# The neutral theory of molecular evolution and the world view of the neutralists[1]

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## Introduction

First of all, I would like to thank the organizing committee of this congress for inviting me to give the plenary lecture named after the late Professor Hitoshi Kihara. For me, this is not only a great honor but also a real pleasure, for Dr. Kihara was one of my former tutors who greatly influenced my choice to become a geneticist. In fact, when I was a student in The 8th National High School back some 45 years ago, I first came across Dr. Kihara's name in a biology course in which the teacher told us, in conjunction with introductory lectures on cytogenetics, that Professor Kihara of Kyoto University was doing world-famous research on "genome analysis" of wheat. This aroused my interest in cytogenetics, and gradually my existing desire of becoming a botanist changed to that of becoming a plant cytogeneticist. I entered Kyoto University toward the end of the Second World War as a student majoring in botany in the Faculty of Science, rather than in the Faculty of Agriculture where Dr. Kihara was then teaching. However, upon graduation, I moved to Kihara's laboratory as an assistant. It was in this laboratory that I came across Sewall Wright's famous 1931 paper on "Evolution of Mendelian Populations." Soon I was captivated by Wright's work on theoretical population genetics, and as time went on, I became more and more absorbed by his writings. This led me to do research work on stochastic processes of gene frequencies by applying the diffusion equation method. This was the beginning of my career as a theoretical population geneticist.

These events make me realize what a great influence I had received from these two great geneticists, Dr. Kihara and Dr. Wright; my career as a scientist would have been totally different if both of them had not existed. I was very fortunate to be well acquainted with both of them. Therefore, I am glad that this Congress pays tribute to them by arranging this Kihara lecture and a symposium dedicated to Sewall Wright.

Today, I want to talk about my research work on evolution, a topic which both Kihara and Wright shared, despite their widely different fields of research in genetics.

## The neutral theory as an evolutionary paradigm

Darwin (1859), in his book *On the Origin of Species*, concludes that "species have changed, and are still slowly changing by the preservation and accumulation of successive slight favourable variations" (see p. 480 of the first edition). His theory of evolution by natural selection states that among "inheritable deviations" (or mutations to use modern terminology) which continuously appear in the species, those that are beneficial for the survival and reproduction of individuals spread through the species by natural selection. Thus each species is well adapted to its environment.

The Darwinian theory of evolution by natural selection has served as a great unifying principle in biology. Augmented by modern genetics, it led in the 1930's and 1940's to the orthodox view of evolution known as the "synthetic theory" (or "neo-Darwinism"). This theory, which reached its heyday during the period from the late 1950's until the early 1960's, was dominated by the view that the speed and direction of evolution are almost completely determined by natural selection, with mutation playing only a small and subsidiary role (see, for example, Stebbins 1966, p. 29). Most evolutionists at that time believed that selectively neutral mutants, which confer neither advantages nor disadvantages, are very rare, if they exist at all. A corollary was that random genetic drift plays no significant role in evolution or in forming the genetic structure of species. A notable exception to this was Wright's "shifting balance theory" of evolution, in which epistatic interaction in fitness and intergroup selection interact with sampling drift within each deme. Wright, however, repeatedly emphasized that his shifting balance theory differs sharply from "pure sampling drift" of selectively neutral alleles in evolution. (For details, see Wright 1978 and Provine 1986; see also Wright 1988 for his latest view.)

In contrast to the Darwinian theory of evolution by natural selection, the neutral theory (Kimura 1968; for details, see Kimura 1983) claims that the great majority of evolutionary changes at the molecular level are caused not by natural selection acting on advantageous mutants, but by random fixation (due to sampling drift) of selectively neutral or very nearly neutral mutants under continued mutation pressure. In other words, the neutral theory emphasizes the predominant role that mutation pressure and random genetic drift play in evolutionary changes at the molecular level.

The theory does not deny the role of natural selection in determining the course of adaptive evolution, but it assumes that only a minute fraction of DNA (or RNA) changes are adaptive. The neutral theory also asserts that most of the intraspecific variability at the molecular level (including protein and DNA polymorphism) is essentially neutral, so that the majority of polymorphic alleles are maintained in the species by the balance between mutational input and random extinction. In other words, it regards protein and DNA polymorphisms as a transient phase of molecular evolution and rejects the notion that the majority of such polymorphisms are adaptive and maintained by some form of balancing selection.

