

Body size, metabolic rate, generation time, and the molecular clock

(mitochondrial DNA/mutation/substitution rates/molecular evolution/oxidative damage)

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ABSTRACT There is increasing evidence for variation in rates of nucleotide substitution among divergent taxonomic groups. Here, we summarize published rate data and show a strong relationship between substitution rate and body size. For instance, rates of nuclear and mtDNA evolution are slow in whales, intermediate in primates, and fast in rodents. A similar relationship exists for poikilothermic vertebrates. However, these taxa have slower mtDNA substitution rates overall than do homeotherms of similar size. A number of physiological and life history variables are highly correlated with body size. Of these, generation time and metabolic rate explain some patterns of rate heterogeneity equally well. In many cases, however, differences in metabolic rate explain important exceptions to the generation time model. Correlation between metabolic rate and nucleotide substitution may be mediated by (i) the mutagenic effects of oxygen radicals that are abundant by-products of aerobic respiration, and (ii) increased rates of DNA synthesis and nucleotide replacement in organisms with higher metabolic rates. Both of these factors increase mutation rate by decreasing the “nucleotide generation time,” the average length of time before a nucleotide is copied either through replication or repair. Reconsideration of the generation time hypothesis to include physiological effects such as metabolic rate improves the theoretical underpinnings of molecular evolution.

Understanding the factors that affect nucleotide substitution in DNA is central to evolutionary biology, population genetics, and mutation research. To explore such factors evolutionary rates are generally determined with reference to absolute or relative divergence time between taxa (1). Rates of evolution estimated in this way often differ among divergent taxonomic groups (2–10), and explanations for this rate variation represent important hypotheses about the factors that determine DNA evolution (11–14).

Many hypotheses have been proposed to account for differences in rates of DNA evolution, including DNA repair efficiency (3), rate of cell division (5), generation time (5, 6), and weight-specific metabolic rate (14). Often, very few data are available to support associations between molecular rate and cellular attributes. For example, DNA repair efficiencies or rates of germ-cell division are poorly known for many taxa, and direct tests of the relationships between these variables and DNA substitution rate have never been performed. Thus, it has been difficult to partition the observed variance in DNA substitution rates in biologically meaningful ways or to estimate the contribution made by cellular, physiological, or life history differences to variation in rates of molecular evolution.

The search for factors that influence molecular evolution is made more difficult because many physiological and life history variables are correlated with one another. Such variables include generation time, life span, age at first

reproduction, intrinsic rate of population increase, population size, and weight-specific metabolic rate (15). Traditionally, these variables have been related to a single, easily measurable biological attribute: body size (15). In the following, we show that when there are differences in rate of DNA substitution between taxa, high rates of DNA evolution often are associated with small body size.

Body size probably does not control the rate of DNA substitution directly but serves as a convenient guidepost for understanding the biological correlates of molecular rate heterogeneity. We attempt to illuminate the underlying causes of the body size effect by separating the influence on evolutionary rate of some of the attributes correlated with body size. We primarily focus on generation time and metabolic rate because the effects of both on mutation have a sound mechanistic basis, have been suggested to play important roles in determining rates of molecular evolution, and represent factors for which large comparative data bases exist (6, 11, 13, 14). Finally, we present an explanation for the influence of metabolic rate on silent substitutions that shows how generation-time effects should theoretically be affected by metabolic rate in just the fashion that we observe.

Experimental Evidence for Body Size Effects

Minimum-evolution analysis of mutations in a globin pseudogene has shown that there is marked heterogeneity in the silent rate among different primates (16). When published measurements of average adult body size are compared to these rates (Fig. 1A; Table 1) we see that much of this variation is explained by smaller body size in taxa with higher rates. Analysis of cytochrome *b* evolution in mammalian mtDNA shows a similar pattern. Evolution along terminal branches leading to a variety of different mammalian orders is markedly heterogeneous. Silent rates calculated from the fossil data available show a decrease with increasing body size (Fig. 1B). Transversion differences in the cytochrome *b* data of Martin *et al.* (14) also show higher substitution rates in smaller elasmobranchs than in genera with larger individuals (21). Analysis of other data sets shows similar patterns (e.g., refs. 21, 23, 24).

