Nucleotide substitution type dependence of generation time effect of molecular evolution

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ABSTRACT

Using DNA sequence data of 18 genes from 14 mammals, we analyzed how the average molecular evolution rate per year per site (V_y) depends on the generation time (g). (I) Assuming the relation $V_y \propto g^{-\alpha}$, the index of generation time effect, (α), was estimated to be about 0.14 for amino acid replacement substitutions (A), and about 0.32 for synonymous substitutions (S). (II) Assuming the relation $V_y = V_g^m g^{-1} + V_y^e$, where V_g^m and V_y^e are constant independent of g , the fraction, $r^e \equiv V_y^e / V_y$, of the mutation rate independent part (V_y^e) in the total evolution rate (V_y) was estimated under the assumptions of the star phylogeny and the constancy of the mutation rate per generation. r^e was smallest for mouse with the shortest generation time among our analyzed species, and it was estimated to be about 0.57 for A and 0.31 for S. Both results do not support the view that V_y is equal to the neutral mutation rate per site both for A and for S. They are in line with the thesis that, at least for A and probably even for S, the molecular evolution rate is influenced by some causes other than the mutation rate, such as changing environment.

1. INTRODUCTION

Analyzing amino acid sequences of proteins and nucleotide sequences of genes, the rate of molecular evolution has been found to be approximately constant *per year* independent of species. This feature of molecular evolution is called a molecular clock, which was first suggested by Zuckerkandl and Pauling (1965). This hypothesis has often been utilized to reconstruct molecular phylogeny (Futuyma, 1986). Kimura (1968, 1983, 1987) proposed in his neutral theory that the rate of molecular evolution was equal to the neutral mutation rate. However, there is so far no measurement of the neutral mutation rate, and the mutation rate is usually believed to be constant *per generation* rather than *per year* (Crow, 1976). If the mutation rate is constant per generation among species, it is expected that the rate of substitutions per year for an organism having shorter generation time would be larger than that for an organism having longer genera-

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tion time. This effect is called generation time effect.

Recently, Wu and Li (1985) reported that the rate of nucleotide substitutions of rodent is 2 times faster than that of human for synonymous substitutions and 1.3 times for amino acid replacement substitutions. The rate of synonymous substitutions of *Drosophila* was found to be approximately 2 times faster than that of rodents and 10 times faster than that of higher primates (Moriyama, 1987). The estimated rates of nucleotide substitutions in rodents were found to be 4 to 8 times faster than those in higher primates and 2 to 4 times faster than those in artiodactyls (Li et al., 1987).

These results suggest that the rate of nucleotide substitutions has negative correlation with generation time of species and that the magnitude of generation time effect depends on the type of nucleotide substitution, such as the synonymous one and the amino acid replacement one.

In this study, we analyze more quantitatively how the rate of nucleotide substitution depends on the generation time of species and on the type of nucleotide substitution.

2. MATERIALS AND METHODS

Genes and species used in our analysis are listed in Table 1. All species analyzed are mammals. We first assume that mammals diverged at the same period during geologic age, that is, star phylogeny, which was first proposed by Kimura (1983) (Fig. 1). This assumption enables us to use the number of nucleotide substitutions per site between two species, i.e., their difference (K), to compare the rates of molecular evolution among species. For the assumption of the star phylogeny to be valid, we only use the *species pair* between different orders in the following analysis.

In Fig. 1, lineage A-S-B for species A and B that have a common ancestor S is looked upon as the lineage of a species pair AB. For the generation time of a species pair AB (g_{AB}), we use the harmonic mean of the generation times of species A (g_A) and B (g_B):

$$1/g_{AB} = (1/g_A + 1/g_B)/2,$$

which represents the mean number of generations per year in the lineage A-S-B. The difference (K_{AB}) per site between homologous DNA sequences of species A and that of species B is computed by the method giving the weight factor on evolutionary pathway among codons (Miyata and Yasunaga, 1980). Multiple substitution effects on a difference (K) are corrected by the following formula (Jukes and Cantor, 1969).