The neutral theory has sufficiently simple assumptions that its population genetical consequences can be worked out using a suitable mathematical method, particularly the diffusion equation method, or the diffusion models as I call them (Kimura 1964).

## Dynamics and statics of neutral mutants in a finite population

Let us consider an evolutionary process in which mutant genes are substituted one after another within the species. Each such substitution is made up of a sequence of events in which a rare mutant form which appeared, usually singly represented in the population, finally spreads through the whole population (Fig. 1). If such substitutions are caused by random fixation of selectively neutral (i.e., selectively equivalent) mutants through random sampling drift in a finite popula-

[1]This is contribution No. 1769 from the National Institute of Genetics, Mishima, Shizuoka-ken, 411 Japan.

Printed in Canada / Imprimé au Canada

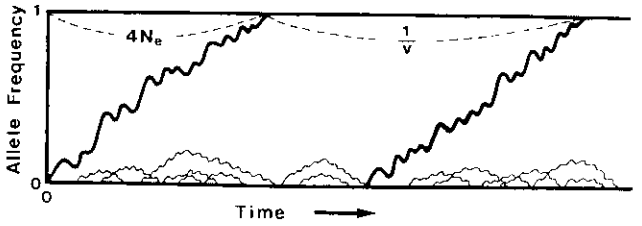


FIG. 1. Diagram illustrating the course of change in the frequencies of mutant alleles following their appearance in a finite population. A great majority of such mutants, including those having small selective advantage, are lost from the population within a small number of generations (say, in 10 generations). Only a tiny fraction, taking a very long time (say 100 000 generations), can spread through the whole population to reach fixation. In this figure, courses of change in the frequency of mutant alleles destined to fixation are depicted by thick lines.

tion, we have the following formula for the rate of evolution per generation:

$$[1] \quad k_g = \nu_0$$

where $\nu_0$ is the rate of production of selectively neutral mutants per gamete per generation. This means that the rate of neutral evolution is equal to the mutation rate to neutral alleles. Note that $k_g$ represents the rate per generation at which molecular mutants are substituted one after another in the long course of evolution. Each of these events also takes a long time, i.e., four times the effective population size (Kimura and Ohta 1969), but we consider a much longer time to measure $k_g$.

Advantageous mutations may occur, but the neutral theory assumes that they are so rare that they may be neglected from our consideration. Thus, if we denote by $\nu_T$ the total mutation rate per gamete, and if we assume that fraction $f_0$ of the mutants at the time of occurrence is selectively neutral, then, we have

$$[2] \quad k_g = \nu_T f_0,$$

where $f_0 = \nu_0/\nu_T$. Here, we assume that the fraction $(1 - f_0)$ of mutants are definitely deleterious, and that they are eliminated from the population without contributing either to evolution or polymorphism. What is remarkable in the above formulation is that the rate of evolution is independent of population size and environmental conditions.

When we estimate the actual rates of evolution through comparative studies of protein or DNA sequences, we usually express the evolutionary rate by taking 1 year as the unit length of time, while mutation rate is usually measured per generation. With this in mind, the formula [2] above may be modified to give the evolutionary rate per year so that

$$[3] \quad k_1 = (\nu_T/g) f_0,$$

where $g$ is the generation span, and therefore $\nu_T/g$ is the total mutation rate per year.

We shall now consider intraspecific variability. If we assume the infinite allele model (Kimura and Crow 1964), namely, that whenever mutation occurs at a locus it leads to a new, non-preexisting allele, then at equilibrium in which mutational input and random extinction of neutral alleles balance each other, we have the following formula for the average heterozygosity per locus:

$$[4] \quad \bar{H}_e = \frac{4N_e\nu_0}{4N_e\nu_0 + 1}$$

where $N_e$ is the effective population size and $\nu_0$ is the mutation rate for selectively neutral alleles, so that $\nu_0 = \nu_T f_0$.

## Features of molecular evolution and the neutral theory

Ample evidence has now accumulated to show that molecular evolution is distinguished from phenotypic evolution by two remarkable features: (*i*) constancy of the rate; i.e., for each protein or gene region, the rate of amino acid or nucleotide substitutions is approximately constant per site per year (known by the term "molecular evolutionary clock"); and (*ii*) the "conservative nature" of changes; i.e., functionally less important molecules, or portions of molecules, evolve faster than more important ones.

