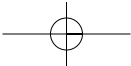
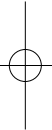
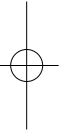
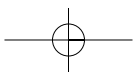
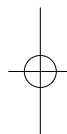
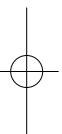
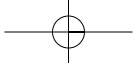


PART I

The genetic machinery of evolution





CHAPTER 1

Near neutrality and its implications for evolution

Tomoko Ohta

By the middle of the twentieth century, neo-Darwinism was very popular among evolutionary scientists and random **genetic drift** was thought to be unimportant except in the context of Wright's shifting balance theory. Then, in the 1960s, Kimura applied population genetics theory to biochemical data. He compared amino acid sequences of cytochrome *ca* and hemoglobin α among mammalian species available at that time, and found that the number of mutant substitutions in evolution was larger than expected according to Haldane's prediction based on **genetic load**. In 1968, Kimura proposed the **neutral theory** of molecular evolution (Kimura 1968). In the following year, King and Jukes published a similar idea based on biochemical considerations (King and Jukes 1969).

In the early 1970s, I thought that the borderline mutations between the selected and the neutral classes ought to be important, and published the slightly deleterious mutation theory (Ohta 1973), that was later called the **nearly neutral theory** (Ohta 1992). In the neutral theory, new mutations are classified into deleterious, neutral, and advantageous classes. In contrast, the nearly neutral theory gives importance to the borderline (i.e. nearly neutral) mutations. Figure 1.1 depicts the relationship between the selection, neutral, and nearly neutral theories.

Through DNA sequence comparisons, particularly through separate estimation of the numbers of **synonymous** and **nonsynonymous substitutions**, the nearly neutral theory is gaining support. The purpose of this chapter is to review progress in the nearly neutral theory and present recent results

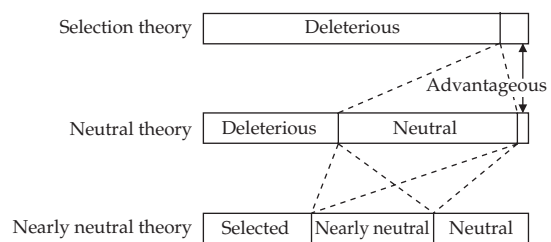


Figure 1.1 A diagram showing the comparison between the selection, neutral, and nearly neutral theories based on the proportion of various classes of mutations.

from simulation studies based on an interaction model.

The molecular clock and near neutrality

It is well known that the rate of molecular evolution is equal to the neutral mutation rate under the neutral theory, which provides a basis for the **molecular clock** of protein and DNA evolution (Kimura 1983). Let the mutation rate be ν_g per gene per generation, and let u be the probability of **fixation** of a mutant gene. In a population of size N_e (diploid), the total number of mutations appearing in the population is $2N_e\nu_g$ per generation. A fraction u of them becomes fixed in the population, and the rate of substitution per generation, k_g , is,

$$k_g = 2N_e\nu_g u. \quad (1.1)$$

The value of u is equal to the initial frequency, $1/(2N_e)$, for a neutral mutant, and the rate of evolution becomes,

$$k_g = \nu_g. \quad (1.2)$$

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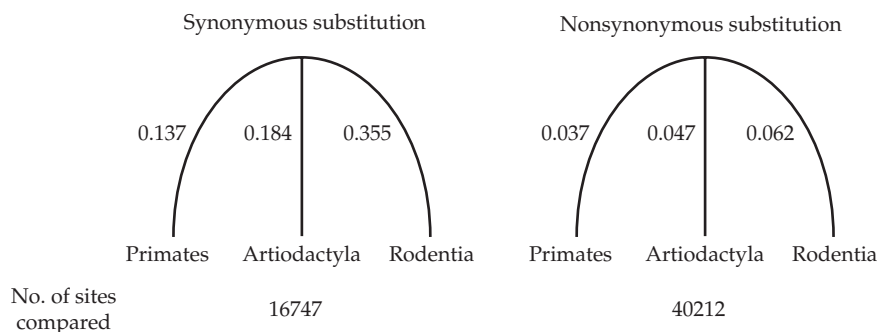


Figure 1.2 Star phylogenies of 49 mammalian genes (from Ohta 1995). The numbers beside each branch are the estimated numbers of substitutions per site. Note that the number of synonymous substitutions may be underestimated, but the general pattern is unchanged (see Yang and Nielsen 1998).

