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Population Size and Rate of Evolution*

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Summary. It is suggested that in evolution there is much substitution of nearly neutral mutations, for which the selection intensity varies from time to time or from region to region. Since the variance among the selection coefficients of new mutants decreases when the environment becomes uniform, the probability of a mutant being advantageous to the species as a whole increases in more uniform environment (Fig. 1). Therefore the rate of gene substitution increases in smaller populations, as smaller populations are likely to be distributed over less varied environments.

The adequacy of the model was discussed in relation with the following facts or plausible postulates. 1. A large number of amino acid substitutions during a period corresponding to the formation of new species. 2. Rapid evolution at the phenotypic level of populations having a small size. 3. Many extinctions and expansions of the species in the past.

Key words: Evolutionary Rate — Environmental Diversity — Nearly Neutral Mutations — Population Size.

Introduction

The hypothesis presented here is that evolution is more rapid in small populations than in large ones. The essential argument that will be developed is that in a stable environment a random mutant need be beneficial only under restricted circumstances to have a selective advantage whereas in a more variable environment a mutant must be beneficial in many circumstances; and the smaller the population, the more restricted the environmental variability.

My theory differs from the theories of both Fisher (1930) and Wright (1929, 1931). Fisher argued that the most favorable population for evolutionary change is a large one, both because of its greater genetic variability and because random processes are less important. Wright, in his shifting balance theory, places great importance on random gene frequency drift but he regards the most favorable structure as being a population subdivided

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into groups with some migration between groups. My theory emphasizes random changes, but considers the evolution within the restricted population rather than the effect of migrants. It also differs from a strict neutral theory in that in the latter, the rate of evolution is independent of the population size.

Although the hypothesis is an ecological one in that its validity depends on assumed relationships between population size, environmental diversity, and the probability of a mutant being favorable, it deals with gene substitutions which are more likely to be detectable by molecular means than by ecological observations. For this reason, the discussion is partly in molecular terms.

In discussing the mechanism of evolution, it is important that we should make a clear distinction between mutant substitution at the population level and the occurrence of mutations at the individual level. It is likely that the majority of mutations are harmful to well-adapted organisms, but that there is a continuous transition from unconditionally deleterious to neutral and even advantageous mutants. We then expect that there are mutations in the neighborhood of neutrality that are subject not only to random genetic drift but also to natural selection. In fact, it is realistic to suppose that the effective selection intensity for such mutants is a random variable rather than a constant and that it has a certain distribution around neutrality. In this regard, it is possible that many amino acid substitutions in protein evolution represent the substitutions of nearly neutral but not strictly neutral mutations. On the other hand, strictly neutral mutations are likely to be more important in nuclear DNA divergence as a whole. This is because there is probably a great deal of non-informational DNA. This will explain the negative correlation between generation length and DNA divergence if the intrinsic mutation rate is dependent on the number of generations as well as on simple chronological time (Ohta, 1972b).

Now, regarding nearly neutral mutations, the very important parameter which influences their behavior is the size of the population. As early as 1931 and also in succeeding papers, Wright (1931, 1935, 1940, 1956) emphasized the importance of a structured population with small local effective size for the evolution of the species. Haldane (1932) and Simpson (1944) agreed with Wright's idea based on paleontological observations. In fact, almost all rapid evolution in the past seems to have occurred while the population size was small or sparse (cf. Simpson, 1944; Wright, 1956). On the other hand, we should expect that evolution of definitely advantageous mutations must be rapid when the population size is large, because such populations contain a large amount of genetic variation of every kind (Fisher, 1930). In the following I shall discuss the relationship between population size and the rate of evolution by considering nearly neutral mutations.

