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Relative roles of mutation and selection in the maintenance of genetic variability

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The extent and pattern of protein and DNA polymorphisms are discussed with emphasis on the mechanism of maintenance of the polymorphisms. Statistical studies suggest that a large proportion of genetic variability at the molecular level is maintained by a mutation-drift balance. At some loci, such as those for histocompatibility in mammals, however, a form of overdominant selection seems to be involved. In the presence of overdominant selection, polymorphic alleles may be maintained for tens of millions of years, so that the number of nucleotide differences between alleles is often very large, as in the case of self-incompatibility alleles in plants. There are also an increasing number of examples in which an adaptive change of a morphological or physiological character is caused by a single nucleotide substitution. Nevertheless, these mutations seem to be a small proportion of the total nucleotide changes that contribute to genetic variability and evolution. Although there are many examples of frequency-dependent selection, this form of selection is apparently unimportant for the maintenance of genetic variability except in some special cases. Observations on the evolutionary change of DNA suggest that the driving force of evolution is mutation rather than selection.

Introduction

I understand that my role in this symposium is to criticize work on frequency-dependent selection and examine whether or not frequency-dependent selection is an important factor in the maintenance of genetic variability in natural populations. I suppose I have been assigned this task because I have claimed that a large proportion of genetic variability is neutral or nearly neutral at the protein or DNA level.

I should first indicate that I have never denied the existence of frequency-dependent selection. There are many examples of frequency-dependent selection in natural populations. There are also many cases in which frequency-dependent selection is expected to occur from theoretical considerations. Nevertheless, frequency-dependent selection may not be an important factor for the maintenance of genetic variability except under special circumstances.

In this paper, I should like to discuss the general picture of the maintenance of genetic variability and consider frequency-dependent selection as one of the factors involved. Obviously, there are many different types of selection that are involved in the maintenance of genetic variability, and in a general discussion of this subject we must consider all of them. Moreover, as everyone knows, the ultimate source of genetic variation is mutation. Therefore the contribution of mutation to genetic variability should also be considered.

Types of selection

In classical population genetics, it is customary to state that any genetic polymorphism due to neutral alleles is unstable and will eventually disappear because of genetic drift. In practice, however, the loss of genetic variability due to drift is offset by new mutations, so that the extent of polymorphism in a population reaches an equilibrium value. If we use the infinite-allele model of neutral mutations, the equilibrium level of genetic variability as measured by average heterozygosity is given by

$$H = 4N_e v/(1+4N_e v),$$

where N_e and v are the effective population size and the mutation rate, respectively (Crow & Kimura 1970).

In the presence of selection, the amount of genetic variability can either increase or decrease compared with the case of neutral alleles. It is therefore convenient to classify various types of selection into two categories, i.e. (1) diversity-enhancing selection and (2) diversity-reducing selection. The former includes any type of selection that increases the genetic variability of an equilibrium population compared with that for neutral mutations. Well-known examples are overdominant selection and frequency-dependent selection with minority advantage. This category of selection is usually very powerful in enhancing genetic variability even if the

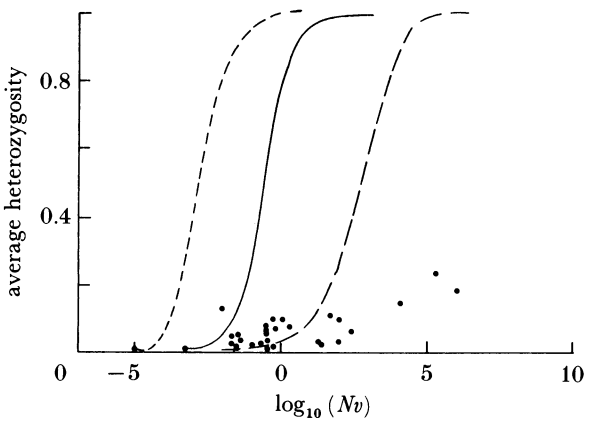

Figure 1. Relation between average heterozygosity (gene diversity) and Nv for 30 species. The mutation rate (v) is assumed to be 10^{-7} per locus per year. Solid line: expected relation for neutral alleles. Right-hand broken line: expected relation for slightly deleterious alleles with a mean selection coefficient of $\bar{s} = 0.002$. Left-hand broken line: expected relation for overdominant alleles with $s = 0.001$. The curve for overdominant alleles does not change appreciably even if s varies from homozygote to homozygote as long as the mean of s remains as 0.001. Essentially the same result was obtained when 77 species were examined (Nei & Graur 1984). From Nei (1983).

selection coefficient is very small (figure 1). Diversity-reducing selection is the type of selection that reduces the amount of genetic variability compared with the neutral level. This category of selection includes selection against deleterious mutations and selection for advantageous mutations that will eventually be fixed in the population. In this case, too, selection with a small selection coefficient may have a drastic effect on genetic variability (figure 1).

