
Marsupialia	Dasyuridae	*Planigale maculata*	10.0	—	10.3	10.3	0.005344	1, 2, 3
		Antechinus flavipes	34.0	—	11.8	11.8	0.008597	1, 2, 3
	Didephidae	*Didelphus virginiana*	4,899.0†,#	6.2	6.4	6.3	0.001858	4, 2, 3
		Monodelphus brevicaudata	84.0†	—	6.5	6.5	0.003874	5, 2, 3
Insectivora	Erinaceidae	*Atelerix albiventris*	212.4†,#	—	5.2	5.2	0.001867	6, 7, 2, 3
	Soricidae	*Blarina brevicauda*	19.7†	2.1	19.8	11.0	0.014010	8, 2, 3
Scandentia	Tupalidae	*Tupaia glis*	150.0	6.8	5.3	6.1	0.004241	9, 2, 10
Chiroptera	Pteropodidae	*Cynopterus sphinx*	90.0†	—	9.2	9.2	0.007027*	11, 2, 3
Primates	Callithricidae	*Callithrix jacchus*	282.9	20.8	21.2	21.0	0.004526	12, 13, 14, 15, 16, 2, 3
		Cebuella pygmaea	113.9	32.7	34.0	33.4	0.005238	12, 13, 14, 15, 16, 2, 3
	Cebidae	*Saimiri sciureus*	665.1	40.6	49.1	44.8	0.005488	12, 13, 14, 15, 16, 2, 3
		Allouatta seniculus	6,380.5	—	54.9	54.9	0.002478*	12, 13, 14, 15, 16, 2, 23, 3
	Cercopithecidae	*Macaca mulatta*	5,704.4	55.2	51.7	53.5	0.002065	12, 13, 16, 2, 24
		Macaca fascicularis	4,209.1	46.0	57.1	51.5	0.002828	12, 13, 16, 2, 25
		Colobus polykomos	6,707.6	—	30.3	30.3	0.001654*	12, 13, 16, 2, 25
	Cheirogaleidae	*Cheirogaleus medius*	211.0	—	13.1	13.1	0.003667	12, 13, 16, 17, 18, 2, 26, 3
		Microcebus murinus	76.0	14.2	11.1	12.6	0.005287	12, 13, 16, 17, 18, 19, 2, 25
	Homonidae	*Homo sapiens*	46,536.9	—	171.3	171.3	0.001183	12, 13, 2, 3
	Indriidae	*Propithecus verreauxi*	3,770.6	—	34.8	34.8	0.001161	12, 13, 16, 18, 20, 2, 25
	Lemuridae	*Lemur macaco*	2,542.8	36.0	28.8	32.4	0.001803	12, 13, 17, 18, 2, 3
	Lorisidae	*Galago crassicaudatus*	1,085.7	—	25.0	25.0	0.002820	12, 13, 16, 18, 21, 22, 2, 3
		Nycticebus coucang	1,003.3	36.0	26.5	31.3	0.001331	12, 13, 18, 21, 2, 3
	Pongidae	*Pan troglodytes*	38,330.5	168.0	136.9	152.4	0.001451	12, 13, 16, 2, 24
		Pongo pygmaeus	15,206.7	132.0	101.7	116.9	0.001674	12, 13, 16, 2, 24
	Tarsidae	*Tarsius syrichta*	118.9	—	18.9	18.9*	0.003941	12, 13, 18, 21, 27, 25
Carnivora	Felidae	*Felis domesticus*	2,617.0	12.0	12.1	12.1	0.003962	7, 2, 11
		Panthera leo	135,500.0	38.5	42.6	40.6	0.009820	28, 2, 29
	Ursidae	*Thalarctos maritimus*	320,000.0	63.6	55.2	59.4	0.000664	28, 2, 29
		Ursus americanus	97,000.0	59.1	43.3	51.2	0.001786	28, 2, 11
Pinnepedia	Phocidae	*Phoca vitulina*	69,694.0†	36.0	62.2	49.1	0.002675	7, 2, 3
		Phoca groenlandica	136,000.0†	—	16.0	16.0	0.001126	30, 2, 3
		Phoca fasciata	90,000.0	—	46.3	46.3	0.002196	30, 2, 3
Sirenia	Dugonidae	*Dugong dugon*	456,990.2†,#	—	132.2	132.2	0.000276	31, 2, 3
Proboscidea	Elephantidae	*Loxondontia africana*	2,766,000.0	182.1	158.0	170.1	0.000915	7, 2, 32
Perissodactyla	Equidae	*Equus caballas*	409,778.0	30.0	40.8	35.4	0.000929*	7, 2, 3
Artiodactyla	Antilocapridae	*Antilocapra americana*	57,358.5†,#	12.0	22.7	17.4	0.001563	33, 2, 10
	Bovidae	*Bos taurus*	636,396.1†,#	—	27.3	27.3	0.000884	33, 34, 35, 3
		Capra hircus	27,700.0	29.0	18.8	23.9	0.000106	36, 2, 37
	Cervidae	*Odilocoileus hemionus*	56,300.0	22.6	24.3	23.4	0.001633	7, 2, 3
		Odilocoileus virginianus	39,700.0	13.8	13.1	13.5	0.001633*	38, 2, 3
	Camelidae	*Camelus dromedarius*	540,832.7†,#	52.2	66.8	59.5	0.000563	33, 2, 3
		Lama pacos	59,791.3†,#	—	22.5	22.5	0.001295	33, 2, 3
	Cervidae	*Cervus nippon*	45,250.0	36.0	19.5	27.8	0.001678	39, 40, 2, 3
		Cervus unicolor	133,195.3*	24.5	—	24.5	0.001678	41, 2, 3
	Suidae	*Sus scrofa*	54,781.0	10.6	7.9	9.3	0.000771	7, 2, 3
	Tayassuidae	*Tayassu tajacu*	18,500.0	—	16.1	16.1	0.001633	42, 2, 3
	Tragulidae	*Tragulus jarvanicus*	2,102.4†,#	9.5	9.6	9.6*	0.003038	33, 2, 3
		Tragulus napu	4,899.0†,#	9.5	9.6	9.6	0.003038*	33, 2, 3
Rodentia	Caviidae	*Cavia porcellus*	1,000.0†	—	4.4	4.4	0.003091	11, 2, 3
	Geomyidae	*Geomys bursarius*	367.4†,#	—	3.6	3.6	0.003959	6, 2, 3
	Gliridae	*Glis glis*	140.0†	11.5	38.6	25.1	0.003289	43, 2, 3
	Hydrochoeridae	*Hydrochaeris hydrochaeris*	49,416.6#	—	20.8	20.8	0.001408	33, 2, 3
	Hystricidae	*Hystrix africaeaustralis*	12,600.0	—	24.1	24.1	0.001166	44, 2, 45
	Muridae	*Rattus norvegicus*	202.8	5.5	4.7	5.1	0.005647	45, 2, 3
		Mus musculus	15.8	3.4	3.2	3.3	0.009412	40, 2, 3

Table 2. Continued.