Concept of Near Neutrality

Nearly neutral mutations are those mutations whose selection coefficients are so small that their behavior is not very different from strictly neutral mutants. Operationally, this is defined by $|N_e s| < 1$ where N_e is the effective population size and s is the selection coefficient. However, since natural selection is very complicated, it is unlikely that the selection coefficient stays constant. Wright (1948) has shown that when the selection intensity fluctuates from generation to generation due to environmental variations, not only the average selection coefficient ($\bar{s}$) but also its between-generation variance (V_s) becomes important. In particular I have shown that when the ratio of $\bar{s}$ to V_s is less than 1/2, the mutant behaves as if it were selectively neutral (Ohta, 1972a). In this context, I have proposed the term, effective selection coefficient in terms of the effectiveness on fixation probability, to treat such phenomena.

It is clear, then, that the above simple definition of near neutrality is insufficient. Under the term "nearly neutral mutations" we should include all those mutations which have a chance to have a non-zero selection coefficient but whose behavior is significantly influenced by random fluctuation of selection intensity and/or by random sampling of gametes. The thorough investigation on cytochrome c by Margoliash and his associates (1970) provides a model case for showing the importance of amino acid substitutions with small effects. The main function of cytochrome c is obviously electron transport. There is no observable difference among the cytochrome c's of many organisms in this function. Yet there are differences in a number of ion binding constants. These authors have speculated that this enzyme may have a subsidiary ion transport function and this minor function may be subject to random drift as well as natural selection.

Perhaps the most important general example of alleles definitely involved in natural selection and definitely having selection coefficients that fluctuate around a mean of zero is the general situation of a polygene under stabilizing selection. If the population mean is at the optimal phenotypic value for the environment, the average selection coefficient for an allele with a small effect is zero. But if there is a difference between the population mean and the optimal phenotypic value, alleles tending to shift the mean toward the optimum will, on the average, be selected for, and others selected against. Presumably the population mean will itself vary around the optimal phenotype, or looking at it another way, the optimum will fluctuate randomly as the environment changes and the genetic mean will never be quite able to keep up with it.

Variation of selection intensity can also be seen from the following examples. According to Ohno (1970), the rabbit is resistant to the alkaloid, atropine, when it has the enzyme atropinesterase. Thus, the gene for

atropinesterase has an obvious selective advantage only when atropine is present in the food souce. The well known case of sickle cell hemoglobin shows that the gene for this abnormal hemoglobin has selective advantage only in presence of malaria. Otherwise, it has a severe disadvantage causing lethality in homozygotes. Such environmental factors may not remain constant but vary from region to region or from time to time.

These are examples of genes with strong selection, but the same phenomena probably exist for genes with weak selection. It is likely that the class of alleles with fluctuating selection coefficients near neutrality includes numerous mutations that can be detected at the molecular level.

Selection Coefficient and Population Size

The examples in the previous section indicate that a mutant can be advantageous under a restricted condition, but generally be disadvantageous. If the environment is very diverse, it is almost impossible for a mutant to find itself advantageous under all conditions required by the environment. On the other hand, if the environment is uniform a mutant will have a better chance to be advantageous.

Generally, the variation of the effective selection coefficient among different mutants must be larger under a more uniform environment, for the following reason. Suppose that there are a series of mutants with selective advantages s_1, s_2, s_3, ... in a restricted environment. If the environment were heterogeneous, each mutant would encounter many environments and its selective advantage would be the average of the values in each of these, $\bar{s}_1$, $\bar{s}_2$, $\bar{s}_3$, ... On the principle that the variance of means is less than the variance of the individual observations, the value in the heterogeneous group should have the lesser variance. Since the overall average of the selection coefficient of various mutants is negative (i.e. the mutants are deleterious), the chance of a mutant being advantageous is larger when the variance is larger. Fig. 1 is a schematic diagram of this relationship. The curve A represents the probability density distribution of the selection coefficients of new mutants in a large population with relatively small variance of s between mutants. The curve B is that in a smaller population with a relatively large variance of s between mutants. The probability density distribution of mutations with a strong disadvantage should be approximately the same both in large and in small populations and are not included in the figure. The mean value of s is negative and unchanged in both populations. However in the smaller population (B) because of the greater variance the proportion of selection coefficients with positive values is larger. Thus, by and large the greater the population size, the greater is the habitat diversity; the greater the diversity, the smaller is the between-mutant variance of selection coefficients; the smaller the

