DARWINIAN AND NON-DARWINIAN EVOLUTION

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1. Introduction

Evolution by natural selection, by survival and differential reproduction of the fittest, is about as firmly established as any broadly general scientific theory could imaginably be. Why then should it be challenged by a rival theory in 1971? The answer is that it is not, for the proponents of non-Darwinian evolution are not questioning that evolution of form and function has occurred in the orthodox neo-Darwinian manner.

So let me first say what non-Darwinian evolution is not. It is not orthogenesis, emergent evolution, inheritance of acquired characters, catastrophism, vitalism, inherent directiveness, or telefinalism. It is not associated with names such as Lamarck, Osborn, or Teilhard de Chardin. Rather it is evolution by random drift of mutants whose effects are so minute as to render them essentially neutral, and a more appropriate name to mention is Sewall Wright.

Random drift is not a new idea. It was considered quite thoroughly by R. A. Fisher [10] and discounted by him as a factor of any great interest in evolution. He regarded it as a calculable amount of random uncertainty that could cause disorderly fluctuations, but would not alter to any great extent either the direction or the rate of evolution, except in very small populations. To Sewall Wright [47], [48], [50], on the other hand, random gene frequency fluctuations became an important part of his shifting balance theory of evolution. Random fluctuations may enable a population to pass to the other side of an unstable equilibrium, or in a structured population permit a particularly favorable gene combination to arise locally and spread through the entire population. In Wright's view, random drift caused by near neutrality, small population size, and fluctuating selective values is part of a basic mechanism that enhances the probability of evolutionary novelty.

Random drift in the present context is different in emphasis. The idea put forth as non-Darwinian evolution is that most DNA changes and most amino acid substitutions in evolution have been so nearly neutral that their fate was determined mainly by random processes. In this view the chief cause of observed molecular evolution is random fixation of neutral mutations. The effect of all this on fitness is regarded as negligible.

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How similar must a mutant be to the gene from which it arose to be regarded as neutral? For its fate to be determined largely by chance, its selective advantage or disadvantage must be smaller than the reciprocal of the effective population number; so the operational definition of a neutral gene is one for which $|s| \ll 1/N_e$, where s is the selective advantage and N_e is the effective population number [47].

The principal reason for not accepting non-Darwinian evolution, I believe, is an unwillingness to believe that any mutational change can be so slight as to have no effect on fitness when considered over the enormous geological times involved. Another reason, perhaps, is that a random theory may discourage a search for other explanations and thus may be intellectually stultifying. Thirdly, a biologist may well say that if these changes are so nearly neutral as to be governed by chance in large populations and over long periods of time they are not really of much interest. He is more interested in processes that affect the organism's ability to survive and reproduce, and which have brought about such exquisite adaptations to diverse environments. To many biologists the evolution of amino acid changes is rather dull compared to that of the elephant's trunk, the bird's wing, the web spinning skills of a spider, the protective resemblance of mimetic butterflies, the vertebrate eye, or the human brain.

On the other hand, the neutral theory leads to a different formulation with new ideas and with quantitative predictions. It is directly concerned with the gene itself, or its immediate product, so that the well-developed theories of population genetics become available. It produces testable theories about the rates of evolution. I have commented elsewhere [4] on the great enrichment to population genetics that has come through molecular biology, which at last makes it possible to apply population genetics theory to those quantities (that is, gene frequencies) for which it was developed.

The original plan of the Symposium was to have two introductory papers, one on Darwinian evolution and one on non-Darwinian evolution. Due to illness this has not been possible, so I am discussing both subjects. This means that there will be many places with the equivocal "on the one hand . . . but on the other," as I endeavor to present arguments that have been given for both views. I shall probably slight the Darwinian arguments somewhat; they are already too well known to need further elaboration.

2. Some recent history

The neutral evolution hypothesis in its present molecular context was foreshadowed by the work of Sueoka [42] and Freese [15]. Both were concerned with the diversity of base content in bacteria of different species despite rather similar amino acid makeup and suggested that this might depend on mutation rates of individual nucleotides with negligible differences in selective values.

The real beginning of the subject was Kimura's daring challenge to evolu-

tionary orthodoxy, published in 1968 [19]. His argument was based on the difficulty of explaining the enormous number of gene substitutions that would occur if all the DNA were evolving by selection at the same rate as that observed for such proteins as hemoglobin. Because of this difficulty, based on Haldane's [17] idea of the cost of natural selection, he argued that most of the changes are in fact not selective, but the result of random fixation.

Kimura's idea was strongly supported in the influential paper by King and Jukes [28], which gave the name to this Symposium, and in a more tentative way by me [4]. King and Jukes presented several more arguments, chief among which were the now familiar ones based on the constancy of amino acid substitution rates, the predictability of amino acid composition from nucleotide frequencies and the genetic code, and the great difficulty of interpreting the apparent indifference of one *Escherichia coli* strain to an inordinately high mutation rate.

3. The continuum of fitness values of new mutants

It has been observed since the beginnings of modern genetics that mutations that have effects conspicuous enough to be noticed are almost invariably harmful. It is of course to be expected from natural selection theory that the great majority of newly arising mutants would be deleterious, or at best neutral, in the environment where the existing genes evolved. When geneticists look for examples of beneficial mutants they customarily think of mutants that are adapted to a new environment, such as mutations for drug resistance in bacteria, DDT detoxification in houseflies, or industrial melanism in moths.

Fisher [10] argued that mutations with large effects should almost always be harmful, but that as the effect of the mutant gene becomes less the probability of its being favorable increases until near the limit of zero effect the probability of being deleterious approaches ½. Muller [38] emphasized another, related point. He noted that mutants with minor effects were more frequent than those with more drastic effects. In particular it was shown experimentally in *Drosophila* that recessive mutants causing a small decrease in viability are some two to three times as frequent as those causing a lethal effect [18], [45]. These findings have been confirmed and extended by Mukai and his collaborators [36], [37] using methods of greater sensitivity by which smaller differences could be detected. From these experiments the mutation rate of genes causing minor effects on viability is estimated to be at least ten times that for lethals, and perhaps considerably higher since the experiment permits only a minimum estimate. In absolute frequency, this amounts to at least 0.15 per gamete. For 10,000 loci this is a rate of 1.5×10^{-5} per locus.

Although this experiment does not detect neutral mutations, the increasing frequency of mutants as the sensitivity of the experiment increases suggests a continuum of fitness values. Presumably mutations range from severely deleterious, through neutrality, to mildly beneficial. The situation is illustrated in

Figure 1. The part that can be substantiated by direct measurement is shown in the solid line. Whether the extrapolation through zero effect is more like A or like B is unknown.

