Letter to the Editor

Generation Time and the Rate of Molecular Evolution

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The importance of generation time in determining the rate of molecular evolution has long been at issue. Resolution of this question has important consequences both for the validity of the neutral theory (as formulated by Kimura 1983) and for the use of molecular data in systematics.

Wu and Li (1985) performed two tests based on comparative analyses of the DNA sequences of a variety of mammalian genes, the results of which, they argue, show clear evidence of a generation-time effect. In the first “relative rate” test the numbers of nucleotide substitutions were compared between homologous genes in humans (a long generation-time species) and “reference-species” with the numbers in the same genes between mice or rats (short generation-time species) and the same reference species. The reference species used were dogs, rabbits, pigs, cows, and goats. The results (summarized in table 1) show that for all categories of nucleotide sites the number of substitutions per site, averaged over 12 genes, was greater in the mouse-reference comparison than in the human-reference comparison. It is argued that this shows that there has been a faster rate of substitution in the shorter-generation-time rodent lineage, than in the longer-generation-time human lineage.

In conducting the test, Wu and Li assumed that rodents and humans are more closely related to each other than either is, on average, to the reference species. This pattern of relationship (fig. 1a) implies the following approximate numbers of substitutions (at the fourfold degenerate sites) in the three branches of the phylogenetic tree: reference species, 0.2; humans, 0.2; rodents, 0.4. Since the number of substitutions in the human and reference-species branches are approximately the same but the reference-species branch is longer than the human branch, this implies that the order of substitution rates among the three lineages has been: rodents > humans > reference. (The fourfold degenerate sites are used here to illustrate the point, which applies equally to the other kinds of sites.) The relative order of generation times among the three lineages, on the other hand, is: rodents < reference < human, there being approximately an order of magnitude difference between each adjacent pair. Thus, when all three lineages under this phylogenetic scheme are considered, there appears to be variation in substitution rate, but this variation is not adequately explained by variation in generation time.

Wu and Li (1985) suggest that this test does not depend on any knowledge of the divergence times of the species. This is true of absolute divergence times, but the test depends critically on a knowledge of relative divergence times. The phylogeny in figure 1a is by no means firmly supported by the fossil record. Most of the major mammalian divergences occurred during the late Cretaceous and the Paleocene. However, the mammalian fossil record during this period is poor, and the relative order of divergences is not clear. An alternative pattern of relationship among the species (figure 1b), one that places humans closer to the reference species than to rodents, can explain the variation in substitution rate among the three branches as resulting from variation in the lengths of the branches. In other words, if the data are analyzed in the context of a different, equally feasible, phylogenetic scheme, they can be taken as evidence that substitutions have occurred at a constant rate that is independent of generation time.

1. Key words: generation time, evolutionary rate, neutral evolution.

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Table 1
Comparison of the Average Number of Substitutions per Nucleotide Site between Humans, Rodents, and “Reference Species” for Various Nucleotide Site Classes

<table>
<thead>
<tr>
<th>NUCLEOTIDE SITE CLASS</th>
<th>Human-Rodent</th>
<th>Human-Reference</th>
<th>Rodent-Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondegenerate</td>
<td>0.17</td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>Twofold degenerate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsynonymous substitutions</td>
<td>0.08</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Synonymous substitutions</td>
<td>0.32</td>
<td>0.18</td>
<td>0.31</td>
</tr>
<tr>
<td>Four-fold degenerate</td>
<td>0.66</td>
<td>0.41</td>
<td>0.60</td>
</tr>
</tbody>
</table>

SOURCE.—Table 1 of Wu and Li (1985).

We do not know the relative order of divergence times, so we cannot distinguish between the two possible interpretations. However, we can say that the relative-rate test applied in this situation does not provide a conclusive demonstration of nonconstancy of substitution rate or of a generation-time effect. The relative-rate test can usefully be used only in situations where the relative order of species divergences is not in question.