Order	Family	Species	Mass (g)	Generation time (mth) A	B	Avg	Metabolic rate (W/g)	Source
	Sciuridae	*Sciurus niger*	809.6	—	13.1	13.1	0.003817*	47, 2, 3
		Spermophilus richardsoni	225.0	12.0	11.8	11.9	0.002710	47, 2, 3
	Capromyidae	*Capromys piliorides*	5,486.3†,#	—	14.2	14.2	0.001278	6, 2, 3
	Erethizontidae	*Erethizon dorsatum*	94.9†,#	—	32.3	32.3	0.002027*	6, 2, 3
Lagomorpha	Leporidae	*Oryctolagus curiculus*	1,546.3	4.0	6.2	5.1	0.003660	7, 2, 3
		Lepus europeaus	3,463.5	—	8.0	8.0	0.003167	48, 2, 3

[a] Species names from the sequence description. Assignment of species to families and orders follows Corbet and Hill 1991. Two measures of generation time (and their average value) are included: A is age at first birth; B is age at sexual maturity plus gestation (see text). An asterisk (*) indicates data from a similar congeneric when no species-specific data was available. A number sign (#) indicates a geometric mean calculated from range data (square root of the product of the maximum and minimum). A cross (†) marks mean values for both sexes, where female mean unavailable. Annotated sources are as follows: 1. Strahan 1983; 2. Hayssen *et al.* 1993; 3. Heusner 1991; 4. Boitani and Bartoli 1986; 5. Eisenberg 1989; 6. Grzimek 1990; 7. Purvis and Harvey 1995; 8. Stephan *et al.* 1981; 9. Boitani and Bartoli 1982; 10. McNab 1988; 11. Eisenberg 1981; 12. Fleagle 1988; 13. Harvey *et al.* 1987; 14. Emmons and Feer 1990; 15. Plavcan and Gomez 1993; 16. Damuth 1993; 17. Dagosto and Terranova 1992; 18. Kappeler 1990; 19. Pagés-Feulliade 1988; 20. Simons 1988; 21. Smuts *et al.* 1987; 22. Nash *et al.* 1989; 23. Crockett and Eisenberg 1987; 24. Elgar and Harvey 1987; 25. Ross 1992; 26. Richard 1987; 27. Bearder 1987; 28. Oftedal and Gittleman 1989; 29. McNab 1989; 30. King 1983; 31. Husar 1978; 32. Langman *et al.* 1995; 33. Macdonald 1989; 34. Adsel 1964; 35. Nowak 1991; 36. Rudge 1990; 37. McNab 1986; 38. Davidson 1990a; 39. Davidson 1990b; 40. Macdonald and Barret 1993; 41. Douglas 1990; 42. Sowls 1984; 43. Corbet and Harris 1991; 44. Skinner and Smithers 1990; 45. Haim *et al.* 1990; 46. Moors 1990; 47. Heany 1984; 48. Flux 1990

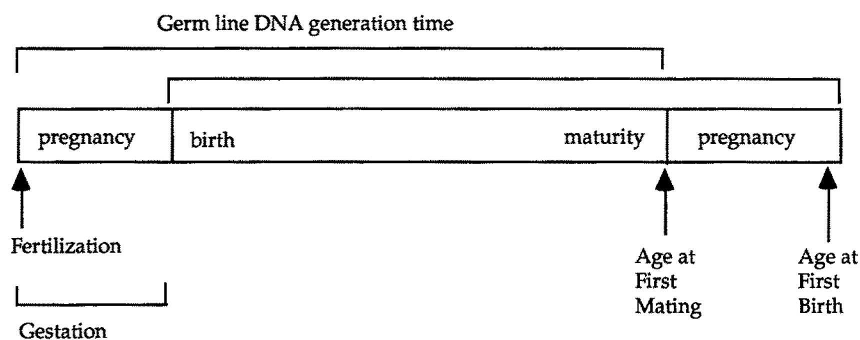

Fig. 1. DNA generation time can be measured birth-to-birth (age at first birth) or fertilization-to-fertilization (age at first mating plus gestation).

employed to find any association between genetic distance and body mass, generation time, and/or metabolic rate.

Results

Of the five sequences tested, cyt*b* and beta-globin showed significant correlations between log genetic distance and log generation time (Fig. 2), and beta-globin introns and epsilon-globin exons showed significant correlations between genetic distance and body mass (Fig. 3). The results summarized below are only those that satisfy the assumptions of the tests (linearity of relationship and homogeneity of error) and which are not attributable to covariation of life-history parameter as assessed by partial correlation analyses. Results of statistical tests are given in the appropriate figure legends. None of the ML analyses produced significant correlations (including the cyt*b* and 12s trees with independent estimates of TS/TV, and the whole globin trees). For the protein-coding sequences, synonymous and nonsynonymous distances were analyzed separately. None of the nonsynonymous distances showed any significant correlations with the biological parameters.

Cytochrome b: A significant correlation between log relative-rates (RR) distance and log generation time was robust to removal of *Loxodontia/Dugong* comparison, a pair for which the genetic distance is particularly high due to the curiously divergent elephant cyt*b* gene (Irwin et al. 1991; Ma et al. 1993; Irwin and Arnason 1994).

12s RNA: No significant correlations beyond spurious associations caused by outliers (e.g., *Equus/Pongo*).

Beta-globin exons: All points but one fit a clear linear relationship between log RR distance and log generation time (Fig. 2B). Without this outlier (*Lemur/Galago*) the correlation coefficient is highly significant.

Beta-globin introns: A significant relationship exists between genetic distance (RR) and log body mass (Fig. 3A). There is no evidence that this relationship was

caused by covariation of body mass with generation time or metabolic rate, as neither variable showed a significant association with genetic distance.

Epsilon-globin exons: A significant correlation between genetic distance and mass exits, which is robust to the removal of extreme points (Fig. 3B). Note, however, the uneven spread of data.

Epsilon-globin introns: No significant correlations.