As to the first feature, the constancy of the evolutionary rate is most apparent in hemoglobin, which has an evolutionary rate of about $10^{-9}$ substitutions per amino acid site per year. I once pointed out the possibility (Kimura 1969), especially if the neutral theory is valid, that hemoglobin and other molecules of "living fossils" have undergone as many amino acid (and therefore DNA base) substitutions as corresponding molecules (genes) in more rapidly evolving species. Since then, much evidence corroborating this possibility has been found. Let me mention an example (Kimura 1983). According to Romer (1968), the Port Jackson shark is a relict survivor of a type of ancestral shark that had numerous representatives in the late Paleozoic days, notably in the Carboniferous period (270–350 million years ago). Thus, this shark is well entitled to be called a living fossil. In Table 1, a result of comparison between the $\alpha$ and $\beta$ chains of the Port Jackson shark (data from Fisher et al. 1977) is presented together with a similar comparison of $\alpha$ and $\beta$ chains of humans. From the two sets of comparisons, it is clear that genes coding for the $\alpha$ and $\beta$ chains of hemoglobin in this shark have diverged to roughly the same extent (or slightly more) as have the corresponding two genes in humans by accumulating random mutations since the origin of the $\alpha$- and $\beta$-globin genes by duplication possibly some 500 million years ago.

Such a constancy of the evolutionary rate may be explained by the neutral theory by assuming that $\nu_T/g$ in eq. 3 remains the same (constant) among diverse lineages and over time for a given protein or gene, for which $f_0$ is assumed to be constant. In other words, for a given gene, the rate of production of neutral mutations per year must be nearly constant among diverse organisms whose generation spans are very different, if the neutral theory is valid. One problem which immediately arises is that traditional mutation studies on "visible" and viability traits (including lethals) strongly suggest that the spontaneous mutation rate per generation, but not per year, is roughly equal among different animals whose generation spans are very different. In fact, this observation has been used by some to criticize the neutral theory.

It now appears, however, that many of these "mutations" are caused or controlled by transposons and insertion sequences (see, for example, Rubin 1983; Mukai and Yukuhiro 1983; Mackay 1984). On the other hand, it is likely that errors in DNA replication and repair are the main causes of DNA base changes which are responsible for molecular evolution. Thus, the mutation rate for nucleotide substitutions may depend on the number of cell divisions in the germ lines, particularly in

TABLE 1. Comparison of amino acid differences between the $\alpha$ and $\beta$ chains of human and shark hemoglobins

| Type of change* | Human $\alpha$ vs. $\beta$ | Shark $\alpha$ vs. $\beta$ |
|---|---|---|
| 0 | 62 | 50 |
| 1 | 55 | 56 |
| 2 | 21 | 32 |
| 3 | 0 | 1 |
| Gap | 9 | 11 |
| Total | 147 | 150 |

*The type of amino acid differences that can be interpreted from the code table as being due to a minimum of 0, 1, 2, and 3 nucleotide substitutions, together with the number of gaps (expressed as equivalents of the number of amino acid sites).

the male line (see Miyata et al. 1987), and this will make the mutation rate for nucleotide substitutions roughly proportional to year. Experimental studies on this subject are much needed. I would like to add that recent reports (Wu and Li 1985; Kikuno et al. 1985) showing that rodents (with a short generation span) evolve faster than human and other primates (with a long generation span) seem to support the neutralist explanation.

The second feature of molecular evolution may be restated as follows: molecules or portions of molecules that are subject to less functional constraint evolve faster (in terms of mutant substitutions) than those that are subject to stronger constraint. In other words, those mutant substitutions that cause less drastic changes in the existing structure and function of a molecule occur more frequently than those which cause more drastic ones. Let me give a few examples showing such a property. Among proteins so far investigated, the fastest evolving are fibrinopeptides, with an evolutionary rate some seven times higher than hemoglobins. It is interesting to note that fibrinopeptides have little known function after they become separated from fibrinogen during the clotting of blood. A similar example is the middle segment (C peptide) of proinsulin. This part is removed when active insulin is formed from its precursor molecule (i.e., proinsulin). It has been shown (Kimura 1983) that the C peptide evolves at a rate approximately six times as fast as that of insulin. Furthermore, in hemoglobins it is known (Perutz and Lehman 1968) that the surface portion is less important in maintaining the structure and function of the molecules than the heme pocket, which is vitally important. It has been found that in both $\alpha$ and $\beta$ hemoglobins, the surface portion evolves about 10 times as fast as the heme pocket (Kimura and Ohta 1973). Such conservatism is easy to understand from the neutral theory, because the less drastic or more conservative the mutational change, the more likely it is to turn out to be selectively neutral. This means that for more conservative changes the values of $f_0$ are larger.