Larger mtDNA data sets with broader taxonomic breadth are available from restriction fragment studies rather than from sequence comparisons. A summary of published genetic distances and fossil dates (Table 2; Fig. 2) shows the same general trend as in Fig. 1; rate of mtDNA evolution decreases with increasing body size. However, in the larger analysis, two groups are evident that separate with regard to energy metabolism: the homeotherms and the poikilotherms. In general, for a given body size, rate of mtDNA divergence is greater for homeotherms than for poikilotherms.

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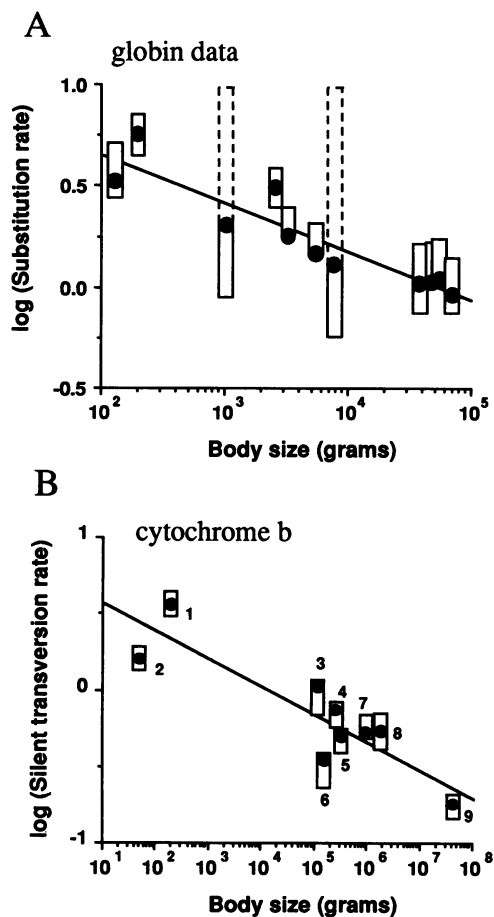


FIG. 1. (A) Relationship between the logarithm of substitution rate (in number of substitutions per 1000 bp) at synonymous sites and body size for lineages of primates. Rates were obtained by dividing branch lengths from neighbor-joining trees by published fossil dates for origination times. Data are from Table 1 and refs. 16, 17–20. Dots are best estimates; vertical boxes represent ranges. Boxes are dotted for spider and owl monkeys, whose minimum divergence times are poorly known. (B) Relationship between the logarithm of silent transversion rate in the cytochrome *b* gene and body size for mammals. 1, Rat; 2, mouse; 3, sheep; 4, pig; 5, dolphin; 6, goat; 7, cow; 8, elephant; 9, whale. Rates are from refs. 21 and 22. Body sizes are from Wootton (17).

Within the homeotherms, the most extreme results are the slow rates recorded for whales and the fast rates for rodents (Table 2; Fig. 2). How robust are these data? For whales, previous reports have suggested that the rate of molecular evolution is slow (ref. 10; C. S. Baker and S.R.P., unpublished data). Comparison of mtDNA fragment patterns based on 15 restriction enzymes shows that humpback and fin whale mtDNAs are 6% different, even though the genera represented by these species diverged by the late Miocene, between 6 and 15 million years ago (35). These fossil and molecular data show that overall mtDNA divergence is between 0.4% and 1.0% per million years in whales.

For rodents, previously published divergence times between rat and mouse range from 5 to 30 million years (36). This 6-fold difference in timing has meant the difference between conclusions of substitution rate constancy or variation (6, 36, 37). Recent detailed studies provide strong evidence for a 10- to 12-million year divergence time between *Rattus* and *Mus* (38) and have established divergence times among species of *Mus* (25, 38, 39). Use of these data suggests that the rodent rate is high (see also refs. 7, 9, 39–41).

The general inverse relationship between rate of DNA evolution and body size shows several intriguing exceptions.