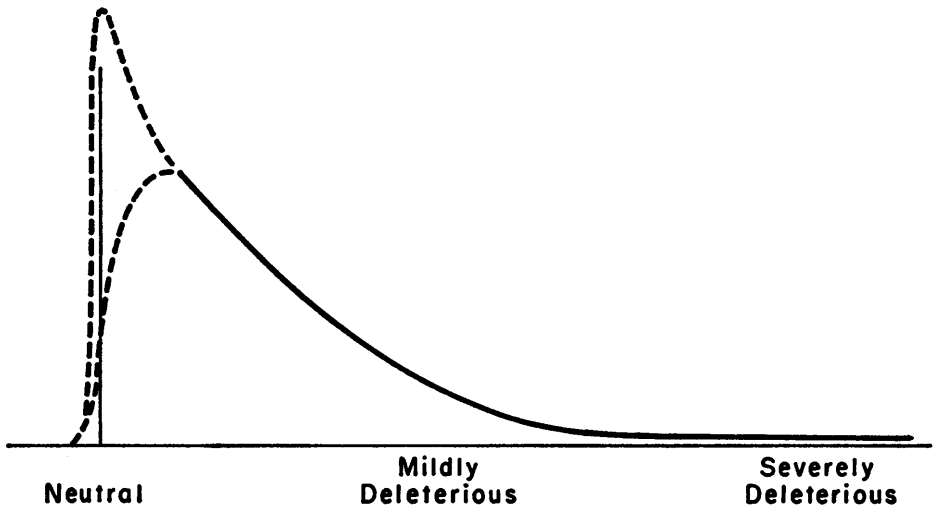

FIGURE 1

Distribution of viabilities of new mutations.
Solid line: from data. Dotted lines: extrapolations.
Farther to the right would be a hump caused by the grouping together of all lethal mutations, regardless of the time of death.

I believe that a debate over whether Darwinian or non-Darwinian evolution is more important is largely fruitless. We know that selection occurs and that some loci are strongly selected. We know further that the main direction of phenotypic evolution is determined by selection, within the limits set by mutational possibility. On the other hand, we know that some loci are so weakly selected that random drift is a major factor in determining their frequency. An elegant laboratory demonstration of random drift of modifying genes affecting the relative viability of inversion types was given by Dobzhansky and Pavlovsky [7]. I suggest that, just as the concept of heritability replaced a meaningless discussion of whether heredity or environment is all important, the right statistical formulation can assign the proper allocation to selection, to mutation, and to random drift as determinants of the evolutionary process.

The subject of this Symposium is mainly molecular evolution rather than evolution of overt traits and processes. Therefore, we are concerned with individual nucleotide changes and their consequences, amino acid replacements. So we ask what fraction of the observed evolution at this level is caused by drift and what fraction by selection.

4. Contrasts between classical and molecular evolution

In selection dominated evolution, as traditionally viewed, the increase or decrease in frequency of a phenotype depends on its survival and fertility relative to competing types. The genetic basis is typically polygenic and essentially continuous. Concealed within a relatively uniform phenotype is a large amount of genetic variability. The amount of variability in the population is very large compared to that which arises in a single generation of mutation, so selection is mainly utilizing variability that is already in the population; in other words, mutation is hardly ever the rate limiting factor. The pattern and direction of evolution are determined by ecological opportunity, diversity of habitat, stability of the environment, and the nature of competing species. Population structure and migration are likely to be important.

Although the general direction of selection is highly deterministic, there may be a large stochastic element in the individual genes involved. There are typically many genetic ways of accomplishing the same phenotypic change, so that the particular genes that increase or decrease in a particular population are largely a matter of chance. It is occasionally true that genetic variability is limiting, as when an insect population that happens to have a mutant gene producing a detoxifying enzyme survives the application of a new insecticide, but this is not thought to be typical.

Neutral molecular evolution, as viewed by its proponents, has quite different kinetics. The stability of the environment, the ecological situation, competing species, population size and such factors are largely irrelevant. There is little effect of the manner of reproduction or of population structure. Species that superficially are evolving very rapidly, like Darwin's finches on the Galapagos Islands, should show no more rapidity of change for neutral amino acid changes than slowly evolving forms. The rate determining factor becomes the rate of mutation of neutral alleles.

The best analogies for non-Darwinian evolution may come from simple asexual systems. For example, there may be phenomena similar to periodic selection in bacteria [40]. As Morton [34] has suggested the amount of polymorphism may be related to the time since a favorable mutant swept through the population, or since a size bottleneck occurred.

Although, in my view, the true situation is an essentially continuous range of fitness values and a range of types of genetic determination from oligogenic to polygenic, it is convenient for classification and discussion to contrast the extreme situations. The evolutionary process can be dichotomized two ways:

(i) on the basis of phenotype:
  (A) morphological and physiological traits,
  (B) molecular changes;
(ii) on the basis of selection:
  (1) selected,
  (2) neutral.

It is likely that many of the component genes in a polygenic system (affecting body size, for example) may be very nearly neutral on the average in a population that is near equilibrium for this trait. For size, as for almost any quantitative trait, the optimum is intermediate rather than at an extreme. Therefore, a gene that increases size is favorable in an individual that is below the optimum and unfavorable in an individual that is too large; the net effect of the gene is neutral.

But this is not the subject of this Symposium. We are here concerned with whether class B2 exists, and if so, what fraction of DNA and amino acid substitutions in evolution are of this type.

5. Arguments for and against neutral evolution

I should like now to list and discuss some of the major arguments that have been put forth for and against the neutral hypothesis. Many of these are discussed in more detail elsewhere in this Symposium.

5.1. *The cost of natural selection.* Haldane [17] first showed that the total amount of selective mortality or differential fertility required for a gene substitution is largely independent of the intensity of selection and depends mainly on the initial frequency. Thus, for a certain excess of reproductive capacity that can be devoted to natural selection, there is a limit to the number of independent gene substitutions that can occur in a given time interval without reduction of the population size.

That a limitation on the rate of gene substitution is inherent in a given pattern of variability in birth and death rates is, I think, generally accepted. But whether the Haldane cost principle provides the most appropriate measure has been seriously questioned. The meaning of a substitution load for an advantageous mutant in a nondeteriorating environment is not clear. Another limitation is the inherent assumption that gene substitutions are independently inherited; linkage may alter this. It is also assumed that the genes being substituted are independent in their effects on fitness. If the genes interact strongly, the principle may be grossly misleading. An extreme model assumes that above a certain level of fitness there is no distinction. By properly adjusting the parameters in such a threshold model, one can demonstrate a system in which a much larger number of gene substitutions can be carried out with the same amount of selection per generation [43], [32], [49].

There are a number of reasons for questioning a strict threshold, or truncation model. For one thing, truncation selection applies to a trait for which there is some underlying variable on which the genes act cumulatively and then selection retains all that are above a certain level on this scale and rejects those that are below. Although selection for yield or performance in livestock and plant breeding approximates this procedure, I doubt that strict truncation applies to much of natural selection. Furthermore, the heritability of fitness must be exceedingly low, which has the effect of blunting the sharpness of the truncation. I suspect

that the truth lies somewhere between a strict application of the Haldane principle and a truncation model.