More than a decade ago, we enumerated (Kimura and Ohta 1974) five principles that govern molecular evolution, one of which states that functionally less important molecules or parts of a molecule evolve (in terms of mutant substitutions) faster than more important ones. When this was proposed, accompanied by its neutralist explanation, much opposition was voiced by the neo-Darwinian establishment, but I am glad to note that it has become a part of common knowledge among molecular biologists. It is now a routine practice to search for various signals by comparing a relevant region of homologous DNA sequences of diverse organisms and to pick out a constant or "consenses" pattern, but to disregard variable parts as unimportant.

Returning to the first feature, I would like to point out, from the standpoint of the neutral theory, that a universally valid and exact molecular evolutionary clock would exist only if, for a given molecule, the mutation rate for neutral alleles *per year* ($v_0/g$) were exactly equal among all organisms at all times. Any deviation from the equality of neutral mutation rate per year makes the molecular clock less exact. Such deviation may be due to two causes: one is the change of the mutation rate per year (such as that due to a change of generation span), and the other is the alteration of the selective constraint of each molecule (due to a change of the internal molecular environment). I must emphasize that departure from exact clockwise progression of molecular evolution by no means invalidates the neutral theory, contrary to the criticism of Gillespie (1987). Recently (Kimura 1987), I presented a discussion on the molecular evolutionary clock and the neutral theory, so I shall not go into details here.

## Evolution of DNA sequences

During the last decade, we have witnessed an outburst of DNA sequence data. This has brought much new information that lends strong support to the neutral theory. It has now been well established that synonymous base substitutions within codons, which do not cause amino acid changes, occur at a much higher rate in evolution than amino acid altering substitutions. It has also been found that evolutionary base substitutions in 'introns' occur at a rate comparable to the synonymous ones or even higher. Considering the fact that natural selection acts on the phenotype of the organism, these observations showing preponderance of synonymous and other silent substitutions suggest that *molecular changes that are less likely to be subject to natural selection occur more rapidly in evolution*. This can readily be explained by the neutral theory because such molecular changes must have a higher chance of being selectively neutral (i.e., selectively equivalent) and therefore neutral evolution occurs at a higher rate (because of larger $f_0$).

I once predicted (Kimura 1977), based on the neutral theory (see eq. 2), that the maximum evolutionary rate is set by the mutation rate ($k_g \leq v_T$) and that the maximum rate is attained when all the mutations are selectively neutral ($f_0 = 1$). A dramatic example vindicating this prediction was the discovery of very high evolutionary rates for pseudogenes (or "dead" genes) that have lost their function. This was first shown clearly by Miyata and Yasunaga (1981) who made a careful analysis of the evolutionary rate of a pseudo $\alpha$-globin gene in the mouse. This was followed by a more elegant statistical analysis of the evolutionary rates of pseudogenes by Li et al. (1981). What is really interesting, as revealed by these studies, is that the rates of substitution are equally high in all three codon positions. The estimated rate in globin pseudogenes is about $k = 5 \times 10^{-9}$ substitutions per nucleotide site per year in mammals. Note that this is roughly twice as high as the rate of substitutions at the third codon position (most of which are synonymous) in the normal globin genes. This suggests that the rate of synonymous substitutions does not represent the real maximum rate predicted by the neutral theory, and that they are subject to weak negative selection (e.g., due to unequal availability of cognate tRNA species; see Ikemura 1981 and Kimura 1983). In addition to base substitu-

tions, pseudogenes accumulate deletions and additions at very high rates, suggesting that they have been liberated from the constraint of negative selection, and that they are on the way of disintegration by accumulating various mutational changes.

## Genetic variability

The neutral theory is also concerned with genetic variability within species at the molecular level (such as enzyme and DNA polymorphism). Although extensive discussion on this topic is outside the scope of this paper (for details, see Kimura 1983), I would like to mention one type of observation that is very important from the standpoint of the neutral theory. As is evident from the comparison of equations 1 and 4, it is expected that genes (proteins) or portions of DNA that evolve more rapidly must show higher intraspecific variability.