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Frequency-dependent selection

One of the most important forms of frequency-dependent selection is minority advantage. In this form of selection, a genotype in low frequency has a selective advantage over the other genotype or genotypes in high frequency, so that it generally leads to a stable polymorphism. Minority advantage is expected to occur when a polymorphism is involved in mimicry, host–pathogen interactions, or predator–prey systems, as is discussed by several speakers in this symposium.

It should be noted, however, that not all types of minority advantage are diversity-enhancing. A good example is the minority advantage that occurs in the influenza A virus. Infection of humans by this virus is mediated by glycoprotein spikes of the virus surface. There are two types of spikes: one is composed of haemagglutinin and the other of neuraminidase. Spikes composed of haemagglutinin are more important for infection than neuraminidase spikes. When a particular strain of the influenza virus infects a human population, people eventually become resistant to the strain. This is because humans produce antibodies against the haemagglutinin of the strain. It is therefore clear that the influenza virus is subject to frequency-dependent selection. That is, when the frequency of a particular strain is low, the strain spreads rapidly, but when the frequency is high, it has a low fitness and is eventually eliminated from the population. In practice, the viral haemagglutinin is subject to a high rate of mutation (about 1% per nucleotide site per year), and a new strain with a mutated haemagglutinin may infect humans with antibodies against the old strain. This generates a new cycle of influenza epidemic.

There are 13 different subtypes of the influenza A virus that infect humans and some other vertebrates. Each subtype has many strains that are mutant descendants from a common origin. The relative frequencies of the 13 subtypes changes with time, but the subtypes have existed for a long time, at least a few hundred years (Saitou & Nei 1986). As mentioned earlier, influenza virus strains are certainly subject to frequency-dependent selection, but this is not diversity-enhancing. Without the high rate of mutation of the haemagglutinin gene, the polymorphism of the 13 subtypes is expected to disappear. The frequency-dependent selection involved is a form of minority advantage, but it does not lead to polymorphism without mutation.

Several speakers in this symposium discuss frequency-dependent selection due to competition. When a pair of alleles are involved in competition for limited resources, selection necessarily becomes frequency dependent (see, for example, Nei 1971). Here again, however, frequency-dependent selection does not necessarily lead to a stable polymorphism. In Nei's (1971) mathematical model, a stable polymorphism is generated only when heterozygotes are competitively stronger than both types of homozygotes (overdominance). Currently, I know of no case in which a pair of alleles controlling competitive ability show such overdominant gene action. As emphasized by Lewontin (1974), overdominant selection seems to be rare even for non-competitive selection.

Kojima & Yarbrough (1967) proposed that a large proportion of protein polymorphisms might be maintained by selection with minority advantage. This proposal was based on the experimental observation that the frequency of allele *F* at the esterase 6 locus of *Drosophila melanogaster* increased rapidly when the initial frequency was low but as the frequency increased the amount of gene frequency change gradually diminished. A similar result was obtained in

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several population cage experiments with *Drosophila* (see, for example, MacIntyre & Wright 1966). These results are certainly consistent with expectations from the minority advantage hypothesis. However, if they are really due to such selection, what kind of mechanism would be involved? No clear answer to this question has been given. Huang *et al.* (1971) reported experimental results that support the idea that different genotypes utilize different nutrients or microniches, but their results were not confirmed in a later experiment by Dolan & Robertson (1975). Clarke & Allendorf (1979) argued that frequency-dependent selection may be generated when the relative velocities of the reactions catalysed by two allozymes vary with substrate concentration. From the theoretical point of view, however, this model also seems to have some problems (Maynard Smith & Hoekstra 1980).

One should note that minority advantage is not the only mechanism by which one can explain Kojima and Yarbrough's experimental results about the frequency changes of allozymes. The rapid changes of allozyme frequency in the early generations of cage experiments could be due to the so-called hitchhiking effect of a linked locus under selection. Because the initial population in a cage experiment is usually started with a small number of different types of chromosomes, the enzyme and selected loci are usually in linkage disequilibrium. In this case, even a neutral pair of alleles may show a rapid frequency change. In a random-mating population, this linkage disequilibrium gradually declines in successive generations, so the hitchhiking effect also diminishes (Nei 1975). The pattern of gene frequency change resulting from this effect is therefore consistent with Kojima and Yarbrough's observation with the esterase 6 locus.

However, a more serious problem with the Kojima–Yarbrough hypothesis is that minority advantage cannot work for a large number of loci. When a large number of polymorphic loci exists, an individual cannot have rare alleles for all loci or common alleles for all loci. All individuals will have rare alleles at some loci and common alleles at other loci. Therefore if there is minority advantage at each locus, the overall fitness will be more or less the same for all individuals (Lewontin 1974).