If a mutant is not strictly neutral, but nearly neutral, u becomes larger or smaller than $1/(2N_e)$ depending upon the selective value. The simplest case is that of a semidominant gene with selection coefficient, s , that is positive or negative. The crucial quantity is the product, $N_e s$, such that fixation probability is an increasing function of $N_e s$ (Kimura 1962). The important point is that u takes a positive value even if s is negative, provided that the absolute value of $N_e s$ is not large.

Under the nearly neutral theory that gives significance to the borderline mutations between the selected and neutral classes, very slightly deleterious mutations are expected to be common. For such mutations, there is a negative correlation between u and population size, N_e . Therefore, the evolutionary rate of a gene by **drift** is higher in a small population than in a large population (Ohta 1973). This may be expressed as follows:

$$k_g \propto \nu_g / N_e. \quad (1.3)$$

This relationship is extremely important for the molecular clock. The mutation rate is thought to depend on the number of cell generations, and hence on generation time. In general, large organisms tend to have short generation times and vice versa (Chao and Carr 1993). Therefore, for nearly neutral mutations, the generation time effect of mutation rate is expected to partially cancel with the population size effect of fixation probability, resulting in the molecular clock (Ohta 1973). In

other words, the generation time effect should be stronger in neutral mutations than nearly neutral mutations.

This prediction can be examined by estimating the numbers of synonymous and nonsynonymous substitutions. Figure 1.2 shows **star phylogenies** for three mammalian orders (Ohta 1995). From the figure, it may be seen that the generation time effect is more conspicuous for synonymous substitutions than for nonsynonymous substitutions, that is, the result agrees with the nearly neutral theory. It is now commonplace to examine the divergence patterns of synonymous and nonsynonymous substitutions between closely related species and within populations. Such analyses are in the same line as the present study.

So far, we have discussed the average pattern of synonymous and nonsynonymous substitutions. Another interesting problem is the variance of evolutionary rate in relation to near neutrality. It has long been known that protein evolution displays large fluctuations in evolutionary rate (Ohta and Kimura 1971; Langley and Fitch 1974; Kimura 1983; Gillespie 1991). My analyses of 49 mammalian genes confirm this large variance. Following Gillespie (1991), attention was paid to the **dispersion index**, R , that is, the ratio of the variance to the mean number of substitutions among lineages. Results of the analysis with the same data as in Fig. 1.2 are given in Table 1.1. As can be seen from the table, the dispersion index is often 5–6 for

Table 1.1 Average dispersion index

	Synonymous		Nonsynonymous	
	Unweighted	Weighted	Unweighted	Weighted
Primate–artiodactyl–rodent (39 genes ^a)	25.01	5.89	8.46	5.60
Primate–lagomorph–rodent (38 genes ^b)	20.73	5.56	6.61	3.95
<i>Drosophila melanogaster</i> – <i>subobscura</i> – <i>pseudoobscura</i> (24 genes ^c)	4.52	4.37	1.65	1.64

^a Ohta (1995).^b Ohta (1997).^c Zeng *et al.* (1998).

both synonymous and nonsynonymous substitutions. These results are supported by the maximum likelihood analyses of Yang and Nielsen (1998).

Zeng *et al.* (1998), also included in Table 1.1, found that the dispersion index is large for synonymous substitutions but not so for nonsynonymous changes in *Drosophila*. In plants, the chloroplast genes of rice, tobacco, pine, and liverwort have large dispersion indexes (Muse and Gaut 1994). However, Kusumi *et al.* (2002) found that the dispersion index is not large for the nuclear genes of conifer trees.

Interaction systems: a simulation study of the NK model

The erratic pattern of evolutionary rates is thought to reflect interactions at various levels. The **mutational landscape model** was proposed to explain the episodic pattern of molecular evolution by Gillespie (1984, 1991). The model is based on strong selection, and environmental shifts are thought to be responsible for bursts of mutant substitutions. More recently, Kauffman (1993) proposed a generalized landscape model, the **NK model**, which may be extended to treat the nearly neutral mutations of a protein.