Table 1. Estimates of silent substitution rate (substitutions per site per billion years) for nuclear DNA (from ref. 16), measurements or estimates of specific metabolic rate (ml of O₂/kg per hr), and generation time (days) for species of primates

| Species | Substitution rate | Metabolic rate | Generation time* |
|-----------------------------------|-------------------|-------------------|--------------------|
| Owl monkey | 2.1 | 450 [†] | 880 |
| Spider monkey | 1.9 | 415 [‡] | 1700 |
| Macaque | 1.8 | 430 [†] | 1095 |
| Gibbon | 1.7 | 370 [‡] | 3410 |
| Orangutan | 1.2 | 230 [‡] | 4290 |
| Gorilla | 1.2 | 200 [‡] | 3438 |
| Chimpanzee | 1.2 | 220 [†] | 3190 |
| Human | 1.1 | 210 [†] | 6200 |
| Kendall's correlation coefficient | | 0.75 [§] | -0.75 [§] |

*Listed as age at first reproduction in Wootton (17).

[†]From McKnabb (19) and Altman and Dittmer (20).

[‡]Calculated from the relationship between metabolic rate and body size (15) using body sizes from Harvey and Clutton-Brock (18).

[§] $P < 0.01$.

Geese have a slower substitution rate than predicted for their size (box 6 in Fig. 2). Whether this is true of all birds is currently unknown, but Kessler and Avice (42) suggested that birds have a slower rate of mtDNA evolution than mammals. In addition, rat appears to have a faster silent mtDNA substitution rate than mouse (Fig. 1B), even though mice are less than half the size of rats.

Competing Explanations for Body Size Effects

Our results indicate that DNA substitutions accumulate at a slower rate in large animals than in small animals. In the past, similar results have been ascribed to generation time effects on rates of molecular evolution (5, 6, 16). As a result, the generation time hypothesis has gained widespread acceptance among evolutionary biologists. Theoretically, an association between generation time and the accumulation of nucleotide substitutions is to be expected if most substitutions are the result of errors during replication and if most species have broadly similar numbers of cell divisions per generation. In essence, species with a short generation time will experience similar numbers of cell replications and the same degree of DNA substitution per generation as do long-generation time species but will accumulate a greater number of DNA changes per year (11). This rationale does not necessarily apply to mutations under weak selection (11).

However, recent evidence from analysis of nucleotide substitution rates of mtDNA (12–14) and nuclear DNA (8, 10) challenges the exclusivity of the generation time hypothesis. In these cases, mammals, reptiles, and fish with relatively short generation times have unexpectedly slow rates of DNA evolution. Departure from the predictions of the generation time hypothesis provides the basis to investigate the role that other variables play in setting the pace of nucleotide substitution.

As noted above, the association between rates of molecular evolution and generation time is confounded by tight correlations between body size, generation time, metabolic rate, and other physiological and life history variables. In this context, a correlation between variables does not prove a causative relationship between them. Multiple regressions of various factors against silent rate in principle could tease apart the relationships between causative and correlated variables as long as silent rates for each lineage are calculated independently.

For comparisons among simian primates (monkeys and apes), generation time and specific metabolic rate data are

Table 2. Estimates of percent sequence divergence, time of divergence from the fossil record, divergence rate, and average adult body size for a variety of vertebrates