Using his principle, Haldane [17] suggested that a reasonable rate of evolution when the population can devote about ten per cent of its reproductive excess to gene substitutions is about one substitution every 300 generations. Kimura [19] pointed out that if all the mammalian DNA is evolving at the same rate as that observed for hemoglobin and cytochrome c, this is equivalent to a gene substitution every year or two, far faster than would be possible if the Haldane limitation applies. Kimura suggested that this contradiction can best be reconciled by the assumption that most molecular evolution is selectively neutral.

There are two other ways around this dilemma. One is the assumption of truncation selection mentioned above. The other is to doubt that the number of genes is as large as direct DNA measurements would suggest. If the number of genes is 10^4 there is no problem with even the strictest interpretation of Haldane's principle. For example, 10^4 loci evolving at a total rate of one substitution every 300 generations would mean a substitution per locus of one in 3×10^6 generations; if there are 300 codons per locus, the per codon rate would be about one substitution per 10^9 generations, a value of the same order of magnitude as the observed rate.

I shall return to a discussion of gene numbers.

5.2. *The remarkable constancy of molecular evolution rates.* Another argument that has been advanced for the neutral hypothesis is the constancy of evolutionary rates in different proteins and in the same protein in different lineages. One example, elaborated by Kimura in this Symposium and elsewhere [21], is hemoglobin. A rate of about 10^{-9} per codon per year is found in several diverse ancestral sequences. Particularly striking, since it does not depend on an estimate of the time involved, is a comparison of β and α hemoglobins following the duplication which started them on separate evolutionary courses. Whereas human β and carp α differ by 77 of 139 amino acid sites, human α and β differ by 75. Furthermore, the human β differs from the α of mouse, rabbit, horse, and cattle by 75 to 79 amino acids. The constancy is for the total number of changes, not the individual changes themselves which are often at different sites or involve different amino acids at the same site. Since the time of the original duplication, the amount of divergence of the chains in the same organism, man, is almost exactly the same as that between two chains, one in man and one in a fish, despite the fact that the lines of descent of man and fish have been separate for most of the time. Despite the enormous differences in evolution of form and function between fish and mammals, some timing mechanism has kept the hemoglobins evolving at the same rate.

The rate constancy is equally impressive if we compare different proteins. Fitch and Markowitz [14] have classified the amino acid sites into constant and variable. The former presumably contain amino acids that are essential for the proper functioning of the molecule and cannot be changed without damage.

The latter are free to evolve, since they can be changed without seriously affecting the function. By estimating the rate of evolution of those codons that are variable at a given time (concomitantly variable codons, or covarions) Fitch [11] has shown that the number of substitutions per variable codon is 0.50, 0.44, 0.80, and 0.72 for cytochrome c, α hemoglobin, β hemoglobin, and fibrinopeptide A, respectively, in the two lines of descent since the pig and horse diverged from a common ancestor. There is reason to think that the β hemoglobin estimate is too high. It is remarkable that these widely diverse proteins with proportions of covarions ranging from ten per cent or less in cytochrome c to 95 per cent in fibrinopeptide differ in their evolution by amounts no greater than might be expected from errors in the estimating procedures.

Finally, from the data of Kohne [29] the rate of evolution of nonrepetitive DNA, based on thermostability of hybrid DNA between new and old world primates, is estimated as about 2×10^{-9} per nucleotide per year. The rate of 6×10^{-9} per three nucleotide codon is roughly the same as that for the most rapidly evolving protein (fibrinopeptide A, with 18 of its 19 amino acids variable) and for the variable parts of other proteins.

It thus appears that, to a first approximation based on limited data and necessarily involving a number of uncertainties, DNA and the variable codons are evolving at roughly the same rate.

On a selection hypothesis there is no obvious reason to expect this rate constancy. Different proteins would be expected to evolve at different rates depending on their functions and their environments. The same protein might also differ in rate in different phylogenies.

On the other hand, with the neutral hypothesis the rate of gene substitution is equal to the neutral mutation rate and quite independent of other factors [19], [4]. An evolutionary rate of 10^{-9} per codon per year would imply, for a 500-amino-acid gene and a five year average age of reproduction, a mutation rate of $500 \times 5 \times 10^{-9} = 2.5 \times 10^{-6}$. Since this is about ten per cent of the usually accepted mutation rate per locus, this implies that if one tenth of mutants were selectively neutral this would be sufficient to account for the observed rate of molecular evolution.

A difficulty with the neutral interpretation is that the amino acid substitution rate seems to be constant per year, not per generation. This is unexpected from classical knowledge of mutation rates, which have been regarded as being more related to generation time than to calendar time. Human, mouse, and *Drosophila* mutation rates for single loci with conspicuous phenotypes are rather similar when the measure is per generation, but widely different when measured in absolute time units, as discussed by King in this Symposium.

Furthermore, calendar equality of rates can be ruled out for some cases. Consider a comparison of *Drosophila* and man. The spontaneous rate of occurrence of recessive lethal mutations in *Drosophila* is about 0.015 per gamete per generation and these persist in the population long enough to reach an equilibrium frequency of about 0.5 per gamete. The human reproduction cycle is about

1000 times that of *Drosophila*, so if lethals were to arise at the same absolute rate in man there would be at least 15 lethals per gamete per generation, making no allowance for the possibly greater gene number in mammals. If these were to accumulate to anything like the extent that they do in *Drosophila*, each of us would carry several hundred recessive lethal genes. This means that the child of a cousin marriage would never survive! There must have been some adjustment of the lethal mutation rate to correspond to the life cycle. Furthermore, the same argument can be applied to mildly deleterious genes having an effect on viability of less than five per cent. In *Drosophila* these equilibrate at a frequency of about 0.25 lethal equivalents [35] per gamete [44]. If these occurred in man with a frequency 1000 times as high, we would be riddled with them and again consanguineous marriages would inevitably lead to lethality.

We must conclude that for genes having deleterious effects on viability, whether mild or lethal, the mutation rate is much more nearly constant per generation than per year. What does this mean for the neutral hypothesis for evolution of amino acids?

There are two ways out of the dilemma. One is to postulate that DNA changes leading to neutral mutations are a different class from those producing deleterious changes. Perhaps the latter are reduced by repair mechanisms that are somehow adjusted to the generation length. But I find it unappealing to assume that there is a fundamental difference in the mutation process between those amino acid substitutions that are nearly neutral and those that are severely deleterious.

The second way is to question the accuracy of the rate measures. It should be mentioned that the best data are for organisms whose life cycles are not greatly different. Comparison between widely divergent organisms, like mammals and wheat, involve so many differences that correction for multiple changes in the same amino acid site become important, and these are subject to error. It may be that when all the data are in there may be a correlation of evolution rate and life cycle. This is suggested by some of the DNA data [30], [29]. See also King's discussion in this Symposium.