The recent observations of DNA polymorphism in natural populations of fruit flies appear to be roughly consistent with this expectation: on the whole synonymous and other silent sites are much more polymorphic than amino acid altering sites (for actual data, see Kreitman 1987 and Schaeffer et al. 1988). More systematic studies based on more extensive DNA data than available at present will be needed for more rigorous test of the neutral theory. In this respect, a study by Ward and Skibinski (1985) is reassuring. Using electrophoretic data for 42 proteins from over 200 invertebrate and 300 vertebrate species, they have found that there is a very strong correlation between protein genetic distance and protein heterozygosity among different proteins and that the observed relationship between the two can be explained quantitatively by the neutral theory.

## Some recent developments

The predominant role played by mutation pressure in molecular evolution (quite in line with the neutral theory) has become increasingly evident from recent studies. One of the most remarkable examples demonstrating this is a very rapid evolutionary change observed in RNA viruses, which are known to have very high mutation rates: RNA viruses show evolutionary rates roughly a million times as high per year as that of DNA organisms.

Saitou and Nei (1986), through a careful analysis of the evolution and polymorphism of influenza A virus genes, found that the rate of nucleotide substitution is of the order of $10^{-3}$ per site per year for most genes studied. Since the mutation rate is estimated to be 0.01 per site per year, and since this is much higher than the average nucleotide substitution rate (0.001) in this virus, the authors conclude that most influenza genes are subject to negative selection (i.e., $f_0 \ll 1$ on average). An extremely high substitution rate and the clocklike progression of substitution in influenza A virus were also reported by Hayashida et al. (1985).

Gojobori and Yokoyama (1985) compared evolutionary rates of the retroviral oncogene of Moloney murine sarcoma virus (*v-mos*$^{MO}$ gene) with its cellular homologues (*c-mos*$^{MO}$), and found that the former evolves nearly 0.8 million times faster than the latter. For *v-mos*$^{MO}$, they obtained the estimates $1.31 \times 10^{-3}$, $0.56 \times 10^{-3}$, and $2.06 \times 10^{-3}$ per site per year for the evolutionary nucleotide substitution rates at the first, second, and third codon positions, respectively. They pointed out that the rapid evolution of this RNA virus is caused by the high mutation rate, which is due to a lack of the proofreading enzymes that ensure accurate replication, coupled with the very high replication rates. These authors also investigated the molecular evolution of AIDS viruses (Yokoyama and Gojobori 1987) and obtained a nucleotide substitution rate on the order of $10^{-3}$ per site per year (see also Yokoyama et al. 1987; Penny 1988; Li et al. 1988).

What is really remarkable in the evolution of these RNA viruses is that not only do the nucleotide substitutions occur at extraordinarily high rates in clocklike fashion, but also that synonymous substitutions (that do not cause amino acid changes) predominate over amino acid altering substitutions, indicating the typical pattern of neutral evolution.

The concept of mutation-driven neutral evolution is also useful in understanding the evolution of the deviant coding system recently discovered by Osawa's group (Muto et al. 1985; Yamao et al. 1985) in *Mycoplasma capricolum*. In this bacterial species, UGA, a stop codon in the standard code table, codes for tryptophan instead of UGG, the ordinary codon for tryptophan. This organism has the characteristics of having a very high A+T content (75%) in its genomic DNA. Such a high A+T content must have been brought about by A/T directed mutation pressure (A/T pressure), that is, by the predominantly high mutation rate from G/C to A/T over the reverse direction. Presumably, this was caused by modifications in the DNA polymerase system. It was pointed out by Jukes (1985) that A/T pressure can lead to the replacement of UGA (as a stop codon) by UAA (another stop codon), followed by a change in the anticodon of one of the duplicated copies of the tRNA gene for tryptophan from CCA to UCA. After these changes, there is nothing to hinder the gradual replacement of UGG by UGA as the major codon for tryptophan under A/T pressure (G→A). Such a "capture of stop codon" should have been brought about by a series of changes, none of which has been deleterious (probably neutral).