The NK model assumes that each amino acid makes a **fitness** contribution that depends on K other amino acids among the N that make the protein. In the original NK model, there are two

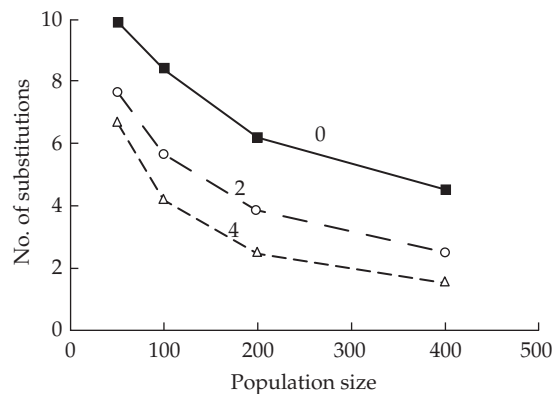


Figure 1.3 The number of mutant substitutions per term ($12/\nu$ generations) as a function of population size (from Ohta 1997). Parameters are $N = 24$ and $\bar{s} = 0.25$. Numbers beside each line are K .

states at each site. For each interacting combination, fitness is a uniform random number, and the fitness of an entire sequence is obtained by taking the average for all sites. In my simulations, I assumed nine states at each site. Also the fitness of each site is assumed to decrease with distance from the first site (for details, see Ohta 1997).

The results for the case of $N = 24$, and $K = 0, 2$, and 4 are given here. Let ν be the mutation rate per gene. The number of mutant substitutions that occurred in the period of $12/\nu$ generations, denoted one “term,” was examined, with $\bar{s} = 0.25$. Figure 1.3 shows the

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Table 1.2 Results of simulations to show the effect of duration time of small size on the dispersion index (R). Parameters are $\nu = 0.0024$, $2N_e = 50$ or 200 , and 100 replications

Duration time of small size (terms)	Neutral ^a		Selected ^b ($\bar{s} = 1/8$)	
	No. of substitutions	R	No. of substitutions	R
X1	3.04	1.90 ± 3.66	3.47	1.75 ± 2.29
X0.8	2.78	1.29 ± 1.61	3.20	1.67 ± 2.80
X(2/3)	2.73	1.39 ± 1.60	2.99	1.33 ± 0.89
X0.5	2.64	1.19 ± 1.24	2.84	1.34 ± 1.82
X0.2	2.65	0.87 ± 0.39	2.53	0.95 ± 0.43
X0.1	2.97	0.99 ± 0.38	2.72	0.99 ± 0.50

^a Term = $3/\nu$.^b Term = $5/\nu$.

number of substitutions in a term as a function of population size. The figure shows that the number of substitutions is a decreasing function of population size, in agreement with the predictions of other models of near neutrality. It is also clear from the figure that, as K gets larger, the number of substitutions decreases, that is, selective constraints becomes stronger as expected. The dispersion index was only slightly larger than unity except for the case of $K = 0$ (no interaction). Hence, it appears that the NK interaction is not important in increasing the index.

Other factors that may inflate the variance of evolutionary rate are shifts of selective value, changing population size, and higher level (i.e. among genes) interactions. The results of simulations that incorporate shifting selective values indicate that the dispersion index becomes larger than in the case of no shifting. Its magnitude is often 2–3, and the shifting is not by itself sufficient to explain the observed values. My simulation study of the effects of changing population size also shows that the dispersion index becomes large such that the weaker the selection, the larger the effect of changing population size. This agrees with the observation that the index is larger for synonymous than nonsynonymous substitutions in *Drosophila* (Zeng *et al.* 1998).

The results of Araki and Tachida (1997) suggest that the effect of changing population size depends on the time spent at the same population size. Therefore, the effect of duration time was also examined by simulation. By keeping the duration

time at a large population size at one term as before, the effect of six levels of duration time at small population sizes in the range of 0.1–1 terms were studied. Other procedures were the same as before. The results are given in Table 1.2. As can be seen from the table, the R value depends on duration time. The longer this time is, the larger the dispersion index becomes. Roughly speaking, if duration time is less than the reciprocal of the mutation rate, the change of population size has no effect on inflating the value of R .

Why does the dispersion index become large when population size changes? When the population is large, more neutral mutations accumulate than when the population is small. When the population size changes from large to small, drift becomes more rapid and the rate of substitution tends to increase (Chakraborty and Nei 1977). Conversely, if population size changes from small to large, drift becomes slow and **substitution rate** decreases on average. Therefore, the index increases by changing population size.