5.3. *Amino acid frequencies and the code.* Kimura [20] and King and Jukes [28] noted that the frequencies of amino acids, averaged over a large number of proteins, agree rather well with random expectations based on the frequency of the nucleotides in these proteins and the genetic code. King and Jukes used this as one of their major arguments for neutral evolution. The methods have been refined since that time and more data have become available. The agreement is remarkably good, with the exception of arginine which is used much less often than would be expected from the number of ways that it can be encoded. I shall discuss this only briefly, since it is considered in other papers in this Symposium.

It is obvious that on the neutral hypothesis the amino acid composition of proteins should be predictable from nucleotide frequencies and the code. There is also a selectionist interpretation, however. Suppose that, perhaps because of a change in internal physiology or environment, a particular protein would function better if its structure were altered. Suppose also that there are several

ways in which this improvement could occur. The first mutant to occur that is of suitable type has the best chance of success. The more likely a particular amino acid is within the restriction of the nucleotide frequencies and the code, the more likely it is that the first mutant is one encoding this particular amino acid. In the long run, those amino acids whose codons occur most often will be most frequently incorporated.

The same argument applies when the selection is among pre-existing mutants. On the average, those mutants with the highest initial frequencies have the best chance and therefore those amino acids that occur with the greatest frequency in the coding system will be most likely to prevail. For these reasons, I think the argument is equivocal and the observation is consistent with either hypothesis.

5.4. *The functional equivalence of homologous proteins from different species.* Another argument for the neutral hypothesis is the apparent physiological equivalence of proteins from diverse sources. For example, bovine and yeast cytochrome c appear to function equally well with bovine cytochrome oxidase, despite a large number of amino acid differences. Furthermore, enzymes in species hybrids seem to function properly even though they differ in several amino acids. This is given as an argument for neutrality. Yet, there is an obvious selectionist answer: functional differences far too small to be detected in any such manner could still create selective differences large enough to be effective in large populations and over the enormous periods of time involved.

5.5. *The Treffers mutant in Escherichia coli.* Another argument for selective neutrality of many DNA changes comes from the mutator gene, studied by Cox and Yanofsky [2]. This produces an enormous number of AT $\rightarrow$ CG transversions throughout the genome. Despite a number of DNA changes equivalent to half a dozen per cell division, this strain had no obvious deterioration in viability after hundreds of generations—enough time that the DNA base change could actually be measured. Furthermore, these produce mutations by purine-pyrimidine interchanges and therefore a smaller fraction are synonymous than if they were purine-purine or pyrimidine-pyrimidine substitutions. The conclusion that the cells are not greatly harmed by these mutations is strengthened by chemostat experiments in which the mutable strain competed effectively with a normal strain; in fact it seemed to do better, perhaps because of being better able to adapt to chemostat conditions [16].

Unless there is some sort of Maxwell's demon that guides all the half dozen mutant genes into the same daughter cell at each division and thus eliminates them from the population in clusters, they must surely accumulate, as in fact shown by direct chemical analysis of DNA. The great majority of these mutants must therefore be very nearly neutral.

5.6. *Correlation between similarity of amino acids and replacement rate.* Clarke [1] has pointed out that there is a correlation between the frequency with which an amino acid substitution occurs in evolution and the smallness of the difference in the two amino acids, as measured by their structural and chemical properties. He argues from this that amino acid substitutions are selective, since those that

have the smallest effect are most likely to be beneficial. But this argument can easily be turned around. As Clarke himself notes, the smaller the difference between two amino acids, the more likely the change is to be selectively neutral. His analysis does imply that only a minority of amino acid changes are neutral, but as I said before, the rate of neutral evolution does not demand that most amino acid changing mutations be neutral; a small fraction is sufficient to account for the observed rate of amino acid substitution.

5.7. *Successive substitutions.* Another argument has been advanced by Fitch [12]. He notes that in the history of cytochrome evolution most double changes have followed in close succession during the relatively short period while the particular codon was variable. About 30 per cent of the changes are double. In the selectionist view, this must mean that the best substitution was often two steps removed, but it also means that the first step was also an improvement (although the second step made things still better). It would seem surprising that if the best mutant were two steps away, the intermediate step would also be beneficial in such a large proportion of cases. Furthermore, as Fitch notes, the genetic code seems to have the property that individual nucleotide substitutions on the average lead to more similar amino acids than multiple changes do. Similar changes are more likely to be beneficial. Why then, he asks, should the best substitution so often be two steps away? This would seem to argue for neutrality.

However, as King has also noted, there is a selectionist interpretation. If, because of an environmental change, the existing amino acid at some site is no longer optimum, it is likely that it can be improved to varying extents by more than one type of replacement. The first to occur is not necessarily the best; hence the way is open for successive steps.

These arguments, when viewed collectively, make a substantial case for non-Darwinian evolution. In my opinion it is a very strong case for DNA as a whole and a case strong enough to be taken seriously as a working hypothesis for amino acids at concomitantly varying codons. The hypothesis raises a number of new questions and makes a number of quantitative predictions that can guide further experimental and observational inquiry. For this reason alone it merits further consideration.

6. How many genes are there?

The amount of DNA in a mammalian cell amounts to about 3 to 4×10^9 nucleotides. If this is all divided into genes of several hundred nucleotides each there are millions of genes. This is hard to square with observed deleterious mutation rates of 10^{-5} per locus, or higher. It also raises problems with the substitution load unless one postulates truncation selection or neutrality as ways out of "Haldane's dilemma."

Another possibility is that most of the DNA is not genic in the sense of carrying information for protein synthesis or for specific RNA sequences. We

have no basis for estimation of gene number in mammals, but there is good evidence in *Drosophila*. The left end of the *X* chromosome around the white eye locus has been studied exhaustively by Judd and his associates [41]. The best analyzed region includes 16 salivary gland chromosome bands. Lethal and visible mutants within this region can be unambiguously located and classified for identity by a complementation test. The region now appears to be exhausted in that no new mutants have been found for some time that do not fall into one of the 16 complementation units. Thus, there seems to be a perfect correspondence between salivary chromosome band number and the number of complementation units. Similar data for another region of the chromosome give results that are consistent with this idea, although the study is not so exhaustive.

There are a few loci scattered throughout the genome that are known to produce visible mutants but not lethals; that is to say, the normal gene (or genes) at these loci is not absolutely necessary. But such loci appear to be a small minority. That there is not a large class of loci that produce no harmful or lethal mutants is indicated by the fact that any deletion of more than about 20 to 30 salivary chromosome bands has highly deleterious heterozygous effects, usually lethal.