The above arguments indicate that although frequency-dependent selection may occur often, it is unlikely to be an important factor for the maintenance of genetic variability at a large number of loci.

Genetic variability at the molecular level

As soon as the genetic study of quantitative characters was started in the early 20th century, geneticists were aware that most natural populations contain a large amount of genetic variability in these characters. At that time, however, it was not possible to answer the questions: what proportion of loci is polymorphic, and how many polymorphic alleles exist at each locus? The answers to these questions were obtained only in the mid-1960s when molecular techniques such as electrophoresis and amino acid sequencing were introduced in the study of population genetics.

We are now aware that 10–70% of loci are polymorphic at the protein level in most natural populations and that the average heterozygosity for protein loci ranges from a few percent to 50% (see Nei 1987). At the nucleotide level, the extent of polymorphism is even greater, though in this case the extent is usually measured in terms of nucleotide diversity, i.e. the heterozygosity at the nucleotide level. The nucleotide diversity has been estimated for many

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different genes from various organisms and ranges from 0.002 to 0.02 in higher organisms (Nei 1987). Suppose that the average nucleotide diversity in an organism is 0.006 and that the average number of nucleotides of the coding region of a gene is 1000. Then, the expected heterozygosity of the coding region of an average gene becomes $1-e^{-0.006\times1000}=0.998$. This indicates that almost all genes in the genome are polymorphic at the nucleotide level. This value will be even higher if we include the introns and flanking regions of genes.

The extent of DNA polymorphism can also be examined by computing the number of heterozygous nucleotide sites in the genome. For example, the human genome is known to have about 3.5×10^9 nucleotides, and the nucleotide diversity has been estimated to be 0.003. We would therefore expect that an average human is heterozygous for about 10 million nucleotide sites. This is a huge number. In addition to these nucleotide polymorphisms, there are a large number of DNA polymorphisms due to deletion and insertion or transposition of genes (see Nei 1987). In the mid-1950s it was a feat if one could discover a single Mendelian genetic polymorphism. Now no-one pays attention to such a discovery unless it has some biological or medical meaning.

How then is such an extensive amount of protein or DNA polymorphism maintained in the population? In the 1950s, whenever a new polymorphism was discovered, it was customary to assume that it was maintained by some kind of selection. Thus Ford (1964) defined genetic polymorphism as 'the occurrence together in the same habitat of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained by recurrent mutation'. This definition implicitly assumes that all polymorphisms are maintained by some kind of selection. In reality, a high degree of polymorphism can be maintained by recurrent mutation alone if there is no selection or only weak selection against mutant alleles.

Protein polymorphism

The past 20 years have witnessed a great controversy over the mechanism of maintenance of protein polymorphism. This controversy has centred around Kimura's (1968) neutral theory. I have been deeply involved in this controversy and done an extensive statistical analysis of data on polymorphism. Our strategy has been to use the neutral theory as a 'null' hypothesis and examine whether the pattern of protein polymorphism agrees with predictions from the neutral theory.

Level of heterozygosity

As mentioned earlier, the average heterozygosity for neutral mutations is determined by the quantity $M\equiv4N_ev$. Therefore, if we know M, it is possible to examine whether the observed heterozygosity agrees with the neutral expectation. Under the 'null' hypothesis of neutral mutations, the mutation rate v can be estimated from the rate of amino acid substitution, because the mutation rate is equal to the substitution rate under this hypothesis (Kimura 1968). Using this approach, Nei (1975) has estimated that the mutation rate for allozymes or electromorphs is 10^{-7} per locus per year. Therefore, the mutation rate per locus per generation (v) is given by $10^{-7}\,g$, where g is the generation time measured in years. For example, g for humans is about 30, so v is approximately 3×10^{-6}.

Estimation of N_e is not easy, but it is possible to have a crude estimate of actual population size (N) in certain species. N is usually larger than N_e, so that H obtained by using N instead

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of N_e in (1) would give an upper bound of expected heterozygosity. Therefore, if the neutral theory is valid, the observed heterozygosity ($\hat{H}$) is expected to be equal to or lower than H.

With this understanding, Nei (1983) and Nei & Graur (1984) examined the relation between $\hat{H}$ and Nv for 77 species, including *Escherichia coli*, *Drosophila*, fishes, reptiles, and mammals. In this study we included only those species in which an estimate of N was obtainable and there were gene frequency data for at least 20 loci. Estimates of N were obtained by census (e.g. man, Japanese macaques) or by the multiplication of the population density by the geographical distribution. The results obtained are presented in figure 1. It is clear that in all species except one the observed heterozygosity is lower than the expected (H; solid line) and thus the data can be accommodated with the neutral theory in most species.