| Taxon | % sequence difference | Divergence time, Mya | Divergence rate, %/Myr | Body size, kg | Generation time, yr | Ref(s). |
|--|-----------------------|----------------------|------------------------|---------------|---------------------|---------|
| Homeotherm | | | | | | |
| Rodents | | | | | | |
| <i>Mus musculus</i> - <i>M. spretus</i> | 11.3 | 1-3 | 3.8-11.3 | 0.020 | 0.2-0.3 | 25 |
| Dogs | | | | | | |
| <i>Canis latrans</i> - <i>C. lupus</i> | 2.7-4.2 | 1-2 | 1.8-3.5 | 10-55 | 2-3 | 26 |
| Horses | 7.8 | 3-5 | 1.5-2.6 | 100-400 | 3-4 | 27 |
| Bears | | | | | | |
| <i>Ursus arctos</i> - <i>U. maritimus</i> | 0.8-1.2 | 0.5 | 1.6-2.4 | 150-300 | 4-5 | 28 |
| <i>U. arctos</i> - <i>U. americanus</i> | 6.9-7.1 | 3.5 | 1.9-2.0 | 150-300 | 4-5 | 28 |
| <i>U. maritimus</i> - <i>U. americanus</i> | 7.6-7.7 | 4.4 | 1.7-1.8 | 150-300 | 4-5 | 28 |
| Primates | | | | | | |
| <i>Homo sapiens</i> - <i>Pan troglodytes</i> | 10.5 | 5 | 2.1 | 33-55 | 8-15 | 29 |
| Whales | | | | | | |
| <i>Megaptera novanglieae</i> - <i>Baleanoptera physalus</i> | 6.0 | 6-15 | 1.0-0.4 | 40,000 | 5-10 | * |
| Geese | | | | | | |
| <i>Branta</i> - <i>Anser</i> | 9.0 | 4-5 | 1.8-2.3 | 1-2 | 2-3 | 30 |
| Poikilotherm | | | | | | |
| Tortoise | | | | | | |
| East vs. west <i>Gopherus</i> | 5.3 | 5.5 | 0.95 | 0.5-3 | 17-20 | 31 |
| <i>Gopherus</i> - <i>Xerobates</i> | 11.2 | 15-23 | 0.48-0.75 | 0.5-3 | 17-20 | 31 |
| Sea turtles | | | | | | |
| Geminate <i>Chelonia</i> | 0.6 | 1.5-3.0 | 0.2-0.40 | 50-70 | >20 | 13, 32 |
| Kemp and Ridley's | 1.2 | 3-4 | 0.30-0.40 | 30-50 | >20 | 13, 33 |
| Amphibians | | | | | | |
| Newts | 8.0 | 8-12 | 0.67-1.0 | 0.020 | 2-3 | 34 |
| Frogs | 8.3 | 11-12 | 0.69-0.75 | 0.050 | 2-3 | 34 |
| Sharks | | | | | | |
| <i>Sphyrna</i> - <i>Galeocerdo</i> | 17 | 55 | 0.31 | 60-100 | 10-15 | 14, 21 |
| Teleost fish | | | | | | |
| Salmon | 5.2 | 6-10 | 0.9-0.5 | 1-4 | 1-4 | 21 |

Percent sequence divergence is estimated from restriction fragment length polymorphism (RFLP) analysis of mtDNA. Mya, million years ago; Myr, million years.

*C. S. Baker and S.R.P., unpublished data.

available. Regression analyses show that both are highly correlated with the silent rate. However, in a multiple regression of the silent rate versus generation time and metabolic rate, only the latter had a significant β coefficient ($P =$

0.009 for metabolic rate versus $P = 0.349$ for generation time). Although multiple regression can be a weak tool when variables are highly correlated, the analysis suggests that the apparent relationship of silent rate to generation time may be an artifact.

For the cytochrome *b* data in Fig. 1B, neither generation time nor metabolic rate shows a significant correlation with silent rate by either parametric or nonparametric tests. However, generation time and metabolic rate together yield a significant multiple regression analysis ($R^2 = 0.673$, $F = 6.187$, $P = 0.035$), indicating that both factors may be playing important roles in a synergistic fashion.

In other cases, inspections of molecular rate data do not follow the predictions of the generation time hypothesis. For example, whales have a slow rate of nuclear DNA evolution relative to primates despite their shorter generation time (10). Rates of single-copy DNA evolution are slower in marsupials than in placental mammals independent of generation time (ref. 8; but see ref. 43). Substitution rates in nuclear ribosomal genes are 8-fold slower in salamanders than in mammals (44), despite their shorter generation times.

For mtDNA rates, Hasegawa and Kishino (12) failed to find an association between generation time and mtDNA substitution rate. Furthermore, the silent rate in shark mtDNAs is 5-7 times slower than in primates or ungulates despite broadly similar ranges of generation times for the three groups (14). Similarly, mtDNA divergence rates of newts and frogs, whose generation times are on the order of 3-5 years (44), are slower than those of primates (34). Rates of mtDNA evolution are