This all suggests that the number of complementation units (genes?) in *Drosophila* is commensurate with the number of salivary chromosome bands. This number in *Drosophila melanogaster* is about 6000. No corresponding information is available for mammals although the chromomere count in some amphibia seems to give about the same number. The amount of DNA in mammals is an order of magnitude greater than that in *Drosophila*, but there is no reason from this fact alone to think that there are more genes. Some of the organisms with the largest amounts of DNA, such as lungfish, are not any more complex or advanced by other criteria. The absence of correlation between DNA amount and any other property is also true of plants.

The DNA in *Drosophila* is enough for several hundred thousand genes, far too much for the 6000 estimated from the salivary chromosome bands (assuming the propriety of defining the gene by a complementation criterion). What is all this DNA doing? Even if we allow for duplication of ribosomal DNA, satellite DNA, and other forms or repetitive DNA there is still far too much.

I would like to join the group who believes, or at least suspects, that the gene number is not large and that most of the DNA has some function other than coding for proteins. It may be purely structural or mechanical. It may be regulatory. It may once have been informational, but have deteriorated after duplication [39]. It may still have a transcribing function, for it is known that some RNA that is produced by transcription stays in the nucleus and does not participate in protein synthesis. Perhaps this has a timing function, as Watson has suggested [46].

If one were looking for an intracellular structural material that had the desirable properties of replicating itself in synchrony with the cell division process so as to maintain a constant amount, that had a mechanism already existing in the

cell for doing this, that had a regular structure of constant shape and rigidity, and (perhaps most important) that maintained its structural integrity and replicative capacity regardless of random chemical alterations in its own composition, he would find that DNA has exactly these properties.

Noninformational DNA, as I would like to designate all DNA whose cellular function does not depend on its exact nucleotide sequence, would have very little mutation load. Its function would depend on average properties, such as the overall AT:GC ratio, but not on the sequence. Mutations increasing the number of AT pairs and those increasing the number of GC pairs would be largely cancelling in their effects. A mutator gene, or simply the ravages of time, could cause a change in overall composition with much less change in function, perhaps none at all. In other words, this kind of DNA would evolve mainly by mutation and random drift. Such changes would show up as differences in DNA hybridization studies (with perhaps no overall change in base ratios), but need not imply any change in function. Note that noninformational DNA need not be repetitive; it can be as varied in sequence as genic DNA.

Thus, the hypothesis of non-Darwinian evolution, or evolution by random drift, can be broken into two parts:

(1) DNA that is noninformational evolves by random drift, or mainly so;
(2) observed changes in amino acid sequences are mainly the result of random drift.

A more general statement of Kimura's original hypothesis is given in terms of total DNA rather than just that part which encodes proteins [39]. In particular, the argument of his 1968 paper [19] based on "Haldane's dilemma," is more convincing for the totality of DNA than for the probably small part of this that codes for proteins.

7. Neutrality versus near neutrality

I should like to return to my original contention, that there is a continuum of fitness values ranging from strongly deleterious through neutrality to slightly beneficial and ask about the rate of substitution of mutants whose advantage or disadvantage is very close to zero.

As I mentioned before, the average number of neutral mutant genes substituted per unit time is equal to the neutral mutation rate. This is independent of the ecological conditions, and of the population structure and size. I should note, however, that the definition of neutrality is dependent on the effective population number. A gene is effectively neutral if its selective advantage or disadvantage is small relative to the reciprocal of the effective population number. This means, then, that a gene that is effectively neutral in a small population may not be in a large population. A slightly harmful gene has a better chance in a small population than in a larger one; a slightly beneficial mutant has a better chance in a large population.

The probability of fixation of a gene with a small selective advantage s in the heterozygote and $2s$ in the homozygote is given by Kimura's formula:

$$u(p) = \frac{1 - \exp\{-4N_e sp\}}{1 - \exp\{-4N_e s\}}, \tag{1}$$

where N_e is the variance effective population number (see [3] and [5], p. 352) and p is the initial frequency of the mutant (see [5], p. 425). Usually the new mutant is present only once in the population, so $p = 1/2N$ in a diploid population of size N. When $p = 1/2N$ and s is small equation (1) becomes

$$u = \frac{2s}{1 - \exp\{-4N_e s\}}, \qquad s \text{ small}, \tag{2}$$

$$u = \frac{1}{2N}, \qquad s = 0, \tag{3}$$

as given by Wright [47]. This is correct even when s is negative. If the actual and effective population numbers are greatly different, the right side of (2) should be multiplied by N_e/N.

Figure 2 is a graph from Kimura and Ohta [27] and shows the probability of fixation $u(p)$ as a function of $4N_e s$. As expected, when $s = 0$ this has a value of $1/2N$. It is smaller when s is negative and greater when s is positive. The point of interest is that there is an appreciable chance of fixation of a slightly deleterious gene as long as $4N_e s$ is greater than -2. Whatever the exact shape of the distribution in Figure 1, it is certain that there are more deleterious than beneficial mutants. Since the prior probability is thus greater for being deleterious than beneficial, there are more mutants to the left of neutrality where the curve deviates less from $1/2N$ than on the right. The result is that the average fixation probability can be rather close to $1/2N$ (or the substitution rate close to the mutation rate) for mutations some distance on either side of $s = 0$. That is to say, the evolution rate for near neutral genes is also equal to the mutation rate, as a rough approximation. For a further discussion of this point, see King's paper in this Symposium.

The gene substitution rate may therefore be somewhat enhanced in a small population for mutations that are slightly deleterious. However, this more rapid substitution of deleterious mutants is at the price of decreased fitness and any such effect in evolutionary time may well be neutralized by the extinction of small populations accumulating too many such mutants.

One other point merits mention in this context. The value of s can hardly be constant, if for no other reason than that even a neutral gene is linked to other genes on a chromosome and somewhere on the chromosome will be one or more genes with selective differences. If s is highly variable, this can have somewhat the same effect as if N_e is small. The probability of fixation of a slightly harmful gene is on the average enhanced whereas that for a favorable gene is slightly depressed. A mathematical treatment of this has been worked out by Ohta (personal communication).