As an experimental study on mutation-driven neutral evolution, I would like to mention a remarkable experiment reported many years ago by Cox and Yanofsky (1967), who used the Treffers' mutator gene in *E. coli*. This gene causes preferentially the transversion from an A-T pair to a C-G pair (i.e., in the opposite direction as the mycoplasma case). According to these authors, the estimated rate of mutation is $3.5 \times 10^{-6}$ per A-T pair per generation. In a strain containing this mutator gene, they observed an increase of 0.2−0.5% in the G-C composition after 80 subcultures, which corresponds to 1200−1600 cell generations. We pointed out (Kimura and Ohta 1971) that the expected change (assuming 50% G-C content of *E. coli* genome and neutrality of mutational changes) is about 0.21−0.28%, and that such agreement of the expectation with the observation supports the neutrality assumption. Recently, a mutator gene (designated *mut Y*) which preferentially induces a change in the reverse direction (i.e., G · C→T · A transversions) has been found in *E. coli* (Nghiem et al. 1988). This and other examples of mutators suggest that evolutionary shift of directional mutation pressure may not be a rare phenomenon.

## Contrasting features of molecular and phenotypic evolution

As I have explained already, molecular evolution is characterized by two features, (*i*) constancy in rate and (*ii*) conservatism in mode. I have also pointed out that these features can readily be explained by the neutral theory. On the other hand, traditional studies of evolution during the past hundred

TABLE 2. Contrasting features of molecular and phenotypic evolution

| Type of evolution | Rate | Mode |
|---|---|---|
| Molecular | Constant (per year) | Conservative |
| Phenotypic | Irregular | Opportunistic |

years or so have revealed that evolution at the phenotypic level is characterized by (*i*) irregularity in rate and (*ii*) opportunism in mode (see Table 2).

As to the first feature of phenotypic evolution, existence of striking differences in the evolutionary rate among animal groups is well known: in some animals, especially those called living fossils, evolution is extremely slow, while in others, particularly in the line leading to man from the jawless fish, evolution was very rapid. Also, in a given lineage the rate is usually irregular. Sometimes "explosive" evolution (tachytely) is succeeded by long phases of very slow change (bradytely) as noted by Rensch (1960).

As to the second feature of phenotypic evolution, the opportunistic nature of evolution is most clearly shown by the phenomena called parallelism and convergence (Simpson 1949). Here the same sorts of opportunities are seized by different groups of animals with similar solutions. A good example is the adaptive radiation of marsupials in Australia. This produced many types that are analogous to the products of adaptive radiation in placental mammals. The former includes such forms as marsupial anteater, mole-like, dog-like, and squirrel-like forms, including marsupial "flying squirrels." These two features can readily be understood if evolution of form and function is largely controlled by positive natural selection that brings about adaptation of organisms to their environments.

The question that naturally arises is this: Why does neutral evolution predominate at the molecular level, even though adaptive Darwinian evolution appears to be so prevalent at the phenotypic level? The answer to this question, I think, comes from the fact that the most common type of natural selection at the phenotypic level is "stabilizing" selection. It eliminates phenotypically extreme individuals and preserves those that are near the population mean (Mather 1953; Haldane 1959). Unlike the type of natural selection which Darwin had in mind when he tried to explain evolution through accumulation of small beneficial changes, stabilizing selection is a conservative force acting to maintain the *status quo* rather than to produce a directional change. Since the early work of Bumpus on the house sparrow and Weldon on the land snail, many examples of stabilizing selection have been reported, including the relationship between the birth weights of babies and their neonatal mortality (see Endler 1986 for a review).

I have shown (Kimura 1981) that extensive neutral evolution can occur under stabilizing selection if a large number of loci or sites are involved in a quantitative character. This applies, for example, to the situation in which each individual in a mammalian species is heterozygous on the average for one million nucleotide sites and the total selection intensity per individual is 50%. Note that selection involved here is not "balancing selection" which has been routinely invoked by the selectionists. Here I would like to mention that careful, large-scale experiments by Mukai and his associates using *D. melanogaster* have produced no evidence for the three types of balancing selection, i.e., overdominance, frequency-dependent selection, and diversifying selection at work in protein polymorphism (see Mukai *et al.* 1982 for a review). Also, there is the possibility that a certain fraction of nucleotide sites (presumably a large fraction) produce no phenotypic effect at all, and that they are completely neutral with respect to natural selection.

A really important problem facing us now is, How can we understand evolution at two levels (that is, molecular and phenotypic) in a unified way? My view on this problem is as follows.

First, we should consider seriously the possibility that many changes at the phenotypic level are so nearly neutral with respect to natural selection that random drift plays a significant role. Indeed, I think it probable that selectively neutral (i.e., equivalent) variations at the phenotypic level are much more common (particularly with respect to "quantitative characters") than we were accustomed to think under the standard "synthetic theory." Furthermore, as pointed out by Nei (1983, 1987), we should pay more attention than before to the role of mutation in evolution. For example, according to Nei, "punctuated equilibrium" (Gould and Eldridge 1977) can easily be understood if we note that some of the key mutations for progressive evolution are so rare that species must wait for a long time for them to appear.