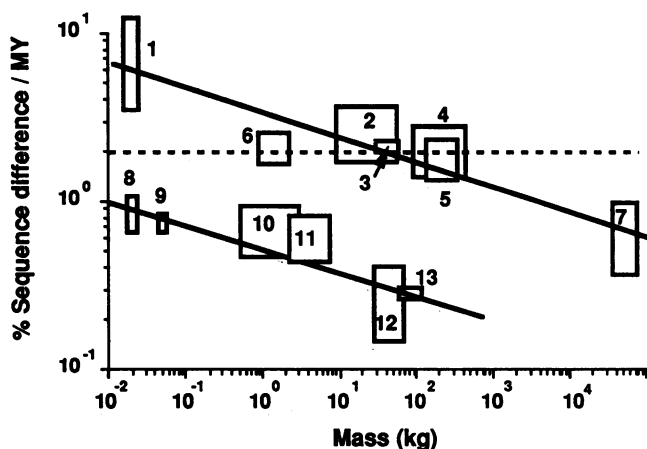


FIG. 2. Relationship between rate of mtDNA sequence divergence (% change per million years) and body size (in kg) for various vertebrates. Data are from Table 2. 1, Mice; 2, dogs; 3, human-chimpanzee; 4, horses; 5, bears; 6, geese; 7, whales; 8, newts; 9, frogs; 10, tortoise; 11, salmon; 12, sea turtles; 13, sharks. Boxes represent the range of rates and body sizes for a given taxon. Solid lines are drawn to pass through the boxes. Dashed line represents the hypothesis of rate constancy.

higher in honey bees than in *Drosophila*, even though generation times for honey bees are longer than *Drosophila* (45).

Finally, the separation of mtDNA rates into two broad groups comprising the homeotherms and the poikilotherms (Fig. 2) is incompatible with a simple generation time hypothesis. We note that weight-specific metabolic rate is significantly lower for poikilotherms than homeotherms (46) and that Fig. 2 is highly reminiscent of classic plots of specific metabolic rate versus body size (46).

Metabolic Rate Hypothesis

Although the generation time hypothesis has successfully explained some of the variation in molecular rates that have been observed, the above section documents instances in which its predictions fail to be met. Other factors in addition to generation time undoubtedly play important roles in molecular evolution. In particular, metabolic rate has been mechanistically related to the molecular process of mutation. In fact, many exceptions to the generation time hypothesis previously noted can be explained by differences in specific metabolic rate.

Rates of DNA damage are proportional to specific metabolic rate (46, 47). This effect appears to be mediated by oxygen radicals, which are highly reactive molecules with free electrons that can damage DNA directly by attacking the sugar-phosphate backbone or the base (48) or indirectly via lipid peroxidates (49). Because about 90% of oxygen in cells is consumed by mitochondria, oxidative damage is greatest to mtDNA (50) and probably partially explains why rates of nucleotide substitution are greater in mtDNA than in nuclear DNA (51). However, oxidative damage to nuclear DNA is also extensive (50), and active oxygen moieties can pass through membranes (52).

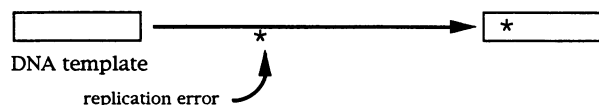
These results predict that species with higher metabolic rates (and therefore higher rates of oxygen radical flux) should have higher DNA substitution rates if there is a relationship between DNA damage and mutation rate. Two mechanisms may account for this relationship. First, DNA subject to constant and high rates of oxidative damage (50) is repaired continuously (47, 48), and mutations may occur by incorrect repair. Such mutations are especially likely when bases are oxidatively modified because such aberrant nucleotides frequently cause mispairing (53).

For mtDNA, there is another possible way for metabolic rate to affect substitution rate. Unlike the nuclear genome, where DNA synthesis is almost exclusively associated with cell division and repair, mitochondrial genomes are degraded and replaced within cells at a high rate independent of cell division. Gross *et al.* (54) showed that rat mtDNAs had half-lives (i.e., the amount of time until half of the mtDNAs were replaced *in vivo*) that ranged from 6 to 31 days. Tissues with the most active aerobic metabolisms, such as heart muscle, tend to exhibit short half-lives. Gross *et al.* (54) attributed these observations to turnover of whole mitochondria in cells and suggested that turnover was higher in cells with higher aerobic metabolisms.

Higher turnover will lead to higher mutation rates as long as replication errors per nucleotide remain constant (Fig. 3). If errors are more likely because of radical-induced damage of DNA temporarily in a single-strand state during replication (W. K. Thomas, personal communication), then DNAs may be exposed to both mechanisms of the metabolic rate effect.