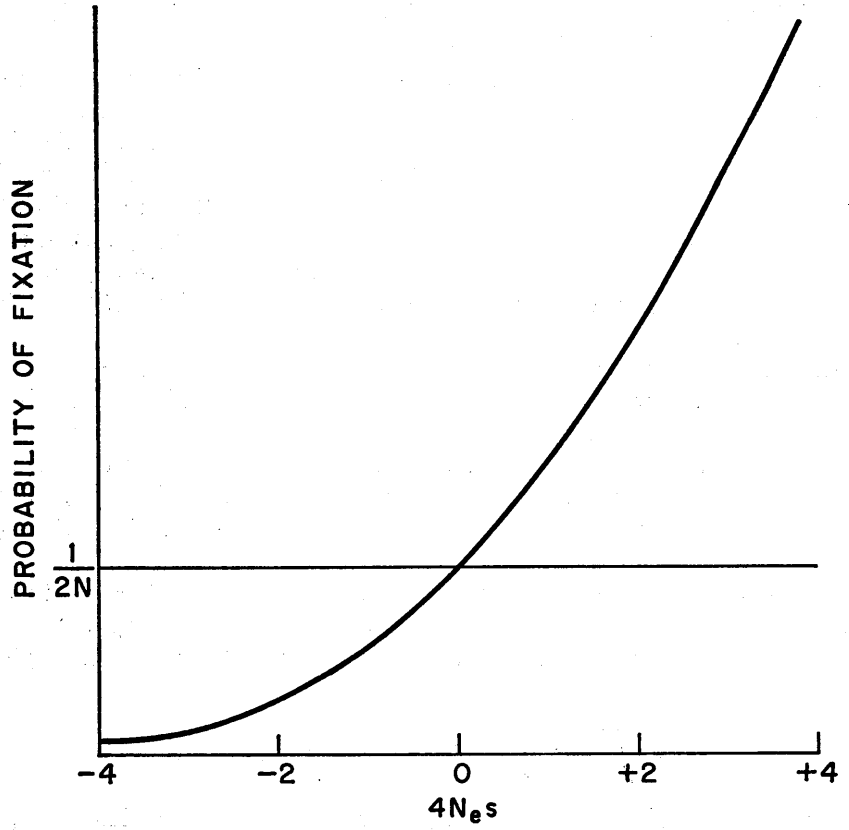

FIGURE 2

Probability of fixation of a new mutant as a function of effective population number N_e and the selective advantage s, where N is the actual number of individuals in the population.

Mutants with very slight effects must surely be of great importance in evolution. Evolutionary fine adjustment depends on having a virtually continous range of differences on many scales. The adjustment of such fine differences is the essence of neo-Darwinian evolution. Surely many genes, if not completely neutral, are near enough to neutrality that their individual chances are very much influenced by random factors. For this reason a comprehensive theory of evolution has to consider both deterministic and random processes.

8. Polymorphism

If any appreciable part of amino acid substitution is by random drift, then at any one time there should be some genes in the process of being substituted at that time provided that the time required for such a substitution is large relative

to the interval between successive substitutions. The latter is the reciprocal of the mutation rate of neutral alleles, as already stated.

Kimura and Ohta [26] showed that the average number of generations between the occurrence and fixation of a mutant, given that it is destined to be fixed rather than lost, is $4N_e$ where N_e is the effective population number. So, if $4N_e$ is large relative to $1/\mu$, the reciprocal of the mutation rate, there will be transient polymorphism due to mutant genes in the process of drifting to fixation. The value of N_e for this calculation is not known for any natural population that I am aware of, but it is clear that the time for a mutant to spread through a species is related to the long term effective number of the entire species, not to any local subdivision thereof.

Random fluctuation in the value of the selection coefficient, even if it is neutral on the average, will have effects similar to those of a small effective population number. Most new mutants are quickly lost from the population through random extinction, even if they are beneficial. Among the minority that are lucky enough to succeed, the average time required for this process is given by the appropriate solution to the Kolmogorov backward equation (see [26] and [5], p. 403).

Consider the case where the average value of s is zero but where there is random variation around this mean with a variance designated by V_s. For the case where the mean is zero, the Kolmogorov equation has the solution giving the average time as

$$\bar{t} = \int_p^1 \frac{2x(1-x)}{V_{\delta x}}\,dx + \frac{1-p}{p}\int_0^p \frac{4Nx^2}{V_{\delta x}}\,dx, \tag{4}$$

where p is the initial frequency and $V_{\delta x}$ is the variance in the change of gene frequency x in one generation. When there is no dominance:

$$V_{\delta x} = \frac{x(1-x)}{2N_e} + x^2(1-x)^2 V_s. \tag{5}$$

If the initial frequency is very small, we can let p approach zero and integrate from 0 to 1. The second term becomes negligible, and the solution is

$$\bar{t} = \frac{8N_e}{C}\log_e \frac{C+K}{C-K}, \tag{6}$$

where $K = 2N_eV_s$ and $C = [K(K+4)]^{1/2}$. When $V_s = 0$, $\bar{t} = 4N_e$, in agreement with the case for random drift of a neutral gene in a population of effective size N_e [26]. The value of $\bar{t}$ in terms of N_e and as a function of $2N_eV_s$ is shown in Figure 3.

A gene can be neutral on the average, but fluctuate in its s value from time to time for at least two reasons. One is that the environment or the background genotype changes so that the gene is sometimes favored and sometimes not in such a way that its average value is neutral; whether such a gene should be classified as neutral is open to debate. On the other hand, a gene that is truly

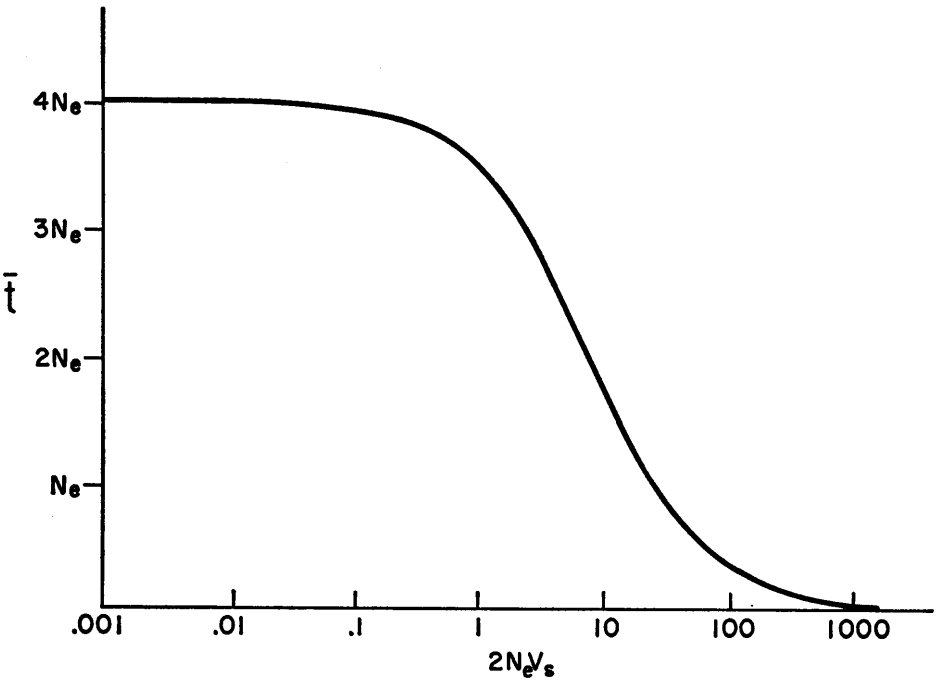

FIGURE 3

Average number of generations until fixation $\bar{t}$ of a new mutant destined to become fixed, where N_e is the effective population number and V_s is the variance in the selective advantage of the mutant.

neutral does not occur in isolation. It has a chromosomal location and therefore is influenced by the selective value of genes linked to it. If a new mutant happens to be on a chromosome that is favored at the time, it has a head start and an increased chance of becoming fixed. This is exactly balanced by the probability of being on a deleterious chromosome, so the mean probability for fixation does not change. The average time until fixation of those that are fixed is shortened however. Equation (6) probably underestimates the influence of this effect since it does not allow for the autocorrelation from generation to generation that is brought about by linkage, for linked combinations persist many generations if the linkage is tight. Equation (6) therefore tends to overestimate the time.