$$K^c = -3/4 \log_e(1 - 4/3 K)$$

Then, K^c must be approximately proportional to the evolution rate per year per

Table 1. Genes and species analyzed and generation time of species

Orders	Primates			Rodentia			Carnivora		Lagomorpha		Artiodactyla			Perissodactyla
	Human	Owl monkey	Rat	Mouse	Guinea pig	Hamster	Dog	Cat	Rabbit	Goat	Bovine	Pig	Sheep	
Species														
G.T.(years)	13.5	4	0.164	0.096	0.164	0.125	0.583	0.875	1	0.625	0.75	0.542	0.625	1
Insulin	+	+	+		+		+		+	+				+
Globin α	+		+					+	+	+				
Globin β	+		+						+	+				
Prolactin	+			+							+	+		
GH	+		+							+				
Glucagon	+				+	+							+	
Albumin	+													
ACHRG	+			+							+			
CCK	+		+								+		+	
Na,K-ATPase	+													
UPA	+			+								+		
Actin	+		+						+					
GPH-A			+								+			
Relaxin	+		+									+		
POMC	+			+							+			
IgG	+			+					+					
IgK	+			+					+					

The abbreviation : G.T. is generation time.
References: Insulin: Human (Ulrich et al., 1980), Owl monkey (Seino et al., 1987), Rat (Cordell et al., 1979), Guinea pig (Chan et al., 1984), Dog (Kwok et al., 1983); Hemoglobin α : Human (Wilson et al., 1980), Rat (Sato et al., 1987), Horse (Clegg, 1987), Cat (Wainwright et al., 1985), Rabbit (Heindell et al., 1978), Goat (Schon et al., 1982); Hemoglobin β : Human (Lawn et al., 1980), Rat (Sato et al., 1987), Rabbit (Kafatos et al., 1977), Goat (Schon et al., 1981); Prolactin: Human (Cooke et al., 1981), Mouse (Harigaya et al., 1986), Bovine (Sasavage et al., 1982), Pig (Schulz-Aellen et al., 1989); Growth hormone: Human (Martial et al., 1979), Rat (Seeburg et al., 1977), Goat (Yato et al., 1988), Bovine (Miller et al., 1980), Pig (Su et al., 1988); Glucagon: Human (White et al., 1986), Guinea pig (Seino et al., 1986), Bovine (Lopez et al., 1983), Hamster (Bell et al., 1983); Albumin: Human (Lawn et al., 1981), Pig (Weinstock et al., 1988), Sheep (Brown et al., 1989). ACHRG (acetylcholine receptor γ subunit): Human (Shibahara et al., 1985), Mouse (Yu et al., 1986), Bovine (Takai et al., 1984); CCK (cholecystokinin): Human (Takahashi et al., 1985), Rat (Deschenes et al., 1985); Na, K-ATPase β -subunit: Human (Kawakami et al., 1986), Pig (Ovchinnikov et al., 1986), Sheep (Shull et al., 1986); UPA (urokinase plasminogen activator): Human (Riccio et al., 1985), Mouse (Belin et al., 1985); Actin: Human (Hanauer et al., 1983), Rat (Zakut et al., 1982), Rabbit (Putney et al., 1983); GPH-A (glycoprotein hormone α subunit): Rat (Godine et al., 1982), Bovine (Nilson et al., 1983); Relaxin: Human, Pig (Hudson et al., 1983), Rat (Hudson et al., 1981); ANF (atrial natriuretic factor): Human, Mouse (Seidman et al., 1984), Bovine (Vlasuk et al., 1986); POMC (pro-opiomelanocortin): Human (Chang et al., 1980), Mouse, Bovine (Uhler et al., 1983); IgG (immunoglobulin constant region gamma chain): Human (Ellison et al., 1982), Mouse (Honjo et al., 1979), Rabbit (Heidmann et al., 1982); IgK (immunoglobulin constant region kappa chain): Human, Mouse (Hieter et al., 1980), Rabbit (Emorine et al., 1983). Generation times of species except that of owl monkey are referred to Nohmura (1986). Generation time of owl monkey is given by personal communication from Fukuoka City Zoological Garden.