Discussion

Of the four genes analyzed, three showed correlations between life-history parameters and substitution rate: the rate of fourfold degenerate substitutions in both cytochrome *b* and beta-globin exons was negatively correlated with species generation time, and the rate of all substitutions in both beta-globin introns and epsilon-globin exons was negatively correlated with average body mass. For the remaining sequences tested (12s rRNA, epsilon-globin introns), there were no significant correlations between substitution rate and mass, generation time, or metabolic rate. These data lend no support to the hypothesis that metabolic rate is a direct causal factor in variation in rate of molecular evolution. Instead, the results point to a relationship between generation time and rate of substitution and also suggest the possibility of a body size effect on molecular evolution.

Although few sequences were able to be tested for this study, the strength of this approach lies in both the number of comparisons used and in the removal of phylogenetic bias through the use of independent contrasts. These two factors allow the identification of broad-scale patterns. The results indicate a broad trend in rate heterogeneity, which cannot be an artifact of comparison of widely divergent groups such as rodents and primates or endotherms and ectotherms. The importance of using a large number of independent contrasts is illustrated by previous dismissals of a generation time effect, based primarily on citing examples that do not fit the expected pattern (Springer and Kirsch 1989; Martin and Palumbi 1993; Rand 1994). Some of these exceptions may be the result of massive biological divergence (e.g., sharks vs primates: Martin and Palumbi 1993; Rand 1994) or inaccurate life-history characteristics (Springer and Kirsch 1989; see Cockburn et al. 1990). While such counterexamples provide interesting cases for further study, the results of the analyses reported here show that these examples are exceptions which do not negate the broad-scale relationship between generation time and substitution rate in mammals. Similarly, while differences between the metabolic rates of highly divergent taxa have been cited in support of the metabolic rate theory, this study finds no evidence of a broadly applicable relationship between the rates of metabolism and molecular evolution in mammals. Similar conclusions have been reached for birds (Mooers and Harvey 1994).

What do the results of this study have to say about mammalian sequence evolution? Clearly there are differences in the rate of substitution between lineages. Rate heterogeneity was evident only for silent changes: fourfold degenerate substitutions in all three protein-coding sequences and all substitutions in the introns of one of two nuclear-encoded proteins. These observations suggest that differences in mutation rate underlie rate variation. So in order to understand the causes of rate heterogeneity, it is necessary to focus on the mutation process.

Mutations can occur in two ways: imperfectly repaired damage to DNA and copy errors during replication. DNA can suffer spontaneous damage by chemical or physical factors in the cell environment, including products of metabolism. A variety of repair mechanisms exist to correct damage, the most commonly used of which is excision repair (Kornberg 1980): The lesion is recognized by special endonucleases which nick the DNA strand upstream of the lesion, a signal to exonucleases to excise and replace the damaged strand. Mutations are created through replication if errors occur and fail to be corrected by a three-stage repair process. First, the correct base must be selected for insertion, possibly by energetic stability or structural geometry. Second, the polymerase has a proofreading function, which may recognize, excise, and replace a newly added nucleotide if it is a mismatch. Third, errors that evade the quality-control and proofreading mechanisms of the replication enzymes may be repaired by post-replicative repair pathways, typically excision-repair (Echols and Goodman 1991; Kunkel 1992). This three stage quality control pathway limits the number of copy errors that are produced per DNA replication. Damage to DNA may also be repaired at replication if it causes a lesion that stalls the polymerase. In such cases the "SOS" repair pathway may be instigated, resulting either in error-free bypass of the lesion or in SOS-induced mutagenesis (Echols and Goodman 1991).

Substitution rate is a net imbalance between mutation (damage or copy error) and repair. For lineages to differ in substitution rate, they must vary in mutation rate, repair efficiency, or both. Is it possible to distinguish the relative contributions of these possible causes of rate differentiation? Interplay of factors makes this difficult: not only do body size, generation time, and metabolic rate vary together, but all of these factors may interact to produce differences in substitution rate.

Mechanisms of repair may differ between groups, notably rodents and primates (Hart and Setlow 1974; Filipski 1988; Holmquist and Filipski 1994), and so are potentially responsible for rate differences. Using independent comparisons across a wide range of mammal species should reduce the effect of phylogenetic patterns of repair mechanisms but does not rule out variable levels of "fine-tuning" repair efficiency. Results of recent studies (see Sullivan 1995) suggest that repair efficiency

Table 3. Comparisons (pair plus outgroup) for the mitochondrial genes cytochrome *b* and 12s rRNA, with differences in mass, generation time, metabolic rate, and distance since common origin (both maximum likelihood [ML] and relative rates tests [RR] for each pair[a])

Gene	Pair		Outgroup
Cyt*b*	*Antechinus flavipes*	*Planigale ingrami*	*Didelphus virginiana*
	Didelphus virginiana	*Monodelphus brevicaudata*	*Antechinus flavipes*
	Loxodontia africana	*Dugong dugon*	*Equus caballas*
	Hystrix africaeaustralis	*Cavia porcellus*	*Monodelphus brevicaudata*
	Rattus norvegicus	*Mus musculus*	*Spermophilus richardsoni*
	Sciurus niger	*Spermophilus richardsoni*	*Rattus norvegicus*
	Panthera leo	*Felis domesticus*	*Thalarctos maritimus*
	Thalarctos maritimus	*Ursus americanus*	*Panthera leo*
	Homo sapiens	*Pan troglodytes*	*Pongo pygmaeus*
	Odilocoileus hemionus	*Cervus nippon*	*Bos taurus*
	Bos taurus	*Capra hircus*	*Cervus nippon*
	Camelus dromedarius	*Lama pacos*	*Tayassu tajacu*
	Sus scrofa	*Tayassu tajacu*	*Lama pacos*
	Equus caballas	*Tragulus jarvanicus*	*Pongo pygmaeus*
	Phoca groenlandica	*Phoca fasciata*	*Thalarctos maritimus*
	Oryctolagus cunniculus	*Geomys bursarius*	*Planigale ingrami*
12s	*Bos taurus*	*Capra hircus*	*Odocoileus virginianus*
	Cervus unicolor	*Odocoileus virginianus*	*Bos taurus*
	Antilocapra americana	*Tragulus napu*	*Equus caballus*
	Homo sapiens	*Pan troglodytes*	*Pongo pygmaeus*
	Atelerix albiventris	*Blarina brevicauda*	*Mus musculus*
	Rattus norvegicus	*Mus musculus*	*Cavia porcellus*
	Hydrochaeris hydrochaeris	*Cavia porcellus*	*Hystrix africaeaustralis*
	Capromys pilioridaes	*Erithizon dorsatum*	*Glis glis*
	Hystrix africaeaustralis	*Glis glis*	*Pongo pygmaeus*
	Equus caballus	*Pongo pygmaeus*	*Didelphus virginiana*

[a] Log values represent the difference of the logged values for each pair (i.e., the difference of the logs, not the log of the difference). ML distances using independently derived estimates of transition:transversion ratio are not included in this table, nor are the relative rates measures for nonsynonymous changes for cyt*b*—these data are available from the authors.

can vary across the genome, so it seems entirely possible that differences in repair fine-tuning could exist between mammalian lineages, possibly in response to selection pressure associated with body size or generation time.