Secondly, regarding evolution at the molecular level, the fact cannot be refuted that positive Darwinian changes have occurred from time to time, even if neutral evolution predominates over adaptive evolution. Perutz (1983) made a detailed stereochemical examination of amino acid substitutions among vertebrate hemoglobins in relation to species adaptation. He concluded that adaptations permitting these molecules to respond to new chemical stimuli have evolved by only a few (one to five) amino acid substitutions in key positions, while most of the amino acid replacements between species are functionally neutral.

Thirdly, gene duplication and subsequent accumulation of new mutations in the duplicated genes (including fixation of the duplicated copy in the population) must have played a very important role in progressive evolution. As a kind of mutation, gene duplication is constantly fed into the population, and many of them may have so little deleterious effect that they become fixed in the population through random drift under "duplication pressure." Because of substantial reduction of selective constraint after gene duplication, some of the mutations that are definitely deleterious and that would have been rejected before duplication can now reach high frequencies by random drift. Such a store of new variations may contain a few variants that will turn out to be useful for adaptation to a new environment. (For a population genetical formulation on the role of gene duplication in evolution, see Ohta 1988.)

At this juncture, I would like to call attention to the importance of a condition that I call "liberation from selective constraint" for our consideration of the causes of macroevolution. If a species is confined for a long time to a constant ecological niche with all other available niches being occupied by other species, further adaptive shift becomes impossible. Only when new vacant niches are presented will the possibility be open for macroevolution to occur. This is clearly shown by spectacular evolution during the early Cambrian, where large-scale evolutionary "experiments" were performed in the then new way of life as multicellular organisms. Similarly, explosive diversification of mammals in the early Cenozoic, immediately following the extinction of the then dominant dinosaurs, is a well-known example illustrating this point.

What I want to emphasize is that "liberation from selective

constraint'' enables extensive neutral evolution to occur, creating new variants that turn out to be useful in a new environment. Although the role of such ''preadaptation'' was once unduly deprecated by advocates of the synthetic theory (see, for example, Simpson 1949), we must reconsider its significance in the light of the neutral theory. A similar point has recently been made by Stebbins and Hartl (1988) who emphasized the importance of ''latent selection potential'' for anagenetic advance (see also ''Dykhuizen–Hartl effect'' as discussed in Kimura 1983).

If intergroup selection or competition between colonies actually occurs to a significant extent in evolution, as dictated by Wright's shifting balance theory, this will make the effection population size (i.e., $N_e$ in eq. 4) much smaller than the actual population size. Also, this will prevent many of slightly deleterious mutations from becoming fixed by random drift in the species as a whole. I think that, in this way, the shifting balance theory and the neutral theory are mutually compatible. Needless to say, quantitative treatments have to be developed in order to verify these points.

## Overwhelming importance of chance in evolution and society

The success of the neutral theory, I believe, calls our attention to the overwhelming importance of chance in evolution, particularly in the form of random frequency drift acting on fortuitous genetic variations. Note that almost every allelic gene in our genome is an outcome of incredibly lucky chance, if the neutral theory is valid.

Just as the Darwinian theory of evolution by natural selection led us to many ramifications, the neutral theory allows us to consider evolution and society in wider perspective than simply the problem of nucleotide substitutions in molecular evolution. In what follows, I would like to discuss briefly some implications of the neutral theory.

The origin of life on the earth has been one of the most outstanding problems in science. Although it is still a formidable problem, with the development of molecular genetics there is a much better hope that substantial progress will be made in near future. I imagine that in the coming century, studies on the origin of life will become more popular and will be regarded as a more respectable field than it is now.

The pioneering work by Eigen (1971), in particular his ''hypercycle'' model on the origin of genetic information, is well known (see Eigen et al. 1981 for a lucid account). His theory, however, is largely based on deterministic treatments incorporating the concept of Darwinian natural selection, but little attention seems to have been paid to stochastic elements. On the other hand, Dyson (1985) proposed a new theory on the origin of life, in which stochastic processes play a crucial role. According to his theory, an active protein evolved first in an Oparin type primitive cell through a process similar to random frequency drift in a finite population. The theory assumes also that the RNA gene emerged later in the cell as a parasite. In developing his theory, Dyson found both the main idea of the neutral theory and my diffusion equation method very useful. Irrespective of whether his new theory is valid or not, it seems to me that chance in the form of random frequency drift must have played some very important role in the process leading to the origin of life, which is intimately related with molecular evolution. In my opinion, the stochastic theory of population genetics will have to be incorporated in the future theoretical studies on the origin of life.