One important message from figure 1 is that although many selectionists tend to believe that the genetic variability of natural populations is too high to be neutral, actually it is too low compared with the neutral expectation obtained under the assumption of mutation-drift balance. It is therefore clear that to explain the level of protein polymorphism properly we must consider the factors that reduce genetic variability. For this, overdominant selection or other types of balancing selection are clearly inadequate because they tend to increase heterozygosity (see Nei (1980) for other problems). The dotted line in figure 1 represents the expected heterozygosity when overdominant selection with a selection coefficient of $s = 0.001$ is operating (Maruyama & Nei 1981). Although the selection coefficient is very small, the expected heterozygosity rises very rapidly as Nv increases and is generally much higher than that for neutral mutations. It should also be noted that the highest gene diversity (equivalent to heterozygosity) so far observed is from *E. coli*, where overdominant selection cannot occur because of haploidy.

Figure 1 indicates that although the observed heterozygosity is certainly lower than the neutral expectation, the difference between them is very large when Nv is large. Under the framework of the neutral theory this can be explained by the following two factors. First, a high value of Nv usually occurs for small organisms such as *Drosophila* and *E. coli*, and in these organisms the effective population size is expected to be much smaller than the actual size because of frequent extinction and replacement of colonies (Maruyama & Kimura 1980). Second, average heterozygosity is affected drastically by the bottleneck effect, and this effect is expected to last for a long time in large popuations, often millions of generations (Nei 1987). Actually, in the Pleistocene ($10^4 - 2 \times 10^6$ years ago) there were several glaciations, and in these glaciation periods many organisms apparently went through bottlenecks. It is known that up to 50 % of mammalian species in North America became extinct in the Pleistocene and many new species appeared (Martin & Wright 1967). Therefore the difference between $\hat{H}$ and the neutral expectation (H) in figure 1 can be explained by the assumption that the long-term effective size is much smaller than the actual size. Of course, the bottleneck effect is not the only factor that can explain the difference between $\hat{H}$ and H. There are at least two other factors, both of which are forms of diversity-reducing selection. One is random fluctuation of selection intensity as formulated by Nei & Yokoyama (1976) and the other is Ohta's (1973) theory of slightly deleterious mutations. However, it is not clear at the moment how important these factors are in reality. The reader who is interested in this problem may refer to Nei (1980, 1983).

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Internal consistency of population parameters

It should be remembered that in the above test we have not really estimated the effective population size (N_e) and thus our conclusion is only qualitative. However, there are several other tests of the neutral theory and its alternatives where estimates of N_e are not required. In these tests the internal consistency of population parameters for a particular theory to be tested is examined. For example, the distribution of allele frequency (x) for neutral genes is a function of $M \equiv 4N_e v$ only. It is given by

$$\Phi(x) = M(1-x)^{M-1}x^{-1} \qquad (2)$$

(Crow & Kimura 1970). Therefore, if we know M rather than N_e and v separately, we can compute the theoretical distribution of x and compare this with the observed distribution. An estimate ($\hat{M}$) of M can be obtained from average heterozygosity by using (1), i.e. by $\hat{M} = \hat{H}/(1-\hat{H})$.

Chakraborty *et al.* (1980) compared the observed distribution of allele frequencies for protein loci with the expected distribution in 138 different species or subspecies. Their results have shown that the observed distribution is U-shaped in all species examined and agrees fairly well with the expected distribution from (2). Figure 2 shows one example obtained from *Drosophila engyochracea*. Clearly, the observed distribution (a) is very close to the neutral expectation (b) but quite different from that for overdominant alleles (c), which was obtained under the condition that the expected heterozygosity is equal to the observed value, i.e. $H = 0.127$.

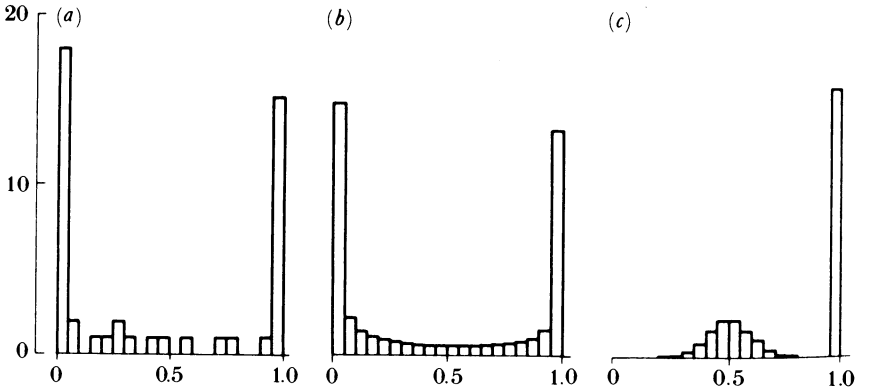

FIGURE 2. Observed and expected distributions of allele frequencies. (a) Observed distribution for *Drosophila engyochracea* ($\hat{H} = 0.127$). (b) Expected distribution for neutral alleles with $\hat{H} = 0.127$. (c) Expected distribution for overdominant alleles with $\hat{H} = 0.127$. From Nei (1983).