So far, we have considered interactions within a gene. The NK model can be extended to deal with the case of a group of linked genes that interact with each other. Each site now represents a gene (locus), and $K + 1$ genes interact among N genes. I conducted a simulation study to examine the number of accumulated mutations at each site. The period for mutant accumulation was longer than before to allow for the appropriate number of

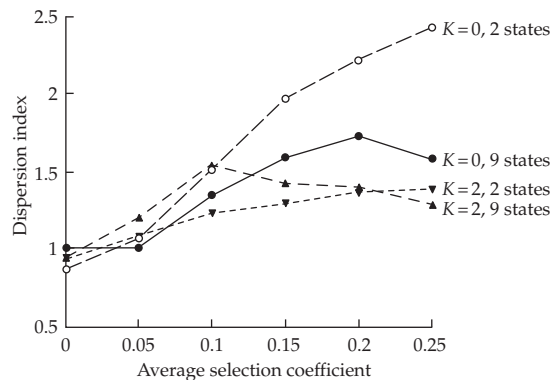


Figure 1.4 Results of simulations of the relationship between selection intensity and dispersion index, with $N = 6$ (six loci). In the simulations, mutants were accumulated in a period of $18/\nu$ generations, so that the expected number of substitutions for the neutral case is three per locus. The dispersion index was obtained from the accumulated number of mutants at each locus with 100 replications. The figures were drawn using average values of six loci.

substitutions to take place, which enabled us to measure the dispersion index at each site. Also the selection model was modified so that the level of selective constraint was the same for all sites. The case of six sites ($N = 6$), or six interacting loci, was studied for $K = 0$ and 2. The number of allelic states of a gene was two or nine. Figure 1.4 gives the results for a dispersion index that is the mean of six loci. In the figure, the intensity of selection was taken as a variable from $s = 0$ to $s = 0.25$ (average value). For $s = 0$, all loci are neutral and the dispersion index is around unity. The condition of two allelic states may correspond to the ON-OFF states of genes, whereas that of nine states may be regarded as a more complicated interaction system. The states could correspond to a level or pattern of gene expression. It may be noted that the dispersion index monotonically increases for 2-state systems by increasing the selection intensity, but not for the 9-state system. Remember that the case of $K = 0$ and 2-state is the situation of an ordinary 2-allele and independent loci system with a smooth fitness landscape. Therefore, the variance becomes larger by increasing selection intensity. If there are nine states, the allelic difference tends to decrease as selection continues, that is, the population fitness approaches the adaptive peak. This effect is stronger

for more intense selection, and the erratic pattern is reduced by increasing selection intensity.

Real examples of interacting systems are now available. Von Dassow *et al.* (2000) present a model which deals with the network of genes that makes body segments in the fruitfly *Drosophila*. This network is an interaction system involving the products of five segment polarity genes, including wingless, engrailed, and hedgehog. The effects of varying parameter values over small ranges are equivalent to those of minor mutations. The results indicate that the segment polarity network is robust to parameter variation and initial conditions. Therefore, the system appears to have properties that are reminiscent of the concept of “self-organization” postulated by the NK model (Kauffman 1993, pp. 198–221). However, Endy and Brent (2001) point out that, based on a model of bacterial chemotaxis, living systems appear to use different ways for the regulation of cellular processes. These authors define regulation as “the processing of information from the genome, from internal events, and from external events, by an amorphous architecture of diffusing molecular components.”

Another notable result of the simulations is that the dispersion index is larger when $K = 0$ than when $K = 2$. This is thought to reflect the ruggedness of the fitness landscape, such that the larger the K value, the more rugged the landscape is (Kauffman 1993). Hence, as the landscape becomes more rugged, the erratic pattern of rate variation by shifting becomes smaller.

The actual process of protein evolution is likely to be compounded, that is, a single step of the multi-loci model described above is composed of several mutant substitutions. Huelsenbeck *et al.* (2000) have shown that the compound Poisson process is appropriate for analyzing mammalian mitochondrial sequences. Gillespie (1991) formulated the compound Poisson process and pointed out that in this case the dispersion index is given by

$$R = \mu_z + \frac{\sigma_z^2}{\mu_z}, \quad (1.4)$$

where μ_z is the mean and σ_z^2 is the variance of the number of substitutions for each step of the Poisson process. In our case, the original process has a

larger variance than the Poisson process, and the above formula may be modified as follows:

$$R = (1 + a)\mu_z + \frac{\sigma_z^2}{\mu_z}, \quad (1.5)$$

where $a > 0$. The formula implies that both a and μ_z contribute to increasing R .