Fig. 1. Phylogenetic relationship of mammals, in which star phylogeny is assumed. Letters from A to D are species of mammals. S is the common ancestor of these mammals. K_{AB}^c is the corrected difference per site between homologous DNA sequences of species A and species B.

site of the species pair.

In order to quantify the magnitude of the generation time effect, we use the following two methods.

(I) Let us assume that the rate of molecular evolution per year per site (V_y) is proportional to generation time (g) to the minus α th power.

$$V_y \propto g^{-\alpha} \quad (1)$$

Here, α is an index of generation time effect on molecular evolution. For $\alpha=0$, we have no generation time effect. The generation time effect is stronger for larger α . If the number of nucleotide substitutions per generation is the same among all mammals, the rate of molecular evolution of the species pair must be inversely proportional to harmonic mean of generation times of the species pairs. In this case, α is expected to be unity.

Since we use the harmonic mean of generation time, the formula (1) also represents how the rate of molecular evolution depends on the generation number per year of species.

Plotting $\log(K^c)$ against $\log(g)$, we have estimated α by the gradient of the regression line. For amino acid replacement substitutions, K^c is 0.001 both between human and hamster and between bovine and hamster in glucagon, 0.001 between human and rat and 0.002 between rabbit and rat in actin. These values of $\log(K^c)$ which are deviated far away from the mean are excluded in the present analysis. On the lineages of these species, these genes may have evolved in a particular manner.

(II) Let us assume that the rate of molecular evolution per year per site (V_y) is written as $V_y^m + V_y^e$, where V_y^m and V_y^e is a part of the evolution rate per year per site regulated by the mutation rate and by the environmental change, respectively.

$$V_y = V_y^m + V_y^e \quad (2)$$

If the mutation rate is constant per generation and the environmental change is independent of the generation time, we may write $V_y = V_g^m g^{-1} + V_y^e$, where V_g^m

and V_y^e are independent of g . If we assume that the divergence time of the star phylogeny is T years ago, we have the relation $V_y = K^c/(2T)$. Therefore, writing $\hat{V}_y \equiv 2TV_y$, $\hat{V}_y^m \equiv 2TV_y^m$, $\hat{V}_y^e \equiv 2TV_y^e$ and $\hat{V}_g^m \equiv 2TV_g^m$, we have statistically estimated $\hat{V}_g^m$ and $\hat{V}_y^e$ by the gradient and K^c -intercept of the regression line, respectively, plotting K^c against g^{-1} . α , $\hat{V}_y^m$ and $\hat{V}_y^e$ are estimated to be average among loci.

3. RESULTS

Figs. 2 and 3 show the relation between the difference (K^c) and the generation time for amino acid replacement substitutions and synonymous substitutions, respectively. Estimated α is 0.140 for amino acid replacement substitutions and 0.318 for synonymous substitutions.

There is a following problem in the method of the analysis. Mammals might not necessarily have diverged at the same time during geologic age. It is impossible to know the accurate divergence time among species of mammals. Therefore, we have first assumed star phylogeny. However, without assuming star phylogeny, we can investigate the generation time effect by studying the ratio, K_a^c/K_s^c , since this is proportional to $g^{\alpha_s - \alpha_a}$ independent of the divergence time. Here, α_s and α_a is the index of the generation time effect for synonymous

Fig. 2. The relation between difference (K^c) and generation time for amino acid replacement substitutions. On the graph, one mark shows one difference (K^c) between homologous DNA sequences of two mammalian species. The regression line is drawn on the graph. The gradient ($-\alpha$) of the regression line is -0.140 .

Fig. 3. The relation between difference (K^c) and generation time for synonymous substitutions. The gradient ($-\alpha$) of the regression line is -0.318 .

Fig. 4. The relation between the ratio (K_a^c/K_s^c) of differences for amino acid replacement substitutions to that for synonymous substitutions and generation time. It shows positive correlation between the ratio and generation time.