In addition to the above study, various other parameters such as the distribution of single-locus heterozygosity, the mean and variance of genetic distance, and the correlation of single-locus heterozygosities between related species have been examined (Nei *et al.* 1976*a*, *b*; Fuerst *et al.* 1977; Chakraborty *et al.* 1978). The results of these studies again showed that observed data do not deviate far from neutral expectations. One can therefore conclude that the pattern of protein polymorphism within and between populations is in approximate conformity with the neutral expectation but substantially deviated from that of several hypotheses involving selection (Nei 1980; Nei & Graur 1984). Of course, the statistical methods so far used are generally very crude and would not detect small differences in

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prediction between alternative hypotheses. This is particularly so when selection coefficients are small. However, it now seems clear that the population dynamics of protein polymorphism is largely controlled by stochastic factors whether some weak selection is involved or not.

DNA POLYMORPHISM

In the study of evolution DNA sequences are much more informative than protein sequences, because a large fraction of DNA does not code for proteins and there is degeneracy in the genetic code. Thus, genetic variation in the non-coding regions of DNA (introns, flanking regions, etc.) or silent nucleotide substitutions can be studied only by examining DNA sequences. Unfortunately, however, the techniques for studying DNA sequences were developed only recently, so that data on DNA polymorphism are still scanty compared with those on protein polymorphism. Nevertheless, many interesting results have already been obtained. In the following I consider only those studies that are directly related to our problem.

Silent polymorphism

From the standpoint of the neutral theory, it is interesting to examine the extent of DNA polymorphism that is not expressed at the amino acid level. In the neutral theory, the level of silent polymorphism is expected to be high compared with the polymorphism revealed at the amino acid level, because silent mutations are subject to purifying selection less often than non-silent mutations. On the other hand, if polymorphism is actively maintained by natural selection and the effect of genetic drift is unimportant, one would expect that the level of silent polymorphism is lower than that of non-silent polymorphism. One way of testing this hypothesis is to examine the polymorphism at the first, second and third positions of codons. All nucleotide changes at the second position of codons lead to amino acid replacement, whereas at the third position only about 28% of changes are expected to affect amino acids because of degeneracy of the genetic code. At the first position, about 95% of nucleotide substitutions result in amino acid changes. Therefore, if the neutral theory is valid, the extent of DNA polymorphism is expected to be highest at the third position and lowest at the second position.

Available data indicate that this is indeed the case except for some immunoglobulin genes. For example, the *F* and *S* alleles at the alcohol dehydrogenase locus in *D. melanogaster* are electrophoretically distinguishable because of the amino acid difference (threonine v. lysine) at the 192nd residue (codon change from ACG to AAG). The nucleotide sequences of about a dozen alleles at this locus indicate that there is no other amino acid substitution between the *F* and *S* electromorphs but that there are many third position substitutions that are silent (Kreitman 1983). Similar examples of a large proportion of silent substitutions are found in the tryptophan operon genes in *E. coli*, the β globin gene complex in man and the histone 4 gene in the sea urchin *Strongylocentrotus purpuratus* (see Nei 1987).

However, this is not necessarily true in immunoglobulin genes. Sheppard & Gutman (1981) showed that two alleles (*LEW* and *DA*) at the rat κ light-chain constant region gene show twelve nucleotide differences in the coding region. Eleven of these twelve nucleotide differences (92%) have resulted in amino acid differences. This is considerably higher than the expected value of 74% under random nucleotide substitution (see Nei 1975). In the case of the constant

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region of the mouse immunoglobulin γ heavy-chain gene (*γ2a*), two alleles, *IgG2a*^{a} and *IgG2a*^{b}, show a total of 111 nucleotide differences plus 15 additional differences due to insertion and deletion when 1114 nucleotides are examined (Schreier *et al.* 1981). Of the 111 nucleotide differences, 18 (16%) were silent and the rest were amino acid altering substitutions. These two observations suggest that in immunoglobulin genes amino acid altering nucleotide substitutions might be favoured by selection because variability is needed in immunoglobulins. Similar selection seems to be involved in the mouse major histocompatibility complex (MHC) loci, where extremely high variability is observed (see Klein & Figueroa 1986).

Allelic variation at loci controlling mating types

Classical genetics has established that genetic loci controlling mating types generally show a high degree of polymorphism. One such locus in plants is the self-incompatibility system that ensures outcrossing. This system is usually controlled by multiple alleles at a locus. Studying the self-incompatibility system of *Oenothera organensis*, Emerson (1939) found 37 different alleles in a population of about 500. A large number of self-incompatibility alleles are also known to exist in many other plants which have this system.