The preceding discussion raises a difficult question. If one observes that the dispersion index is larger than one at a certain locus, it is difficult to determine whether this large R value is caused by shifting on the fitness landscape or by changes in population size. Additional data are urgently needed to clarify the mechanisms of evolutionary change in genes and proteins.

Polymorphisms and near neutrality

An important prediction of the nearly neutral theory regarding polymorphisms is the excess of less frequent alleles in a population compared with the neutral prediction. In 1970s, I investigated this problem by analyzing electrophoretically detectable polymorphisms (Ohta 1975). I found that the observed and theoretical distributions of alleles agree well except for the excess of rare alleles in *Drosophila* and humans. Also, a theoretical analysis using the stepwise mutation model implied that the distribution depends on the values of $N_e\nu$ and $N_e s$. When these values are much less than unity, the allele distribution is indistinguishable from the neutral case, whereas it approaches the deterministic equilibrium as they get larger. Actual data suggested that these values are larger in *Drosophila* than in humans (Ohta 1975). However, the issue of selection versus drift was not settled by these studies, because expanding population size may explain these deviations from neutrality (Nei 1987).

In the present DNA era, one can make more definitive statements about weak selection, particularly by separately measuring synonymous and nonsynonymous polymorphisms. A popular approach for detecting selection is to compare the pattern of within-population divergence with that of between-population divergence separately at synonymous and nonsynonymous sites (McDonald

and Kreitman 1991). For mitochondrial genes, an excess of nonsynonymous within-population divergence is often found (e.g. Ballard and Kreitman 1994; Nachman *et al.* 1994; Rand and Kann 1996). Such an excess is thought to be due to slightly deleterious amino acid substitutions. For nuclear genes, both this pattern and the opposite, that is, an excess of nonsynonymous between-species divergence, are found (e.g. McDonald and Kreitman 1991; Long and Langley 1993). The latter pattern is probably caused by advantageous mutant substitutions. However selection may be weak and may include nearly neutral cases if there is shifting on the fitness landscape.

Rand and Kann (1996) defined a statistic, the **Neutrality Index** (N. I.) for measuring departures from neutrality according to the McDonald and Kreitman (1991) test. The index is

$$\text{N.I.} = \frac{\frac{\text{no. polym. replacement sites}}{\text{no. fixed replacement sites}}}{\frac{\text{no. polym. synonymous sites}}{\text{no. fixed synonymous sites}}}$$

Using this index, Weinreich and Rand (2000) found contrasting patterns between nuclear and mitochondrial genes of various animals.

It is now thought that even synonymous substitutions are not completely neutral. Akashi (1995) estimated that the selection intensity on codon bias is $N_e s \approx -1$, for *Drosophila simulans*, and that the value may be smaller for *D. melanogaster*. In particular, he counted "preferred" and "unpreferred" **silent substitutions** separately for segregating genes of *D. simulans*, and found that the former segregates at higher frequencies and is more often fixed between species than the latter (Akashi 1999). He applied the statistics of Sawyer *et al.* (1987) and showed the presence of weak selection such that $2N_e s \approx 1$ for the preferred codon substitutions and $2N_e s \approx -1$ for the unpreferred ones. Eyre-Walker (1999) has also shown that very weak selection on codon usage bias has been at work for mammalian genes.

Li and Sadler (1991) analyzed the polymorphic pattern of 49 human genes and found low heterozygosity at nonsynonymous sites compared with the other sites. The result implies a high prevalence of

slightly deleterious amino acid substitutions. Others have conducted similar analyses using data from the human genome diversity project. Recent genome diversity studies indicate that nonsynonymous **single nucleotide polymorphisms** (SNPs) are rarer than the random expectation as compared with synonymous SNPs, and therefore nonsynonymous SNPs are likely to be often slightly deleterious.

Note here that nonsynonymous SNPs were found in samples of 100 or so genomes. As such they can not be definitely deleterious, and are excellent examples of nearly neutral mutations or slightly deleterious mutations.