The lack of an observable relationship between genetic distance and metabolic rate argues against an important role for ongoing oxidative damage in raising the mutation rate and thus increasing the rate of substitution. It is possible, however, that the mutagenic effects of higher metabolism are "lost" in the generation time effect: If most mutagen-induced damage occurs at DNA replication when DNA, unwrapped and unzipped, is relatively unprotected, then an increase in mutagen-induced substitutions would be linked to the number of DNA replications per unit time. If this was so, then species with a high metabolic rate for their generation time would suffer more damage per replication and so have a higher substitution rate. Partial correlations of generation time and metabolic rate provide no support for this hypothesis. However, given the relatively small number of independent comparisons available for this study (and all previous studies), type 1 error rates are likely to be quite high. That is, any influence of metabolic rate on molecular evolution would have to be strong for us to be able to reject the null hypothesis of no correlation. That being said, significant correlations were found with both generation time and body mass.

Differences in levels of mutagens or repair efficiency could cause variation in the number of substitutions per DNA replication. Absolute differences in substitution rate—the number of substitutions per lineage per unit time—could also be caused by frequency of opportunities for substitutions. The more DNA replications per unit time, the greater the chance for mutations caused at replication (copy error or damage) to accumulate. This possibility is often cited as the cause of a generation time effect: Small animals have more generations per unit time and therefore copy their DNA more often, and so collect more mutations. However, the relationship between species generation time and number of DNA replications is not so straightforward. Replications per unit time is a product of organism generation time, number of germ-line cell divisions per organism generation, and number of DNA replications per cell division. Species can differ not only in generation length but also in number of cell divisions per generation (Chang et al. 1994; Li et al. 1996). Number of DNA replications per cell division is a function of gene location, whether in the nucleus or a cytoplasmic organelle, which may divide independently of cell division.

Table 3. Continued

Mass		Generation times		Metabolic rate		Distance			
(g)	Log mass	(mth)	log GT	(W/g)	log MR	ML	log ML	RR	log RR
24	1.224	1.49	0.135	0.0033	0.476	−0.077	−0.470	0.069	0.112
4815	4.066	−0.20	−0.031	−0.0020	−0.735	0.024	0.128	−0.192	−0.342
2309010	1.800	37.82	0.252	0.0006	1.199	0.146	0.738	0.462	1.032
11600	2.534	19.69	1.7	−0.0019	−0.975	−0.099	−0.569	−0.568	−1.154
187	2.552	1.82	0.438	−0.0038	−0.511	−0.029	−0.243	−0.223	−0.567
584.6	1.280	1.22	0.098	0.0011	0.343	0.103	0.660	—	—
132883	3.947	28.50	1.213	−0.0030	−1.395	0.009	0.114	−0.320	−0.946
223000	1.194	8.20	0.149	−0.0011	−0.989	−0.006	−0.093	0.403	—
8206.4	0.194	18.87	0.117	−0.0003	−0.205	0.019	0.258	−0.007	−0.044
11050	0.218	−4.32	−0.169	−0.0001	−0.027	0.002	0.022	0.045	0.183
608696.1	3.134	3.43	0.134	0.0008	2.121	0.019	0.200	0.049	0.195
481041.4	2.202	37.05	0.975	−0.0007	−0.833	0.020	0.190	0.131	0.422
36281	1.086	−6.85	−0.554	−0.0009	−0.750	−0.040	−0.323	−0.012	−0.036
407675.6	5.273	25.85	1.31	−0.0021	−1.185	−0.066	−0.377	−0.037	−0.061
46000	0.413	−30.25	−1.061	−0.0011	−0.668	0.034	0.893	0.137	1.779
1178.9	1.437	1.50	0.348	−0.0003	−0.079	−0.067	−0.260	0.127	0.173
608696.1	3.130	3.43	0.13	0.0008	2.121	−0.002	−0.046	0.006	0.102
93495.35	1.210	11.03	0.6	0.0001	0.027	−0.015	−0.547	−0.034	−1.070
52459.54	2.460	7.80	0.6	−0.0015	−0.665	0.001	0.016	−0.012	−0.101
8206.41	0.190	18.87	0.12	−0.0003	−0.205	−0.005	−0.286	0.007	0.373
192.67	2.380	−5.75	−0.74	−0.0121	−2.016	0.041	0.425	0.105	0.706
187	2.550	1.82	0.44	−0.0038	−0.511	0.001	0.022	0.035	1.234
48416.6	3.900	16.44	1.56	−0.0017	−0.786	−0.035	−0.469	−0.053	−0.407
5391.48	4.060	−18.05	−0.82	−0.0008	−0.461	−0.023	−0.275	−0.095	−0.707
12460	4.500	−0.98	−0.04	−0.0021	−1.037	0.027	0.270	0.164	0.966
394571.3	3.290	−81.47	−1.19	−0.0008	−0.589	−0.102	−0.855	−0.274	−1.060

Shimmin et al. (1993) suggest that mutations occurring at replication are the primary cause of substitution. They employ ''male-driven evolution'' as a test of the contribution of replication errors to the generation time effect. In mammals, more cell divisions are required to produce a male gamete than a female gamete, so the more time a sequence spends in males, the higher its rate of accumulation of replication errors. If autosomal genes (which spend an average of half their time in males) show a greater generation time effect than X-linked genes (which spend only one-third of their time in males), then the generation time effect is likely to be a product of replication errors. If the difference between autosomal and X-linked genes is small, then the generation time effect is due to other causes (Shimmin et al. 1994). Chang et al. (1994) argued that agreement between the male-to-female ratio of number of cell replications per organism generation and the ratio of rates of evolution of Y- and X-linked genes suggests that errors in replication are the main source of mutation in rodents.