Fig. 5. The relation between difference (K^c) and reciprocal generation time for amino acid replacement substitutions.

Fig. 6. The relation between difference (K^c) and reciprocal generation time for synonymous substitutions.

and amino acid replacement substitutions, respectively. The obtained $\alpha_s - \alpha_a$ is 0.160 (Fig. 4). Thus, α_s is found larger than α_a by 0.16 without assuming star phylogeny.

Table 2. Values of $\tilde{V}_g^m$ and $\tilde{V}_y^e$

	A	S
$\tilde{V}_g^m$	0.009	0.084
$\tilde{V}_y^e$	0.125	0.401

A and S represents amino acid replacement substitutions and synonymous substitutions, respectively.

Figs. 5 and 6 show the relation between difference and reciprocal generation time for amino acid replacement substitutions and synonymous substitutions, respectively. We have tabulated estimated $\tilde{V}_g^m$ and $\tilde{V}_y^e$ in Table 2.

4. DISCUSSION

We have found that estimated index of the generation time effect (α) is about 0.14 for amino acid replacement substitutions and about 0.32 for synonymous substitutions. These values indicate quantitatively how the rate of molecular evolution per year is more strongly dependent on the generation time of organism for synonymous substitutions than for amino acid replacement substitutions. This result is almost unaffected by removing the assumption of star phylogeny (Fig. 4).

The strict neutral hypothesis asserts that the molecular evolution is mainly caused by the neutral mutations (Kimura, 1983). According to this hypothesis, α should be the same without distinction of the type of substitution. However, our obtained values of α are different for synonymous and amino acid replacement substitutions.

In order to explain the similar difference as our finding in the above, Wu and Li (1985) invoked Ohta's slightly deleterious mutation theory of molecular evolution (1973). The evolution rate of slightly deleterious mutants is lower for larger population in a constant environment, while organisms having shorter generation time tend to have larger population size. Therefore, Ohta (1987) considered that by this cancellation the smaller generation time effect of amino acid replacement substitution would be explained, since the amino acid replacement mutants would be more deleterious than the synonymous ones. Recently, Ohta (1993) reported that the generation time effect is more conspicuous for synonymous substitutions than for amino acid replacement substitutions and persisted that this result is in accord with the nearly neutral theory of molecular evolution. However, the adequate cancellation is a subtle problem and no quantitative evidence for the above premises has been obtained (Gillespie, 1987).

It has been found that the rate of synonymous substitutions in rodents is 4 to 10 times higher than that in higher primates, 3 to 6 times higher than that in monkeys, and 2 to 4 times higher than that in artiodactyls (Li and Tanimura,

1987). They suggested that it is primarily due to the generation time effect. Using our first assumption, $V_y \propto g^{-\alpha}$ and obtained $\alpha=0.32$ for synonymous substitutions, we can estimate the ratio of the evolution rate of rodents (V_r) to that of human (V_h), to that of monkeys (V_m) and to that of artiodactyls (V_a). As the generation time of species, we use 0.164 years for rodents, 13.5 years for human, 4 years for monkeys and 0.636 years for artiodactyls. For rodents and human, $V_r/V_h=(0.164/13.5)^{-0.32}=4.10$. For rodents and monkey, $V_r/V_m=(0.164/4)^{-0.32}=2.78$. For rodents and artiodactyls, $V_r/V_a=(0.164/0.636)^{-0.32}=1.54$. These are almost consistent with Li and Tanimura's results.

Seino et al. (1992) provided the evidence for the hominoid-slowdown hypothesis, analyzing DNA sequences of primate insulin genes. It was found that the evolution rate for synonymous substitutions is about 1.5 times higher in the Old World monkey lineage than in the human and chimpanzee lineage. They asserted that this ratio may be due to the difference of the generation time of these species. We can estimate the ratio by obtained $\alpha=0.32$. As generation time of the Old World monkey and human is about 4 and 13.5 years, respectively, the ratio of the evolution rate of the Old World monkey (V_o) to that of human (V_h) is estimated to be $V_o/V_h=(4/13.5)^{-0.32}=1.48$. This value is well consistent with their result.