Further thoughts on drift vs. selection

One decisive piece of evidence supporting the prevalence of nearly neutral mutations is the rapid sequence divergence found in endosymbiotic bacteria. Moran (1996) found accelerated sequence evolution in *Buchnera* compared to its free-living relative, and suggested that slightly deleterious mutant substitutions occurred because of the small population size. However, even in this case, another explanation is possible, that is, the relaxation of purifying selection. In order to discriminate between the two possibilities, Funk *et al.* (2001) studied intraspecific polymorphisms in *Buchnera*, and found a very low level of polymorphism. They, therefore, conclude that the rapid sequence divergence is the result of accumulation of slightly deleterious mutations, because one expects high polymorphisms under the hypothesis of relaxed constraints (see van Ham *et al.*, Chapter 9). In general, however, drift and selection cannot be separated at the molecular level. In particular, since interactions are so prevalent, the fitness of a mutation is dependent on its genetic as well as its environmental backgrounds, such that its overall effect would be small. On the other hand, even synonymous substitutions are weakly selected (Akashi 1995), so that very many mutations fall in the nearly neutral class.

In the mitochondrial genome of *Caenorhabditis elegans*, synonymous sites seem to be under strong

selection. Denver *et al.* (2000) directly measured the mutation rate of the mitochondrial genome by accumulating deleterious mutations, and found that the rate is two orders of magnitude higher than the rate estimated from molecular evolution studies. They argue that purifying selection is responsible for the difference. Their results imply that only a minor fraction (*ca* 1 percent) of new mutations are accepted during evolution. If the mutants' effects are continuously distributed from definitely deleterious to selective neutrality, the distribution is such that only 1 percent of mutations can spread in the population. This selection is much stronger than the previous estimate based on polymorphism data (Akashi 1995). The difference probably reflects the effect of population structure such that local population size affects polymorphisms. Furthermore, the distribution should differ between various classes such as synonymous and nonsynonymous substitutions, and **deletions** and **duplications**. The strong selection on *C. elegans* mitochondrial genome seems to be exceptional, since the proportion of accepted mutations is much larger in genomes of other organisms (e.g. Kondrashov 1995).

An interesting case of the effect of genetic background on near neutrality is the activity of Hsp 90, a heat-shock protein from *D. melanogaster*. Rutherford and Lindquist (1998) reported that Hsp 90 has a large effect on genetic variation. Hsp 90 is a chaperone molecule that helps restore the native folding of proteins that have been disrupted by high temperatures. These authors argue that Hsp 90 helps in the accumulation of cryptic variations in ordinary conditions, but that under stress, these variations are expressed for selection to work. This may be related to the nearly neutral model. If high temperature is the stress, cryptic mutations may be expressed only when temperature is above some critical value. Therefore, selection coefficients would vary from time to time or from region to region. As I have argued elsewhere (Ohta 1972), the variance among the selection coefficients of mutants increases when the environment becomes more uniform, such that the probability of a mutant being advantageous or neutral becomes larger in more uniform environments. Also, the smaller the population size is, the more uniform

the environment becomes. Therefore, the theory of near neutrality (Ohta and Tachida 1990; Tachida 1991) is applicable to the evolution of cryptic mutations via Hsp 90. Consequently, nearly neutral mutations and morphological evolution may be connected.

It has been argued that most mutant substitutions at the molecular level are neutral, and are irrelevant to adaptive evolution (e.g. Nei 1987, pp. 415–16). However, in view of the intricate interactions between drift and selection, some nearly neutral mutant substitutions could have been responsible for the critical shifting of adaptive peaks in evolution, and hence for morphological changes. This may be a modern version of Wright's shifting balance theory (Wright 1938).

The concept of near neutrality needs to be understood in relation to these processes. Of course, drift or random sampling of gametes at reproduction is the basis of near neutrality. As emphasized already, genetic backgrounds as well as ecological factors influence the effectiveness of natural selection.

Summary

The nearly neutral theory contends that borderline mutations, whose effects are so small that both **random drift** and selection influence their behavior, are important. The theory predicts that there is a negative correlation between evolutionary rate and population size. This prediction was confirmed by separately estimating divergence patterns of synonymous and nonsynonymous substitutions of mammalian genes. The variance of the evolutionary rate was also examined by data analyses and by simulations based on an interaction model. Together with other available observations, the results suggest a prevalence of nearly neutral mutations at the molecular level. The pattern of changing evolutionary rates reflects interactions of gene function at various levels, and therefore the interplay of drift and selection is important for organismal evolution.

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