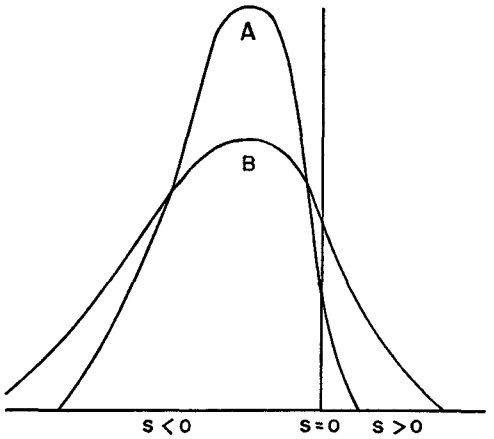

Fig. 1. Schematic diagram showing the probability density distribution of the selection coefficients (s) of new mutants in a large population with relatively small variance of $s(A)$ and in a small population with large variance of $s(B)$. The mean value of s is negative and unchanges in both populations, however in B the proportion of s with positive values is much larger and the mean selection coefficient of all beneficial mutants is also larger

variance of s, the smaller is the probability that a new mutant will behave as if it were advantageous. Therefore, the probability that a mutant will be advantageous is inversely correlated with the total population size of the species.

A greater fraction of advantageous mutants in a small population in a homogeneous environment is not sufficient to guarantee that evolution is faster under this circumstance, however. Random drift is more important in a small population and selection is less efficient. So it is necessary to examine the situation in more detail.

Let N be the population number. Since this examination is only roughly quantitative, I shall make no distinction between actual and effective population sizes, and assume that they are the same. Let p be the probability of a mutant being advantageous. As a first approximation I assume that this probability is given by

$$p = A\,(1/N)^x \tag{1}$$

where A and x are constant, although actually they would be influenced by the effect of the mutant. As Fisher (1930) pointed out, the larger the effect of the mutant, the less is the probability of its being beneficial.

Let us denote by u the probability of fixation of a mutant, and by v the rate of occurrence of nearly neutral mutations per gamete per generation. The total rate of such mutation per species becomes $2Nv$. Then, the rate per generation of advantageous gene substitutions in the population which

we denote by k_+ is given by $k_+ = 2Nuvp$. Substituting from (1),

$$k_+ = 2vuA(1/N)^{x-1}. \tag{2}$$

The fixation probability u can be expressed as follows, assuming semi-dominance,

$$u = \frac{1-e^{-2s}}{1-e^{-4Ns}} \approx \frac{2s}{1-e^{-4Ns}} \tag{3}$$

where s is the average selection coefficient of advantageous mutants (Fisher, 1930; Kimura, 1957).

Let us again compare two curves A and B in Fig. 1. The probability of a mutant being advantageous (p) is the area under the curve for $s>0$. From the figure, one can see that p is at least proportional to the standard deviation (σ_s) among selection coefficients of new mutations around neutrality. On the other hand, we can generally assume that the variance of selection coefficients σ_s^2 is inversely correlated with the environmental diversity D_E, $\sigma_s^2 = A_1/D_E$ where A_1 is a constant. If we further assume that the population density remains constant, the environmental diversity is proportional to N^2 by considering the diversity through space so that, $D_E = A_2N^2$ where A_2 is a constant. Thus we have $\sigma_s \approx A_3/N$ where A_3 is a constant. Hence p is at least proportional to $1/N$ and we have $x \geqq 1$.