The second test involved comparisons of three paralagous human and mouse β-globin genes (designated A, E, and F). Since these genes appear to have diverged shortly before the human-mouse divergence, comparison of the numbers of their nucleotide differences in mice and in humans provides a test for generation-time effect, provided that no lateral gene conversion has occurred among the genes since humans and mice diverged. The results show that the numbers of synonymous-site substitutions between pairs of the human genes (A-F, 0.731; A-E, 0.623; F-E, 0.562) are all less than the numbers between the respective pairs of mouse genes (0.904; 0.974; 0.960) (Wu and Li, 1985). It is suggested that this demonstrates that the substitution rate has been faster in the mouse lineage than in the human lineage and that there has therefore been a generation-time effect. However, when the numbers of substitutions between all genes, both paralagous and orthologous, are considered, this explanation does not appear to be adequate.

The gene phylogeny assumed in the analysis is shown in figure 2. The numbers of substitutions shown by reciprocal comparisons of paralogous genes between different species should be the same if the substitution rates are not substantially different among genes and if no lateral gene conversion has occurred, irrespective of whether or not there has been a generation-time effect. Thus, the differences between mouse

![Fig. 1](image-url)

**Fig. 1.**—a. Phylogenetic relationships assumed in Wu and Li’s (1985) rate test, showing the implied numbers of substitutions per site (for the fourfold degenerate sites) on the different branches; b. alternative phylogenetic scheme, which explains the differences in substitution rate by differences in branch length.
A and human E or F are expected to be the same as those between human A and mouse E or F, since all genes being compared diverged at the same time and the lengths of time during which the genes have evolved separately in the human and in the mouse lineages are the same in each reciprocal comparison. However, the analysis shows that there are 1.081 and 0.902 synonymous substitutions/site between human A and mouse E and F, respectively, but only 0.674 and 0.660 substitutions in the respective comparisons of mouse A with human E and human F.

When human A and mouse A are compared to all E and F genes (table 2), it can be seen that the number of substitutions is independent of which A gene is being compared. It depends only on whether or not the comparison involves mouse or human E and F genes. This implies that there has been no generation-time effect in the evolution of the A gene and that therefore the difference between the human and mouse E and F genes is attributable to another cause (unless one is prepared to postulate that generation time can affect one gene while not affecting another). The similarity of substitution rate in the E and F genes in both the human and mouse lineages suggests that the variations in rate cannot be attributed to variation between genes. One simple explanation for the rate variation is that partial or complete lateral gene conversion(s) has occurred between some other gene(s) in the globin-gene family and the mouse E and F genes since the divergence of humans from mice, making these E and F genes more different from the A genes than are the human E and F genes.

Such gene-conversion events cannot explain all the variation in substitution rates apparent among the six genes. However, there is evidence that complete or partial lateral gene conversion among β-globin genes has occurred in the human lineage quite frequently relative to the divergence time of humans and mice (Slightom et al. 1980; Scott et al. 1984). It is thus quite possible that many gene-conversion events have occurred in the phylogeny outlined in figure 2. Although a detailed analysis is beyond the scope of this note, there are possible combinations of partial and complete conversions occurring in different lineages and at different times that could explain the apparent differences in substitution rate.

**Table 2**

<table>
<thead>
<tr>
<th>A Gene</th>
<th>Mouse Gene</th>
<th>Human Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td>Human</td>
<td>0.902</td>
<td>1.081</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.974</td>
<td>0.904</td>
</tr>
</tbody>
</table>

**SOURCE.**—Table 3 of Wu and Li (1985).
There are thus (1) a direct indication of substitution-rate constancy in the orthologous evolution of the A gene and (2) plausible ways of explaining the heterogeneity of substitution rates apparent in the overall analysis without our having to postulate a generation-time effect.

The above considerations show that the results of both of Wu and Li's (1985) analyses are compatible with the constant-rate hypothesis and that they do not provide a convincing demonstration of a generation-time effect. The neutral-theory prediction of a generation-time effect is based on the assumption that mutation rates are a function of generation time. The prediction is neither valid nor necessary if this assumption, which has little empirical basis, is dropped. A constant rate of nucleotide substitution that is independent of generation time is not necessarily incompatible with the neutral theory of molecular evolution.

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LITERATURE CITED


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