The confusion of interacting factors that may potentially affect the substitution rate can be illustrated by the mitochondrial-encoded protein gene, cytochrome *b*. The link between replication of this gene and species generation time is far from clear. While mitochondria must replicate before cell division, cycles of division and replacement of mitochondria occur throughout the lifetime of the cell (Brown 1983). Because mitochondrial turnover per cell generation could vary, depending perhaps on the life span and energy expenditure of the cell, the potential exists for mitochondrial replication to be effectively decoupled from cell division. In addition, mitochondria are expected to be highly responsive to the effects of oxidative damage from metabolism. Free oxygen radicals can concentrate in mitochondrial membranes, and turnover of mitochondria is greatest where metabolic requirements are highest (Rand 1994). Moreover, mitochondria may lack many of the repair pathways available to the nuclear genome (Brown 1983). However, cytochrome *b* does not show a relationship between substitution rate and metabolic rate. Instead, a correlation between generation time and substitution rate is evident. Why?

There are two possible explanations for a generation time effect in mitochondrial genes. The first is that the potential for decoupling of mitochondrial replication from cell division is not realized in mammalian germline cells. Like the relationship between generation length and number of cell divisions per generation, variable numbers of mitochondrial divisions per cell division may serve to dampen but not negate a generation time effect. Alternatively, the generation time effect may be an artefact of a correlation between generation time and the true casual factor. This may also explain the correlation between genetic distance and mass for beta-globin intron, over and above the effects of generation time.

Table 4. Comparisons (pair plus outgroup) for the nuclear genes beta- and epsilon-globin, with differences in mass, generation time, metabolic rate, and distance since common origin (both maximum likelihood [ML] and relative-rates tests [RR]) for each pair[a]

	Pair			Mass	
Gene	(g)	Log mass	Outgroup	(g)	Log mass
Beta-globin	*Homo sapiens*	*Pan troglodytes*	*Macaca fascicularis*	8206.4	0.194
	Colobus polykomos	*Macaca fascicularis*	*Pan troglodytes*	2498.5	0.466
	Lemur macaco	*Galago crassicaudatus*	*Tarsius syrichta*	1457	0.851
	Rattus norvegicus	*Mus musculus*	*Oryctolagus cuniculus*	187	2.552
	Lepus europeaus	*Oryctolagus cuniculus*	*Rattus norvegicus*	1917	0.806
	Bos taurus	*Sus crofa*	*Mus musculus*	581615	2.452
	Didelphus virginiana	*Tarsius syrichta*	*Tachyglossus aculeatus*	4780.1	3.719
Epsilon-globin	*Callithrix jacchus*	*Cebuella pygmaea*	*Samiri sciureus*	169	0.910
	Allouatta seniculus	*Semiri sciureus*	*Cebuella pygmaea*	5715.3	2.261
	Cheirogaleus medius	*Microcebus murinus*	*Lemur macaco*	135	1.201
	Galago crassicaudatus	*Mycticebus coucang*	*Cheirogaleus medius*	82.5	0.079
	Homo sapiens	*Pan troglodytes*	*Pongo pygmaeus*	8206.4	0.194
	Propithecus verreauxi	*Lemur macaco*	*Cheirogaleus medius*	1227.9	0.394
	Pongo pygmeaus	*Macaca mulatta*	*Lemur macaco*	9502.2	0.980
	Tupaia glis	*Cynopterus sphinx*	*Oryctolagus cuniculus*	60	0.511

[a]Log values are the differences of the logged values for each pair (i.e., the difference of the logs, not the log of the difference). Distances for exons and introns are given separately. ML distances for the whole sequences (exons plus introns) and RR distances for nonsynonymous changes in the exons are not shown (but are available from the authors)

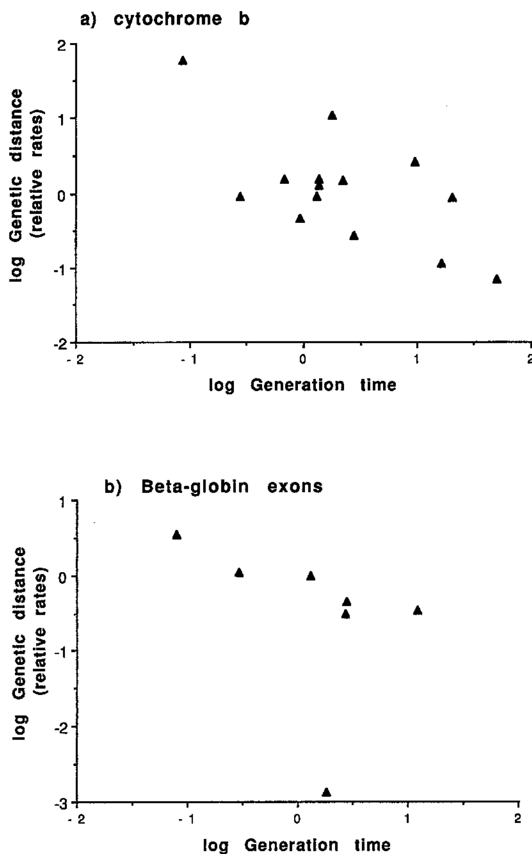

Fig. 2. Plots of log genetic distance (calculated by relative-rates tests) against log generation time for two sequences. Each point represents the difference between an independent pair of species. **A** Cytochrome b ($R^2 = -0.67$, $P < 0.01$). The *point at the top left* of the graph is *Loxodontia/Dugong*. If removed, the relationship is still significant ($R^2 = -0.71$, $P < 0.01$). **B** Beta-globin exons. The *point just above the X-axis* represents the *Lemur/Galago* comparison, a conspicuous outlier. Without this comparison, the association between log genetic distance and log generation time is significant ($R^2 = -0.93$, $P < 0.01$).