The formula (2) for the rate of advantageous gene substitution can be analyzed further as explained below. The probability of fixation (u) is a function of the selection coefficient. By selection coefficient, we mean the effective selection coefficient since its random variation (between-generation and not between-mutant) may not be negligible. For the same reason that the probability that s will be positive increases with decreasing environmental complexity, the mean value of all positive s's will also increase with decreasing environmental complexity. Therefore, the expected value of the selection coefficient (s_+) given that it is positive is assumed to be

$$s_+ = B(1/N)^y \tag{4}$$

where B and y are constant as before. For sufficiently large s_+ and assuming semi-dominance,

$$u \approx 2s_+ = 2B(1/N)^y. \tag{5}$$

By substituting formula (5) into (2), we obtain,

$$k_+ = 4vAB(1/N)^{x+y-1}. \tag{6}$$

Formula (6) says that the rate of advantageous gene substitution k_+ decreases with increasing N if $x+y$ is greater than one. As explained above, x is not less than 1 provided that the population density remains constant. Also it is evident that the value of y is likely to be positive. Therefore the condition of $x+y>1$ is likely to be satisfied. Hence we have negative

correlation between the population size and the rate of gene substitution under the present hypothesis.

Extending this, the change in fitness (Δg) is proportional to the selection coefficient.

$$\Delta g = C s_+ k_+ = 4 v A B^2 C (1/N)^{x+2y-1} \tag{7}$$

where C is a constant. Here, only the change in fitness due to advantageous mutant substitution has been considered. If we assume that the phenotypic change is proportional to the change in fitness, from formula (7), the rate of phenotypic change increases with decreasing N if $x+2y$ is greater than one. By comparing formulae (6) and (7), one can predict that the inverse relation between the rate of phenotypic (fitness) change and N is greater than that between the rate of substitution and N if y is positive.

Similarly the rate of disadvantageous gene substitution (k_-) per generation can also be estimated.

$$k_- = 2Nv(1 - A(1/N)^x)u. \tag{8}$$

The fixation probability u is now a very small quantity in the following form, again assuming semi-dominance,

$$u = \frac{e^{2s} - 1}{e^{4Ns} - 1} \tag{9}$$

where s is the average selective disadvantage (Kimura, 1957, 1962). As seen from formulae (8) and (9), k_- also decreases with increasing N.

Statistical Evolution

The important conclusion obtained from the above analysis is that not only disadvantageous gene substitutions but also advantageous ones can increase when the population size gets small in our model. This fits well the plausible speculation that small populations have a large chance to evolve rapidly, although the risk of extinction is also large, whereas large populations stay unevolved with much less extinction. It has been known that cases of rapid evolution are almost always accompanied by deficiencies of the fossil record. For the explanation of this fact, Simpson (1944) has stated that "it can be shown that this postulate is consistent with all pertinent facts and, indeed, is almost demanded by them".

Wright (1929, 1931, 1943) interpreted the rapid evolution of apparently very rare species as due to their sparsity rather than to absolutely small numbers. He attributed evolution to a large extent to differential growth and diffusion of local populations which happened to have acquired especially favorable sets of gene frequencies, whereas gene substitutions are considered to be most important for evolution in my hypothesis. Also in Wright's model, a small amount of migration is better than complete isolation,

whereas migration must be practically prevented for a sufficient period of time to allow differentiation in my model. Thus, his theory differs from ours, although both predict the statistical nature of evolution.

Our hypothesis can be stated in another way: Specialized evolution should be faster than generalized i.e., it should be easier for a species to adopt to a specialized environment (a parasite, for example) than to a more general environment. It also predicts that the gene substitution is faster on a small island than on the main land. It is our future task to test these predictions.

At present, we do not have sufficient data to test the hypothesis, however, let us examine it in the light of recent data on molecular evolution. Although the rates of amino acid substitutions in each protein, such as cytochrome c or hemoglobins, are on the whole quite uniform among diverse lines, there are some exceptions. From the phylogenetic trees of cytochrome c by Fitch and Markowitz (1970) and by Uzzell and Corbin (1971), we notice three lines which evolved exceptionally rapidly. They are *Saccharomyces*, *Crotalus* and primates. Although the primate line evolved rather slowly in the later period after the divergence of *Homo* and *Macaca*, the amino acid substitutions must have been rapid in the earlier period, since this line is most distantly related to the other mammals. This can easily be explained by assuming that the population size was very small in the earlier period. *Saccharomyces* and *Crotalus* can also be considered to have had very small population sizes due to their specialized habitat.