Similarly, Cairns-Smith (1986) invoked the ''common ancestor effect'' to explain the problem of why organisms use (mainly) L-amino acids. In his paper, he considers random fixation of organisms with L-amino acids starting from a mixture of two types of organisms, one using L-amino acids and the other using D-amino acids. He treats this problem similarly to that of random fixation of one of the two alleles in a finite population.

All these discussions lead me to believe that the most prevalent evolutionary changes that have occurred at the molecular level since the origin of life on the Earth are those that have been caused by random genetic drift rather than by positive Darwinian selection.

At the opposite end of the spectrum of topics relating to evolution, I would like to take up the problem of social implications. The British philosopher and Darwin's contemporary Herbert Spencer, and others, proposed a theory that later came to be called social Darwinism. According to this theory, struggle for existence and ''survival of the fittest'' (a term introduced by Spencer) inevitably lead to social progress. In other words, strong competitive ability is more or less equated with moral good (for a detailed discussion see Richards 1988). Such a view was used, as I understand, to support imperialist and racist policies. Fortunately, however, social Darwinism has declined much during this century, due to the progress of biological and cultural knowledge.

The term ''survival of the fittest'' is often equated with the Darwinian theory of natural selection. Paraphrasing this, I would like to propose, from the standpoint of the neutral theory, the term ''survival of the luckiest,'' in order to emphasize the importance of good fortune for any success in evolution and in society. Here I want to add that there is a possibility that random spreading and extinction of ''neutral'' characters are common in social customs, including fashion in clothes.

Finally, a word about our human existence. In his article entitled ''The lottery of life'', the well-known British science writer Nigel Calder (1985) makes the following remark, which I think is quite cogent in the present context. The evolution of fully fledged intelligence on our own very peculiar planet has depended on a succession of bizarre accidents. Therefore, the odds against anyone else existing within communicable distances in our universe must be extremely long. This will make us feel that we must cherish human life more carefully ourselves.

Until the mid-20th century, our scientific world view had been largely made based on our knowledge in physical sciences. With the rise of molecular genetics, however, things have changed, and the relative importance of biological sciences has been much enhanced. Already, studies of molecular evolution, including population genetics at the molecular level, have started to supply some important knowledge in forming our world views.

I would like to close my talk by quoting from Dr. Kihara. He was really far-sighted when he wrote the following words (originally in Japanese), as early as 1947, in relation to his outstanding cytogenetical work on the origin of cultivated wheat:

> The history of the earth is recorded in the layers of its crust; the history of all organisms is inscribed in the chromosomes.

## Summary

The main tenet of the neutral theory is that the great majority of evolutionary changes at the molecular level are caused not

by Darwinian selection but by random fixation of selectively neutral (or very nearly neutral) alleles through random sampling drift under continued mutation pressure. The theory also asserts that the majority of protein and DNA polymorphisms are selectively neutral, and that they are maintained in the species by mutational input balanced by random extinction rather than by "balancing selection." The neutral theory is based on simple assumptions. This enabled us to develop mathematical theories (using the diffusion equation method) that can treat these phenomena in quantitative terms and that permit theory to be tested against actual observations. Although the neutral theory has been severely criticized by the neo-Darwinian establishment, supporting evidence has accumulated over the last 20 years. In particular, the recent burst of DNA sequence data helped to strengthen the theory a great deal. I believe that the neutral theory triggered reexamination of the traditional "synthetic theory of evolution."

In this paper, I review the present status of the neutral theory, including discussions of such topics as "molecular evolutionary clock," very high evolutionary rates observed in RNA viruses, a deviant coding system found in *Mycoplasm* together with the concept of mutation-driven neutral evolution, and the origin of life. I also present a worldview based on the conception of what I call "survival of the luckiest."

## Acknowledgements

I thank Drs. K. Aoki, W. B. Provine, and J. F. Crow for having gone over the manuscript, or parts thereof, to suggest improved presentation. This work was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture of Japan.

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