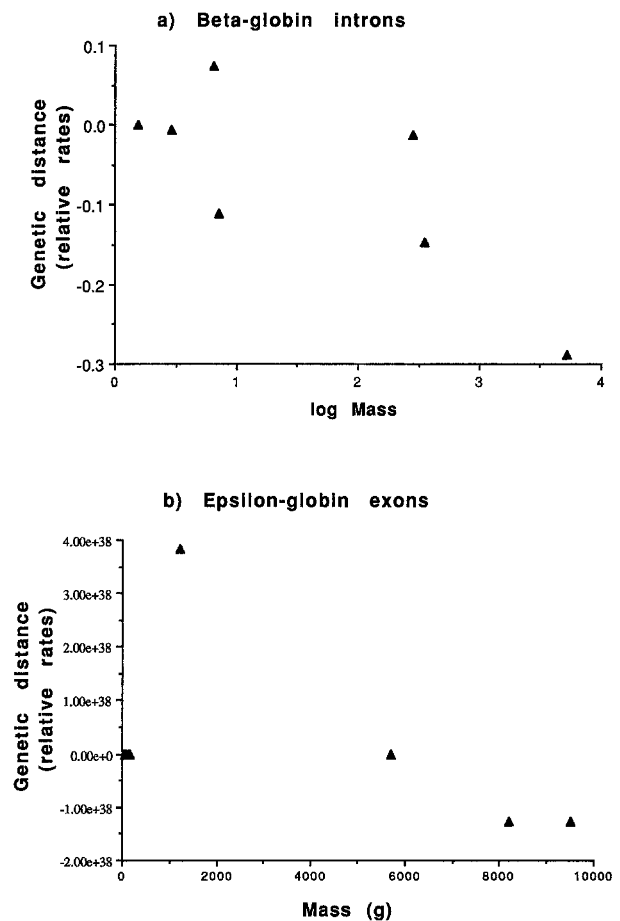

Fig. 3. **A** Genetic distance (relative-rates method) against log body mass for beta-globin introns ($R^2 = -0.77$, $P < 0.05$). **B** Genetic distance (relative-rates method) against mass for epsilon-globin exons ($R^2 = -0.87$, $P < 0.05$). The correlation is robust to the removal of the *point in the top left quadrant* ($R^2 = -0.86$, $P < 0.05$), although this relationship might be questioned due to the uneven spread of points.

Table 4. Continued

Generation time		Metabolic rate		Distance: exons		Distance: introns					
(mth)	log GT	(W/g)	log MR	ML	log ML	RR	log RR	ML	log ML	RR	log RR
18.9	0.117	−0.00027	−0.20452	0.000	—	0.000	0.000	0.000	0.000	0.000	—
−21.2	−0.530	−0.00117	−0.53595	−0.019	−1.001	0.002	0.045	0.000	−0.017	−0.006	−0.545
7.4	0.259	−0.00102	−0.44753	0.061	0.789	−0.225	−2.876	0.056	0.507	−0.110	−2.115
1.8	0.438	−0.00376	−0.51083	−0.071	−0.933	−0.060	−0.505	−0.108	−0.852	−0.147	−0.733
2.9	0.450	−0.00049	−0.14467	−0.011	−0.864	−0.006	−0.339	0.089	1.727	0.074	—
18.1	1.083	0.00011	0.13622	−0.002	−0.020	−0.061	−0.457	−0.057	−0.597	−0.013	−0.066
−12.6	−1.100	−0.00208	−0.75209	0.086	0.454	0.213	0.553	0.226	0.603	−0.289	−1.641
−12.4	−0.463	−0.0071	−0.14605	0.000	—	0.000	0.000	−0.001	−0.225	0.056	—
10.1	0.202	−0.00301	−0.79522	0.006	0.409	0.019	0.425	0.102	0.379	0.001	0.036
0.5	0.038	−0.00142	−0.32643	−0.014	−0.833	−0.018	−0.653	−0.016	−0.353	−0.018	−0.368
−6.3	−0.224	0.00149	0.75099	0.004	0.170	0.003	0.124	0.000	0.009	0.023	0.428
18.9	0.117	−0.00027	−0.20452	0.000	—	0.000	0.000	0.004	0.510	0.003	0.403
2.4	0.072	−0.00069	−0.47937	0.011	0.543	—	—	−0.004	−0.116	−0.006	−0.143
63.4	0.782	−0.00039	−0.20972	−0.006	−1.191	−0.016	−0.143	−0.004	−0.127	−0.008	−0.268
−3.2	−0.419	−0.00279	−0.50491	0.041	0.768	0.121	0.854	0.003	0.012	−0.067	−0.175

One candidate variable for which generation time may be a surrogate is body size. Maintenance of a large body, with many more cells and cell generations than a small body, should demand a higher level of DNA copy fidelity and repair. In order to test for a body-maintenance effect, correlation between DNA repair efficiency and body size must be established. Promislow (1994) finds little support for this relationship, but his findings are based upon reanalysis of several studies of maximum recorded life span and in vitro measurements of ability to repair UV-induced DNA damage. Maximum recorded life span is a problematic measure of longevity: The value may be atypical for the species, and can only increase with further study. Repair of UV-induced damage is an important mechanism for cell survival, but it is only one specific aspect of damage repair, and as such it is possibly a poor indicator of the fine-tuning of repair and copy fidelity. A correlation between average age of adult mortality and substitution rate would be a more appropriate test of the hypothesis.

The results from this study indicate that rate of accumulation of fourfold degenerate substitutions in protein sequences and substitutions in introns may be influenced by the generation length (or some other covarying factor). Fourfold degenerate substitutions are the closest approximation to neutral sites included in this analysis and thus are expected to most closely reflect the neutral mutation rate. Both the metabolic rate hypothesis and the generation time effect explain rate heterogeneity by variation in the mutation rate (either by increased mutagenesis or greater opportunity for replication errors), so neutral sites are an appropriate test of these theories. ML trees included all substitutions not just neutral changes which may explain the lack of relationships between ML rate estimates and biological parameters. A relationship between generation time and non-neutral changes has been proposed (e.g., Rohde 1992). Evolution of pesticide resistance has been cited as an example of the effect of generation time on adaptive evolution (May 1993), though this relationship is disputed (Rosenheim and Tabashnik 1991, 1993). The absence of significant correlation between rate of nonsynonymous substitutions and generation time for the protein genes included in this study shows no link between generation time rate of non-neutral evolution for mammals for these sequences.

The concept of the "nucleotide generation time" has been proposed to explain differences in the rate of molecular evolution in terms of the probability of a given nucleotide being replaced by replication error or damage repair (Martin and Palumbi 1993). This concept allows for the interplay of factors affecting the substitution rate, many of which may be difficult to assess separately. This study indicates that for the mammalian genes studied, metabolic rate does not appear to make a large contribution to nucleotide generation time: Mammals with a high metabolic rate for their size or generation time do not have a noticeably higher substitution rate. Instead, some aspect of the biology of mammals that scales with generation time is affecting the substitution rate. It may be possible with further research to tease apart the potential causes of rate variation, or it may be that their interconnectedness will thwart attempts to further simplify our understanding of molecular evolution.