Self-incompatibility represents a case of strong overdominant selection because the homozygote for an allele is not produced. Therefore, one would expect that a high degree of polymorphism is generated even if $4N_e v$ is small (see, for example, Wright 1939; Fisher 1958; Yokoyama & Nei 1979) and that the polymorphic alleles persist in the population for a long time (Maruyama & Nei 1981). This expectation was recently confirmed by Nasrallah *et al.* (1987), who studied the nucleotide sequences of three alleles at the incompatibility (glycoprotein) locus of *Brassica oleracea*. Deducing the amino acid sequences from the DNA sequences obtained, they showed that the proportion of amino acid difference between alleles is more than 20%. This is probably the highest degree of allelic differences so far observed in higher organisms; it is higher than the proportion of amino acid differences between the human and horse haemoglobins.

This observation suggests that in the presence of strong overdominant selection polymorphic alleles persist in the population for tens of millions of years. I believe the same situation occurs with the sex-determining alleles in honey-bees and some other species of Hymenoptera. However, it should be remembered that the number of loci involved in this type of mating system is very small and that the number of amino acid differences between different alleles is usually one or two.

Adaptive evolution

These studies suggest that genetic variability at the molecular level is maintained mainly by mutation-drift balance and that if there is any selection, it is usually of the diversity-reducing form. Of course, this does not mean that selection plays no positive role in evolution. On the contrary, there must be positive selection at some loci; otherwise no adaptive evolution would occur. Every biologist is aware that most morphological and physiological characters are well-adapted to the environment in which the organism lives. How then can we reconcile these two types of observation? There are two ways. One is to assume that although most molecular mutations behave just like neutral genes in populations, they are not strictly neutral and their cumulative effects for many loci are responsible for the adaptive change of organisms. For

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convenience, I call this the minute gene effect hypothesis. The other way of reconciliation is to assume that most non-deleterious mutations are more or less neutral but that there are a small proportion of mutations that are responsible for adaptation. I call this the major gene effect hypothesis (Nei 1987).

The minute gene effect hypothesis was originally proposed by Latter (1972), but it has been formalized by Kimura (1981, 1983) and Milkman (1982). According to this hypothesis, most important morphological or physiological characters show continuous variation and are subject to centripetal (stabilizing) selection. It is assumed that a character is normally distributed and controlled by a large number of loci each of which has a very small additive contribution to the character. The fitness of an individual is determined by its phenotypic value, x, and as x deviates from some optimum value, the fitness declines disproportionately.

One might think that this type of selection leads to a stable polymorphism at each locus, but actually it results in homozygosity in the absence of mutation, as shown by Wright (1935). That is, this is a kind of diversity-reducing selection. In the presence of mutation, however, a large amount of genetic variability may be maintained. In this case, one can show that although the total amount of selection (genetic load) for a quantitative character is substantial, the selection coefficient for each locus is very small if the number of loci involved is large (Kimura 1981; Milkman 1982). For example, if we consider an abstract character that determines the Darwinian fitness of an individual and assume that all nucleotides in the mammalian genome (3.5×10^9) are concerned with fitness, the selection coefficient (s) for a nucleotide site can be as small as 3.3×10^{-7} if the total amount of selection is 0.5 (Kimura 1983). From this type of computation, Kimura concluded that 'neutral molecular evolution is an inevitable process under stabilizing phenotypic selection when a large number of nucleotide sites are involved.' In this view, 'neutral alleles' are not really completely neutral but have very small phenotypic effects, the accumulation of which is significant for adaptive evolution.

The major gene effect hypothesis was originally presented by Kimura (1968) and has been supported by a number of authors (see, for example, Nei 1975; Wilson 1975). In this hypothesis, the majority of nucleotide substitutions are neutral or nearly neutral, and adaptive evolution occurs by a relatively small proportion of gene substitutions. We have seen that at the DNA level most loci are polymorphic and that there are many alleles at each locus. Thus, even if only a small percentage of gene substitutions is adaptive, there are still a large number of substitutions that can result in adaptive evolution. Therefore there is no problem in explaining adaptive evolution. The selective advantage conferred by a mutation is assumed to be generally quite large, so that a rather small number of gene substitutions are required for a particular event of adaptive evolution. This hypothesis does not preclude the existence of many modifier genes that have been identified in classical genetics but claims that even if these modifier genes are included, advantageous mutations are in the minority.