Doolittle, Chen, Glasgow, Mross, and Weinstein [8] noted that no variation at all was found among 125 persons whose fibrinopeptides were analyzed. Fitch and Margoliash [13] noted that if the effective population number is large there should be some polymorphism and the size of the sample should have been large enough to detect this. Perhaps the answer lies along the lines that I have been discussing; random fluctuations around neutrality have the same consequences as lowering the effective population number.

9. Equilibrium distribution of neutrals in a structured population

The equilibrium distribution of neutral alleles in a finite population has been given earlier [24], [20], [22]. Assume that there are k possible allelic states and the neutral mutation rate is μ; that is, we assume that the mutation rate from any state to a particular one of the remaining states is $\mu/(k-1)$. Then, at equilibrium the probability that two alleles drawn at random from the population are identical is approximately

$$f = \frac{4N_e\mu\frac{1}{k-1}+1}{4N_e\mu\frac{k}{k-1}+1} \approx \frac{1}{4N_e\mu+1} \tag{7}$$

when k is large. The reciprocal of f may be regarded as the effective number of alleles. It is equal to the actual number when they are equally frequent; otherwise it is less. The average heterozygosity is $1-f$.

If the alleles are neutral, the distribution is strongly skewed. Many alleles are represented only once or twice in the population while one or a few drift to comparatively high frequencies. The actual number is then considerably larger than the effective number.

The probability that a neutral locus is polymorphic at equilibrium is determined by whether the mutation rate is larger or smaller than the reciprocal of the effective population number. If $4N_e\mu$ is much larger than 1, the population is mainly heterozygous; if it is much less than 1, the population is mainly homozygous. It should be recalled that if the population size fluctuates, N_e is influenced very much by the smaller values, since it is the harmonic mean of the value at various times.

The equilibrium neutral hypothesis can be tested by seeing how well the distribution of allele frequencies fits the theoretical distribution which is given by a diffusion approximation as

$$\phi(x) = 4N_e\mu(1-x)^{4N_e\mu-1}x^{-1}. \tag{8}$$

The sampling distribution of the number of alleles has been worked out by Ewens [9] and this can be used to test whether a sampled population is in agreement with this expectation.

If the population is geographically structured, the probability of identity of two alleles in individuals a specified distance apart is a very complicated function of the mutation rate, the total size of the population, and the structure of the population. One approach to the problem has been given by Morton [34] who has tried various distributions with actual human data. Maruyama [31] has studied theoretical models, including several patterns of migration between partially isolated colonies and also a population of continuous structure with random migration.

One equilibrium relation appears in all these models regardless of the structure of the population or the number of dimensions [6]. It is the extension of equation (7) to a structured population. The relationship is

$$\bar{f} = \frac{1 - f_0}{4N_e\mu} \tag{9}$$

for a large number of potential alleles, with a slight modification if the number of possible alleles is small. In this formula, f_0 is the probability of identity for two alleles drawn from the same locality or from the same individual, $\bar{f}$ is the probability for alleles drawn at random from the entire population, and N_e is the effective population number not taking structure into account.

One conclusion from this, also noted by Maynard Smith [33], is that two alleles drawn from a pair of individuals widely separated from each other geographically should rarely be identical, regardless of the structure of the population. If the population has very little migration, then each part will come to have its own alleles. If there is free migration, the same alleles will be maintained throughout the population, but there will be many of them with individually low frequencies and the probability that any two will be identical is small. This assumes that the total effective number is large relative to the reciprocal of the mutation rate and that the number of potential neutral alleles is large. For a discussion see Kimura and Maruyama [25].

Finally, the global effective number taking the structure into account (N_{es}) is related to the effective number not taking this into account (N_e) by the relation

$$N_{es} = N_e \frac{1 - \bar{f}}{1 - f_0} \tag{10}$$

regardless of the number of alleles. This shows that with a structure of any sort the total effective number is enhanced.

10. Conclusions

I have tried to present the main arguments for and against the hypothesis of evolution by random drift of neutral mutations, or non-Darwinian evolution. I have devoted most of the discussion to non-Darwinian evolution rather than Darwinian since the latter is so well known. The theory of natural selection needs no further description or defense from me.

Mutants range from severely harmful, through neutral, to rare beneficial types. A proper theory would treat the entire range of values with an appropriately greater emphasis on stochastic elements near neutrality.

Despite this continuum of values, it is convenient for discussion to consider the possibility of a distinct class of mutants whose effect is so slight that their fate is mainly determined by random processes. Operationally, this means that the selective advantage or disadvantage is small relative to the effective population number. We then ask whether any substantial fraction of DNA and

amino acid changes in evolution, or of polymorphisms, have this explanation.

I suggest that the great majority of DNA is noninformational in that it does not code for proteins or for unique sequence RNA, and that this DNA changes for the most part by mutation and random drift. The possibility that amino acid substitutions observed in evolutionary lineages have this explanation seems promising enough to deserve the exploration that it is clearly getting. At a minimum it has heuristic value, for it lends itself to theoretical developments, quantitative predictions, and testable hypotheses that will surely lead to a deeper understanding of evolution, whatever the outcome of this particular question. Whether any appreciable fraction of molecular polymorphism is neutral is an open question.

REFERENCES

[1] B. CLARKE, "Selective constraints on amino-acid substitutions during the evolution of proteins," *Nature*, Vol. 228 (1970), pp. 159–160.

[2] E. C. Cox and C. YANOFSKY, "Altered base ratios in the DNA of an *Escherichia coli* mutator strain," *Proc. Nat. Acad. Sci. U.S.A.*, Vol. 58 (1967), pp. 1895–1902.

[3] J. F. CROW, "Breeding structure of populations. II. Effective population number," *Statistics and Mathematics in Biology*, Ames, Iowa State College Press, 1954, pp. 543–556.

[4] ———, "Molecular genetics and population genetics," *Proceedings of the Twelfth International Congress on Genetics*, Idengaku Fukyukai, Mishima, Shizuoka-ken, Japan, 1969, Vol. 3, pp. 105–113.

[5] J. F. CROW and M. KIMURA, *An Introduction to Population Genetics Theory*, New York, Harper and Row, 1970.