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References

Adachi J, Cao Y, Hasegawa M (1993) Tempo and mode of mitochondrial DNA evolution in vertebrates at the amino acid sequence level: rapid evolution in warm blooded vertebrates. J Mol Evol 36:270–281

Adsel SA (1964) Patterns of mammalian reproduction. Cornell University Press, New York

Avise JC, Bowden BW, Lamp T. Meylan AB, Bermingham E (1992) Mitochondrial DNA evolution at a turtles pace—evidence for low genetic variability and reduced microrevolutionary rate in the testudines. Mol Biol Evol 9:457–472

Bailey WJ, Fitch DHA, Tagle DA, Czelusniak J (1991) Molecular evolution of the psi-eta-globin gene locus: gibbon phylogeny and the molecular clock. Mol Biol Evol 8:155–184

Bearder SK (1987) Lorises, bushbabies and tarsiers: diverse societies in solitary foragers. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW (eds) pp 11–25

Boitani L, Bartoli S (1982) Simon and Schuster's guide to mammals. Simon and Schuster, New York

Boitani L, Bartoli S (1986) Macdonald encyclopedia of mammals. Macdonald, London

Britten RJ (1986) Rates of DNA sequence evolution differ between taxonomic groups. Science 231:1393–1398

Brown WM (1983) Evolution of animal mitochondrial DNA. In: Nei M, Koehn RK (ed) Evolution of genes and proteins. Sinauer, Sunderland, MA, pp 62–88

Brown WM, Prager EM, Wang A, Wilson AC (1982) Mitochondrial DNA sequences of primates: tempo and mode of evolution. J Mol Evol 18:225–239

Bulmer M, Wolfe K, Sharp PM (1991) Synonymous nucleotide substitution rate in mammalian genes: implications for the molecular clock and the relationship of mammalian orders. Proc Natl Acad Sci USA 88:5974–5978

Burt A (1989) Comparative methods using phylogenetically independent contrasts. Oxf Surv Evol Biol 6:33–53

Chang BH-J, Shimmin LC, Shyue S-K, Hewett-Emmett D, Li W-H (1994) Weak male-driven molecular evolution in rodents. Proc Natl Acad Sci USA 91:827–831

Cockburn A, Mansergh IM, Broome MS, Ward S (1990) Molecular clocks and generation time in Burramyid marsupials. Mol Biol Evol 7:283–285

Corbet GB, Harris S (1991) The handbook of British mammals. Blackwell Scientific, Oxford

Corbet GB, Hill JE (1991) A world list of mammalian species. Oxford University Press, Oxford

Crockett CM, Eisenberg JF (1987) Howlers: variations in group size and demography. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (eds) Primate societies. University of Chicago, Chicago, pp 54–68

Dagosto M, Terranova CJ (1992) Estimating the body size of Eocene primates—a comparison of results from dental and postcranial variables. Int J Primatol 13:307–344

Damuth J (1993) Copes rule, the island rule and the scaling of mammalian population density. Nature 365:748–750

Davidson MM (1990a) White-tailed deer. In: King CM (ed) The handbook of New Zealand mammals. Oxford University Press, Auckland, pp 507–514

Davidson MM (1990b) Sika deer. In: King CM (ed) The handbook of New Zealand mammals. Oxford University Press, Auckland, pp 468–477

Douglas MJW (1990) Sambar deer. In: King CM (ed) The handbook of New Zealand mammals. Oxford University Press, Auckland, pp 477–483

Easteal S, Collett C (1994) Consistent variation in amino-acid substitution rate, despite uniformity of mutation rate: protein evolution in mammals is not neutral. Mol Biol Evol 11:643–647

Echols H, Goodman MF (1991) Fidelity mechanisms in DNA replication. Annu Rev Biochem 60:477–511

Eisenberg JF (1981) The mammalian radiations. The Athalone Press, London

Eisenberg JF (1989) Mammals of the neotropics: the northern neotropics. University of Chicago Press, Chicago

Elgar MA, Harvey PH (1987) Basal metabolic rate in mammals: allometry, phylogeny and ecology. Funct Ecol 1:25–36

Emmons LH, Feer F (1990) Neotropical rainforest mammals: a field guide. University of Chicago, Chicago

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125:1–15

Filipski J (1988) Why the rate of silent codon substitutions is variable within a vertebrate's genome. J Theor Biol 134:159–164

Fleagle JG (1988) Primate adaptation and evolution. Academic Press, San Diego

Flux JEC (1990) Brown hare. In: King CM (ed) The handbook of New Zealand mammals. Oxford University Press, Auckland, pp 161–174

Garland T, Janis CM (1993) Does metarsal/femur ratio predict maximal running speed in cursorial mammals? J Zool (London) 229:133–151

Grzimek B (ed) (1990) Grzimek's encyclopedia of mammals. McGraw-Hill, New York

Haim A, van Aarde RJ, Skinner JD (1990) Metabolism and thermoregulation in the cape porcupine, *Hystrix africaeaustralis.* Physiol Zool 63:795–802

Hart RW, Setlow RB (1974) Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammal species. Proc Natl Acad Sci USA 71:2169–2173

Harvey PH, Pagel M (1991) The comparative method in evolutionary biology. Oxford University Press, Oxford

Harvey PH, Martin RD, Clutton-Brock TH (1987) Life histories in comparative perspective. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (ed) Primate societies. University of Chicago, Chicago, pp 181–198

Hasegawa MH, Kishino H, Yano T (1989) Estimation of branching dates among primates by molecular clocks of nuclear DNA which slowed down in Hominidae. J Hum Evol 18:461–476

Hayssen V, van Tienhoven A, van Tienhoven A (1993) Asdell's patterns of mammalian reproduction: a compendium of species-specific data. Cornell University Press, New York

Heaney LR (1984) Climatic influences on life-history tactics and behavior of North American tree squirrels. In: Murie JO, Michener GR (ed) The biology of ground-dwelling squirrels. University of Nebraska Press, pp 43–78

Heusner AA (1991) Size and power in mammals. J Exp Biol 160:25–54

Holmes EC (1991) Different rates of substitution may produce different phylogenies of eutherian mammals. J Mol Evol 33:209–215

Holmquist GP, Filipski J (1994) Organization of mutations along the genome: a prime determinant of genome evolution. Trends Evol Ecol 9:65–68

Husar SL (1978) *Dugong dugon.* Mamm Species 88–1–7

Ina Y (1995) New methods for estimating the numbers of synonymous and nonsynonymous substitutions. J Mol Evol 40:190–226

Irwin DM, Arnason U (1994) Cytochrome b gene of marine mammals: phylogeny and evolution. M Mamm Evol 2:37–55

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome b gene of mammals. J Mol Evol 32:128–144

Kappeller PM (1990) The evolution of sexual size dimorphism in prosimian primates. Am J Primatol 21:201–214

King JE (1983) Seals of the world. Oxford University Press, Oxford

Kornberg A (1980) DNA replication. W.H. Freeman, San Francisco

Kumar S, Tamura K, Nei M (1993) MEGA: molecular evolutionary genetics analysis version 1.01. The Pennsylvania State University, University Park, PA