From the formula (1) and (2), we get $\alpha = -\partial(\log V_y)/\partial(\log g) = V_y^m/V_y$. Therefore, α represents an average fraction of evolution rate taken place by the mutation, and $1-\alpha = V_y^e/V_y \equiv r^e$ represents an average fraction by the environmental change. From our obtained α , the average environmental fraction, r^e , is 0.86 for amino acid replacement substitution and 0.68 for synonymous substitution. These results indicate that the effect of environmental change is stronger for amino acid replacement substitutions than for synonymous substitutions. By the assumption (II), r^e is an increasing function of g , whereas by the assumption (I), $(1-\alpha)$ is a constant independent of g . However, since we have estimated the value of α as a regression coefficient in the plot of $\log(K^c)$ against $\log(g)$, the value of r^e thus estimated through α can be regarded as some gross average of r^e over species with various generation times.

Using the assumption (II), we have obtained that $\tilde{V}_g^m$ is 0.009 for amino acid replacement substitutions and 0.084 for synonymous substitutions, and $\tilde{V}_y^e$ is 0.125 for amino acid replacement substitutions and 0.401 for synonymous substitutions (Table 2). By the equation (2) since we have assumed that V_g^m and V_y^e are independent of g , the evolution rate of organism having shorter generation time becomes more strongly affected by the mutation rate. This is more conspicuous for synonymous substitutions since V_g^m is larger for synonymous substitutions than for amino acid replacement substitutions. We have estimated the ratio, $r^e = \tilde{V}_y^e/(\tilde{V}_y^m + \tilde{V}_y^e)$, of mouse and human. For mouse having the shortest generation time (0.096 years) in our present analysis, $\tilde{V}_y^m$ is 0.094 for amino acid replacement and 0.875 for synonymous substitutions, using $\tilde{V}_y^m = \tilde{V}_g^m/g$. Then, r^e for mouse is 0.571 for amino acid replacement substitutions and 0.314 for

Table 3. The ratios(r^e), $r^e = \tilde{V}_y^e / (\tilde{V}_y^m + \tilde{V}_y^e)$

	A	S
Mouse ($g=0.096$ years)	0.571	0.314
Human ($g=13.5$ years)	0.992	0.985
$1-\alpha$	0.860	0.682

A and S represents amino acid replacement substitutions and synonymous substitutions, respectively.

synonymous substitutions (Table 3). It should be noted that even for synonymous substitutions about 30 percent of the molecular evolution rate for mouse is influenced by the environmental change. For human having the longest generation time (13.5 years) in our present analysis, $\tilde{V}_y^m$ is 0.001 for amino acid replacement substitutions and 0.006 for synonymous substitutions. Both for amino acid replacement and synonymous substitutions, r^e 's for human are over 0.98 (Table 3).

Gillespie (1989) studied the index of dispersion, $R(t)$, of molecular evolution, which is the ratio of the variance in the number of substitutions to the mean number. He showed that the average $R(t)$ is 7.75 for amino acid replacement substitutions and 3.3 for synonymous substitutions, thence he claimed that replacement substitutions are more selected than synonymous substitutions. His claim is in line with our assertion.

By the present data analysis, we have tried to find how the factors which regulate the rate of molecular evolution are different according to the type of nucleotide substitution. Through the analysis, we have found that the contribution to the molecular evolution rate from other causes than the mutation rate is stronger for amino acid replacement substitution than for synonymous substitution, and that it is not negligible even in the latter substitution. Of course, one may question whether the mutation rate is really constant per generation even if it is constant per cell replication. One may argue whether generation times of today well represent those along the phylogenetic line since mammalian divergence. In spite of such questions, the substitution type dependence of generation time effect is important for elucidating the population genetical mechanisms of molecular evolution and we believe that our method is worthwhile as one of possible approaches toward it. However, the number of data used in the present analysis is still limited. Further extension of analysis would give a more conclusive result on the causes of molecular evolution.

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