The volume and efficiency of oxygen utilization may be important in setting the pace of DNA damage and mutation. Sohal *et al.* (ref. 55, p. 213) write that "species-specific characteristics of mitochondria, rather than the basal metabolic rate alone, play a role in determining rates of oxygen radical production." Differences in the organization and efficiency of aerobic respiration in the mitochondria between

Low metabolic rate - slow turnover and infrequent repair



High metabolic rate - high turnover and frequent repair

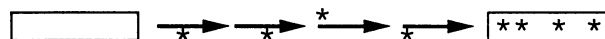


FIG. 3. Generalized effect of metabolic rate on DNA substitution. Replication errors (*) occur at a constant rate per nucleotide generation and will accumulate more quickly when nucleotides are copied frequently because of high turnover or vigorous repair of oxidative damage.

divergent taxonomic groups may account for departures from the general positive relationship between specific oxygen consumption and silent substitution rate. The lower substitution rate in geese than predicted (Fig. 2) may reflect the existence of more efficient oxygen metabolism and lower levels of prooxidant stress in birds than exist in mammals (56). Similarly, absolute rates of oxygen radical production in rat liver mitochondria are greater than in mouse (55). We noted earlier that substitution rates in rat were higher than those of mouse. These results suggest that differences in the efficiency of oxygen metabolism may play a role in setting the pace of molecular evolution.

A Generalized Substitution Model

Two sources of mutation to DNA, errors during replication and DNA damage from oxygen radicals, are interrelated: damage increases the rate of repair as well as increasing the error rate per replicated base (46, 47, 53). An increase in metabolic rate will increase turnover or repair of DNA and thereby increase replication errors via these two mechanisms (Fig. 3).

These considerations focus attention on the concept of a "nucleotide generation," which can be defined as the amount of time needed for a given nucleotide position to be copied, either because of DNA replication or repair. Nucleotide positions that have short generation times are those that are replicated most often, due to either consistent repair or high turnover. We note that when many types of DNA damage are repaired, the damaged base plus the flanking sequences are excised and recopied (57). Thus, repair-mediated replication is often not limited to the single damaged base but can affect regional nucleotide generation times as well. Nucleotide generation time will also be short in rapidly dividing cell lineages, such as those in the germ lines of species with short organismal generation times.

Thus species with higher metabolic rate may have high levels of repair and a larger number of nucleotide generations per organismal generation. This should lead to higher absolute substitution rate. Species with short organismal generation times may also have high substitution rates because of short nucleotide generations in rapidly proliferating germ lines. In both cases, the basic mechanism that leads to higher substitution rates is the same—higher rates of DNA replication yield higher rates of error. Because metabolic rate and the rate of germ-cell division increase in smaller species, the association of fast rates of molecular evolution with small body size should be particularly apparent.

Exceptions

No single factor is likely to completely explain variation in rates of DNA evolution, and it comes as no surprise that not all previous empirical studies follow the predictions of the metabolic rate or the generation time hypotheses. Probably the most interesting finding has been that not all DNA sequences show identical rate heterogeneity in the same group of species (6). Among primates, for example, not all genes show a rate decrease along the human lineage (24).

In addition, there is evidence that the silent rate for different genes in the same genome can be different. Wolfe *et al.* (58) showed higher substitution rates in genes with intermediate G+C contents at silent sites. This discovery suggests that differences in nucleotide composition among genes may account for substitution rate heterogeneity (however, see ref. 9).

Differences in the degree of rate heterogeneity between different genes among taxa and differences in the rate of substitution among genes within taxa suggest that any organism-wide physiological factor like metabolic rate or generation time is likely to have complex effects on DNA evolution. It is reasonable to assume that some regions of DNA are less susceptible to DNA damage than others because of secondary structure, presence of proteins complexed with DNA, or differences in nucleotide composition. Perhaps some parts of the genome have shorter nucleotide generation times due to differential repair.

Despite this complexity, physiological and life history differences often correlate with rates of DNA evolution. We have argued that metabolic rate and generation time effects might ultimately rely on a common molecular mechanism and that the heart of this mechanism is the concept of nucleotide generation time. Other factors may also be at work within complex genomes, and, in all probability, there is a hierarchy of mechanisms that impinge on DNA evolution. Future research should focus on *multiple* causes of rate heterogeneity.

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