Kunkel TA (1992) DNA replication fidelity. J Biol Chem 267:18251–18254

Langman VA, Roberts TJ, Black J, Maloiy MO, Hegland NC, Weber J-M, Kram R, Taylor CR (1995) Moving cheaply; energetics of walking in the African elephant. J Exp Biol 198:629–632

Li W-H, Graur D (1991) Fundamentals of molecular evolution. Sinauer, Sunderland, MA

Li W-H, Tanimura M, Sharp PM (1987) An evaluation of the molecular

clock hypothesis using mammalian DNA sequences. J Mol Evol 25:330–342

Li W-H, Gouy M, Sharp P, O'Huigin C, Yang Y-W (1990) Molecular phylogeny of Rodentia, Lagomorpha, Artiodactyla and Carnivora and molecular clocks. Proc Natl Acad Sci USA 87:6703–6707

Li W-H, Ellesworth DL, Krushkal J, Chang BH-J, Hewett-Emmett D (1996) Rates of nucleotide substitution in primates and rodents and the generation-time effect hypothesis. Mol Phylog Evol 5:182–187

Ma D-P, Zhakikh A, Graur D, Vandenberg JL, Li W-H (1993) Structure and evolution of opossum, guinea pig and porcupine cytochrome b genes. J Mol Evol 36:327–334

Macdonald D (ed) (1989) The encyclopedia of mammals. Unwin Hyman, London

Macdonald D, Barrett P (1993) Field guide to British and European mammals. Harper Collins, London

Martin AP (1995) Metabolic rate and directional nucleotide substitution in animal mitochondrial DNA. Mol Biol Evol 12:1124–1131

Martin AP, Palumbi SR (1993) Body size, metabolic rate, generation time and the molecular clock. Proc Natl Acad Sci USA 90:4087–4091

Martin AP, Naylor G, Palumbi SR (1992) Rates of mitochondrial DNa evolution in sharks are slow compared with mammals. Nature 357: 153–155

May RM (1993) Resisting resistance. Nature 361:593–594

McNab BK (1986) The influence of food habits on the energetics of eutherian mammals. Ecol Monogr 56:1–19

McNab BK (1988) Complications inherent in scaling the basal rate of metabolism in mammals. Q Rev Biol 63:25–54

McNab BK (1989) Basal rate of metabolism, body size, and food habits in the Order Carnivora. In: Gitlleman JL (ed) Carnivore behavior, ecology and evolution. Cornell University Press, New York, pp 335–354

Mooers AØ, Harvey PH (1994) Metabolic rate, generation time and the rate of molecular evolution in birds. Mol Phylog Evol 3:344–350

Moors PJ (1990) Norway rat. In: King CM (ed) The handbook of New Zealand mammals. Oxford University Press, Auckland, pp 192–206

Nash LT, Bearder SK, Olson TR (1989) Synopsis of *Galago* species characteristics. Int J Primatol 10:57–80

Novacek MJ (1992) Mammal phylogenies: shaking the tree. Nature 356:121–125

Nowak RM (1991) Walker's mammals of the world. John Hopkins University Press, London

Oftedal OT, Gittleman JL (1989) Patterns of energy output during reproduction in Carnivores. In: Gittleman JL (ed) Carnivore behavior, ecology and evolution. Cornell University Press, New York, pp 355–378

Ohta T (1993) An examination of the generation time effect on molecular evolution. Proc Natl Acad Sci USA 90:10676–10680

Pagés-Feuillade E (1988) Modalités de l'occupation de l'espace et relations interindividuelles chez un prosimien nocturne malagache (*Microcebus murinus*). Folia Primatol 50:204–220

Plavcan JM, Gomez AM (1993) Dental scaling in the Callitrichinae. Int J Primatol 14:177–192

Promislow DEL (1994) DNA repair and the evolution of longevity: a critical analysis. J Theor Biol 170:291–300

Purvis AP (1995) A composite estimate of primate phylogeny. Philos Trans R Soc Lond Biol 348:405–421

Purvis AP, Harvey PH (1995) Mammal life-history evolution—a comparative test of Charnov's model. J Zool 237:259–283

Purvis AP, Bromham LD (in press) Estimating the transition/transversion ratio from independent pairwise comparisons with an assumed phylogeny. J Mol Evol

Rand DM (1994) Thermal habit, metabolic rate and the evolution of mitochondrial DNA. TREE 9:125–131

Richard AF (1987) Malagasy prosimians: female dominance. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (eds) Primate societies. University of Chicago, Chicago, pp 25–33

Rohde K (1992) Latitudinal gradients in species diversity: the search for the primary cause. Oikos 65:514–527

Rosenheim JA, Tabashnik BE (1991) Influence of generation time on the rate of response to selection. Am Nat 137:527–541

Rosenheim JA, Tabashnik BE (1993) Generation time and evolution. Nature 365:791–792

Ross C (1992) Basal metabolic rate, body weight and diet in primates. Folia Primatol 58:7–23

Rudge MR (1990) Feral goat. In: King CM (ed) The handbook of New Zealand mammals. Oxford University Press, Auckland, pp 406–423

Sarich VM, Wilson AC (1973) Generation time and genomic evolution in primates. Science 179:1144–1147

Seino S, Bell GI, Li W-H (1992) Sequences of primate insulin genes support the hypothesis of a slower rate of molecular evolution in humans and apes than in monkeys. Mol Biol Evol 9:193–203

Shimmin LC, Chang BH-J, Li W-H (1993) Male-driven evolution of DNA sequences. Nature 362:745–747

Shimmin LC, Chang BH-J, Li W-H (1994) Contrasting rates of nucleotide substitution in the X-linked and Y-linked zinc-finger genes. J Mol Evol 39:569–578

Simons EL (1988) A new species of *Propithecus* (Primates) from Northeast Madagascar. Folia Primatol 50:143–151

Skinner JD, Smithers RHN (1990) The mammals of the Southern African subregion. University of Pretoria

Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (eds) (1987) Primate societies. University of Chicago, Chicago

Sowls L (1984) The peccaries. University of Arizona Press, Tucson

Springer MS, Kirsch JAW (1989) Rates of single-copy DNA evolution in phalangeriform marsupials. Mol Biol Evol 6:331–341

Stephan H, Baron G, Frahm HD (1981) Insectivora: with a stereotaxic atlas of the hedgehog brain. Springer Verlag, New York

Strahan R (1983) Complete book of Australian mammals. Angus and Robertson, Sydney

Sullivan DT (1995) DNA excision-repair and transcription—implications for genome evolution. Curr Opin Genet Dev 5:786–791