At present, it is difficult to decide which of the above two hypotheses is correct. I prefer the second hypothesis for the following reasons. (1) It is not clear whether there is any character to which a very large number of 'nearly neutral loci' contribute additively. For Kimura's model of stabilizing selection to be realistic, the phenotypic character must be completely developed before selection operates (Robertson 1968). This is because a selection coefficient cannot be assigned to each individual unless the phenotypic character is completed. In reality, a large proportion of natural selection seems to occur independently in various stages of

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development before maturity, as was emphasized by Nei (1975). In addition, if there are many quantitative characters each of which is controlled by a different set of relatively small numbers of loci, the overall pattern of selection would be close to the case of independent selection rather than to stabilizing selection. (2) There is an increasing amount of data indicating that genetic change of morphological and physiological characters is caused by a few major genes.

In microorganisms, it has long been known that an adaptive change in enzymes (change in substrate specificity) may occur by one or a few amino acid substitutions. For example, β-lactam antibiotics kill bacteria by inactivating a set of penicillin-binding proteins (PBPs) that are essential for cell division. Some mutants of *E. coli* are resistant to these antibiotics because of the reduction in affinity between antibiotics and PBPs. Hedge & Spratt (1985) have shown that this reduction in affinity is caused by one to four amino acid substitutions in the active centre of a PBP and that the majority of other amino acid substitutions do not affect the susceptibility to antibiotics.

Examples of adaptive change of enzymes or proteins are not confined to bacteria but are available from many other groups of organisms (table 1). One interesting case is the adaptation

Table 1. Examples of molecular changes that affect adaptive characters

character	gene (substitutions)	reference
1. resistance to antibiotics	β-lactamase (PBP) (1–4 amino acid substitutions)	Hedge & Spratt (1985)
2. resistance to herbicides	chloroplast *psbA* gene (1 nucleotide substitution)	Hirschberg & McIntosh (1983)
3. crocodilian Hb	haemoglobin (5 amino acid substitutions)	Perutz *et al.* (1981)
4. stomach lysozyme in ruminants	lysozyme c (small proportion of amino acid substitutions)	Jolles *et al.* (1984)
5. courtship song rhythm in *Drosophila*	period (*per* gene) (1 nucleotide substitution)	Yu *et al.* (1987)
6. heterochrony in *Caenorhabditis elegans*	cell division (single mutation)	Ambros & Horvitz (1984)
7. swimming speed of fish	*Ldh* locus (1 amino acid substitution?)	DiMichele & Powers (1982)

(by change in substrates) of crocodilian haemoglobin to the increased blood acidity that occurs when crocodiles stay under water for a long time. This adaptation can be explained by five amino acid substitutions. This number is a small fraction of the total number of amino acid differences (123) that exist between the crocodilian and human haemoglobins (Perutz *et al.* 1981). The functional change of stomach lysozyme of ruminants can also be explained by a small proportion of amino acid changes (Jolles *et al.* 1984).

Another interesting example is herbicide resistance in plants. Many commercially important herbicides kill plants by inhibiting photosynthesis. This inhibition occurs because herbicides bind to a thylakoid-membrane protein encoded by a chloroplast gene, *psbA*. Several plant species have mutant strains that are resistant to herbicides. Hirschberg & McIntosh (1983) sequenced the *psbA* genes from a normal and a mutant strain of the pigweed *Amaranthus hybridus* and found that there is only one codon difference between the two genes: the normal gene has a serine codon (AGT) at the 264th codon, whereas the mutant gene has a glycine codon (GGT). Interestingly, the serine codon AGT is evolutionarily conserved and is shared by many

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plants (e.g. pea, tobacco, spinach), and the same A → G mutation has occurred in other species (*Solanum* and *Brassica*). However, some lower plants and cyanobacteria have different codons, though they code for the same amino acid (serine), and the mutant codons are for alanine (see Nei 1987). These findings suggest that the serine at the 264th residue is responsible for herbicide binding and that the replacement of this serine by some other amino acid prevents herbicides from binding to the chloroplast thylakoid membranes.

The above examples and those in table 1 indicate that a single nucleotide (or amino acid) substitution may have a drastic effect on gene function and consequently on the fitness of an individual. It is expected that the number of such examples will increase as more polymorphic alleles are studied at the DNA level. However, this type of change occurs only at a limited number of sites, and a large proportion of nucleotide changes seem to have little effect on morphological and physiological characters.