From the recent studies of molecular evolution, that the rate of amino acid substitution is about 1.6 paulings (1.6×10^{-9}/amino acid site/year) on the average (cf. King and Jukes, 1969). Assuming 3×10^4 genes (cistrons) each with 300 amino acids in the mammalian genome (cf. Ohta and Kimura, 1971; Crow 1972), the rate of gene substitution per genome per year becomes

$$1.6 \times 10^{-9} \times 300 \times 3 \times 10^4 = 1.44 \times 10^{-2}.$$

Assuming further that the average generation length in the course of mammalian evolution is 2 years, the rate of gene substitutions becomes about 3×10^{-2} per generation. As to the length of time required for the evolution of a new species, Haldane (1957) considered, based on the paleontological studies made by others, that it takes about a half million years for mammals. Mayr (1963) who examined extensive data on the rates of speciation, considered that it might well take 10^5 or perhaps 10^6 years for the completion of the speciation process even if production of new subspecies after isolation sometimes takes only 10^4 years or less. If we tentatively take 10^5 years for the length of time necessary for speciation and using the rate of gene substitution 1.44×10^{-2} per year obtained above, we get roughly 1500 as the total number of gene substitutions during this period. This large number of substitutions should include every kind;

from advantageous through completely neutral to slightly disadvantageous ones. Their selection coefficient has a certain statistical distribution around neutrality which depends strongly on environmental diversity.

The paleontological fact that extinction and expansion of species has occurred quite frequently in the past suggests that important substitutions with large selection coefficients should be small in number. Otherwise such great variation of the rise and fall of the species can not be accounted for.

For example, if 1500 gene substitutions with $s_+ = 0.01$ occur on the average, the variance, V, in the adaptive gain among the populations, assuming Poisson distribution for the number of substitutions; becomes

$$V = s_+^2 \times 1500 = 0.15.$$

Therefore the coefficient of variation (C) becomes,

$$C = \sqrt{V}/(s_+ \times 1500) = 1/\sqrt{1500}.$$

More generally, C is $1/\sqrt{n}$, where n is the average number of gene substitutions. Since this is quite small, for a large n all populations would grow at more or less the same rate with little extinction and little expansion. Actually, as suggested in this paper, the selection coefficients may be very different among different gene substitutions including both positive and negative values. Then, the coefficient of variation of adaptive gain among populations becomes,

$$C = \sqrt{1 + c_s^2}/\sqrt{n} \tag{10}$$

where $c_s (= \sigma_s/\bar{s})$ is the coefficient of variation among selection coefficients of the mutants used for substitutions. C can be large only when c_s is very large. Considering many extinctions and expansions of the species in evolution, it is likely that C may have a value of the order of 1, in which case c_s must be nearly $\sqrt{n}$, that is around 40 with $n = 1500$. This means that the standard deviation of selection coefficients among gene substitutions is at least a magnitude larger than the mean. Thus we conclude that since the mean selection coefficient is nearly zero, individual substitutions include both positive and negative selection.

According to the present theory, the value of c_s is larger when the population size is smaller. This is because, the mutants with larger effect can replace the old allele if the population is smaller and this makes c_s larger. Such a random factor may be very important for the evolution of the species.

Group selection may also play an important role for the evolution of the local population; however the success of a population depends at least partly on successful gene substitutions, and hence on chance. Although we have emphasized the importance of small population size for the evolution, the extinction of the species must be inevitable if it is too small. Thus, the

balance between positive and negative selections can be a crucial factor for evolution.

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