Role of mutation in evolution

The final issue I discuss in this paper is the importance of mutation in evolution. In the past several decades, neo-Darwinism or the synthetic theory of evolution has dominated our thought of evolution. In this theory, natural selection plays the most important role in determining the extent of genetic polymorphism and the rate of evolution. Although mutation is regarded as the ultimate source of genetic variation, its role in evolution is considered to be minor. This is because mutation occurs repeatedly at the phenotypic level and most natural populations seem to carry a sufficient amount of genetic variability, such that almost any genetic change can occur by natural selection whenever the change is needed. That is, natural selection plays a creative role (see, for example, Mayr 1963; Dobzhansky 1970). This view was formed apparently because whenever artificial selection was applied to a quantitative character, a quick response was observed in many organisms. This gave the early population geneticists the impression that a randomly mating population contains almost all kinds of genetic variation and that the only force necessary to achieve a particular evolutionary change is natural selection. However, artificial selection is quite different from natural selection. The response to artificial selection is usually large in the early generations but gradually diminishes as generations proceed. Without further input of new genetic variability, the mean value of a character under selection usually reaches a plateau within a few dozen generations. Note also that the initial response to selection or the heritability of a quantitative character is usually greater in those characters that are remotely related to fitness than in those that are closely related (see, for example, Falconer 1981). This suggests that the former characters have not been subjected to strong natural selection, so that the amount of genetic variability accumulated in the population is large. Therefore if artificial selection is applied to these characters, a rapid response occurs. In the latter characters, however, natural selection seems to have reduced the genetic variation, and thus artificial selection is less effective. If this interpretation is correct, artificial selection does not provide an accurate picture of long-term evolution by natural selection.

It is often said that genetic polymorphism is beneficial to the population because in the presence of genetic variability the population can adapt easily to new environments (Dobzhansky 1970). Thus any mechanism that increases genetic variability is selected for (Ford 1964). I am sceptical about this teleological explanation. The present genetic variability

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of a population is simply a product of evolution in the past. It may happen to be useful for future evolution, as in the case of industrial melanism, but I doubt that genetic variability is stored for future use. In many cases, a population may not have the genetic variability that is needed for new adaptation. In this case, the population may stay unchanged until new mutations occur or may simply become extinct. Note that over 99% of all species have become extinct over geological time. Note also that rapidly evolving species do not necessarily have a high level of genetic variability. For example, many carnivore and primate species (e.g. cheetahs, weasels, chimpanzees and macaques) are depauperate in protein polymorphism, despite the fact that they are among the most highly evolved animals (see Nei & Graur 1984).

In my view, natural selection is a consequence of the existence of two or more functionally different genotypes in the same environment. For example, if one genotype is more efficient in obtaining food or more resistant to a certain disease compared with others, it will have a higher survival value or reproductive success. The functional efficiency of a genotype is determined by the genes possessed by the individual, and natural selection automatically follows when there is a difference in functional efficiency between two different genotypes. Therefore the most fundamental process of adaptive evolution is the creation of better (functionally more efficient) genotypes by mutation or gene recombination. Mayr (1963) and Dobzhansky (1970) have argued that natural selection has creative power just as a sculptor or a composer does. Their argument seems to be based on Muller's (1929) computation that the chance of combining many favourable mutations into one individual is enhanced tremendously if there is selection for all of them. Moreover, in the presence of gene interaction a new phenotype may appear when different alleles are combined at different loci. However, it should be noted that natural selection operates only when there are different genotypes that are produced by mutation or recombination. In other words, mutation is not only the ultimate cause of variation but also the driving force of evolution, whether the evolution is adaptive or not (see Nei (1987) for a more detailed discussion on this problem).

One might wonder if this view is contradictory with Charles Darwin's theory of evolution. Darwin certainly emphasized the importance of natural selection in evolution. However, he was also aware of the importance of generation of variation. In his time, neither the mechanism of inheritance nor the cause of genetic variation was known. Although he was vague about the cause of genetic variation and considered climatic change and use and disuse as possible factors, he clearly separated mutation from selection. He was aware that natural selection operates only in the presence of genetic variation and never stated that natural selection is creative. Therefore, the view presented here is not incompatible with Darwin's. It is also essentially the same as Morgan's (1932) mutation-selection theory, though he was concerned only with morphological or physiological characters (see Nei (1987) for historical details).

At this point, I should like to emphasize that I am not saying that the study of natural selection is useless. Although natural selection is a sieve to save beneficial mutation or eliminate harmful mutations (Morgan 1932), its actual process is complicated. Its effectiveness depends on reproductive system, population size, population structure, etc. As Endler (1986) recently emphasized, the detailed mechanism of natural selection is not well understood, and there is an urgent need to study this. The theme of this symposium is frequency-dependent selection and is concerned only with a restricted form of selection. However, even the mechanism of this type of selection is not well understood as is clear from the previous papers.

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As I mentioned earlier, frequency-dependent selection may not be an important factor for the maintenance of genetic variability except under special circumstances. However, for evolution of certain characters such as secondary sex features it is clearly important (O'Donald 1980). Competition for limited resources is also expected to generate frequency-dependent selection. Therefore, I conclude that it is generally an important form of selection for adaptive evolution. In the future study of natural selection, however, more emphasis should be given to identification of the gene or genes involved, and the biochemical and physiological basis of the adaptive difference between alleles should be studied. Molecular biology is now so highly developed that we can clone any gene of any organism and study the mechanism of selection at the molecular level. In my view, this is the shortest route to an understanding of the process and meaning of evolution.

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