[6] J. F. CROW and T. MARUYAMA, "The number of neutral alleles maintained in a finite, geographically structured population," *Theor. Pop. Biol.*, Vol. 2 (1971), pp. 437–453.

[7] TH. DOBZHANSKY and O. PAVLOVSKY, "An experimental study of interaction between genetic drift and natural selection," *Evolution*, Vol. 11 (1957), pp. 311–319.

[8] R. F. DOOLITTLE, R. CHEN, C. GLASGOW, G. MROSS, and M. WEINSTEIN, "The molecular constancy of fibrinopeptides A and B from 125 individual humans," *Hum. Genet.*, Vol. 10, (1970), pp. 15–29.

[9] W. J. EWENS, "The sampling theory of selectively neutral alleles," *Theor. Pop. Biol.*, Vol. 3 (1972), pp. 87–112.

[10] R. A. FISHER, *The Genetical Theory of Natural Selection*, Oxford, Clarendon Press, 1930; New York, Dover Press, 1958 (revised ed.).

[11] W. M. FITCH, "Rate of change of concomitantly variable codons," *J. Molec. Evol.*, Vol. 1 (1971), pp. 84–96.

[12] ———, "Does the fixation of neutral mutations form a significant part of observed evolution in proteins," *Brookhaven Symp. Biol.*, in press.

[13] W. M. FITCH and E. MARGOLIASH, "The usefulness of amino acid and nucleotide sequences in evolutionary studies," *Evol. Biol.*, Vol. 4 (1970), pp. 67–109.

[14] W. M. FITCH and E. MARKOWITZ, "An improved method for determining codon variability in a gene and its application to the rate of fixation of mutations in evolution," *Biochem. Genet.*, Vol. 54 (1970), pp. 579–593.

[15] E. FREESE, "On the evolution of base composition of DNA," *J. Theor. Biol.*, Vol. 3 (1962), pp. 82–101.

[16] T. C. GIBSON, M. L. SCHLEPPE, and E. C. COX, "On fitness of an *E. coli* mutation gene," *Science*, Vol. 169 (1970), pp. 686–690.

[17] J. B. S. HALDANE, "The cost of natural selection," *J. Genet.*, Vol. 55 (1957), pp. 511–524.
[18] J. KERKIS, "Study of the frequency of lethal and detrimental mutations in *Drosophila*," *Bull. Acad. Sci. U.S.S.R.*, Vol. 1 (1938), pp. 75–96.
[19] M. KIMURA, "Evolutionary rate at the molecular level," *Nature*, Vol. 217 (1968), pp. 624–626.
[20] ———, "Genetic variability maintained in a finite population due to mutational production of neutral and nearly neutral isoalleles," *Genet. Res.*, Vol. 11 (1968), pp. 247–269.
[21] ———, "The rate of molecular evolution considered from the standpoint of population genetics," *Proc. Nat. Acad. Sci. U.S.A.*, Vol. 63 (1969), pp. 1181–1188.
[22] ———, "The number of heterozygous nucleotide sites maintained in a finite population due to steady flux of mutations," *Genetics*, Vol. 61 (1969), pp. 893–903.
[23] ———, "Theoretical foundation of population genetics at the molecular level," *Theor. Pop. Biol.*, Vol. 2 (1971), pp. 174–208.
[24] M. KIMURA and J. F. CROW, "The number of alleles that can be maintained in a finite population," *Genetics*, Vol. 49 (1964), pp. 725–738.
[25] M. KIMURA and T. MARUYAMA, "Pattern of neutral polymorphism in a geographically structured population," *Genet. Res.*, Vol. 18 (1971), pp. 125–132.
[26] M. KIMURA and T. OHTA, "The average number of generations until fixation of a mutant gene in a finite population," *Genetics*, Vol. 61 (1969), pp. 763–771.
[27] ———, "On the rate of molecular evolution," *J. Molec. Evol.*, Vol. 1 (1970), pp. 1–17.
[28] J. L. KING and T. H. JUKES, "Non-Darwinian evolution," *Science*, Vol. 164 (1969), pp. 788–798.
[29] D. E. KOHNE, "Evolution of higher-organism DNA," *Quart. Rev. Biophys.*, Vol. 3 (1970), pp. 327–375.
[30] C. D. LAIRD, B. L. MCCONAUGHY, and B. J. MCCARTHY, "Rate of fixation of nucleotide substitutions in evolution," *Nature*, Vol. 224 (1969), pp. 149–154.
[31] T. MARUYAMA, "On the rate of decrease of heterozygosity in circular stepping stone models of populations," *Theor. Pop. Biol.*, Vol. 1 (1970), pp. 101–119.
[32] J. MAYNARD SMITH, "Haldane's dilemma, and the rate of evolution," *Nature*, Vol. 29 (1968), pp. 1114–1116.
[33] ———, "Population size, polymorphism, and the rate of non-Darwinian evolution," *Amer. Natur.*, Vol. 104 (1970), pp. 231–237.
[34] N. E. MORTON, "The future of human population genetics," *Prog. Med. Genet.*, Vol. 8 (1972), pp. 103–124.
[35] N. E. MORTON, J. F. CROW, and H. J. MULLER, "An estimate of the mutational damage in man from data on consanguineous marriages," *Proc. Nat. Acad. Sci. U.S.A.*, Vol. 42 (1956), pp. 855–863.
[36] T. MUKAI, "The genetic structure of natural populations. I. Spontaneous mutation rate of polygenes controlling viability," *Genetics*, Vol. 50 (1964), pp. 1–19.
[37] T. MUKAI, S. I. CHIGUSA, L. E. METTLER, and J. F. CROW, "Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*," *Genetics*, 1972, in press.
[38] H. J. MULLER, "Our load of mutations," *Amer. J. Hum. Genet.*, Vol. 2 (1950), pp. 111–176.
[39] T. OHTA and M. KIMURA, "Functional organization of genetic material as a product of molecular evolution," *Nature*, Vol. 223 (1971), pp. 118–119.
[40] F. J. RYAN, "Natural selection in bacterial populations," *Atti del VI Cong Int. Microbiol. (Italy)*, Vol. 1 (1953), pp. 1–9.
[41] M. P. SHANNON, T. C. KAUFMAN, and B. H. JUDD, "Lethality patterns of mutations in the *zeste-white* region of *Drosophila melanogaster*," *Genetics*, Vol. 64 (1970), p. 58.
[42] N. SUEOKA, "On the genetic basis of variation and heterogeneity of DNA base composition," *Proc. Nat. Acad. Sci. U.S.A.*, Vol. 48 (1962), pp. 582–592.
[43] J. A. SVED, "Possible rates of gene substitution in evolution," *Amer. Natur.*, Vol. 102 (1968), pp. 283–292.

[44] R. G. TEMIN, H. U. MEYER, P. S. DAWSON, and J. F. CROW, "The influence of epistasis on homozygous viability depression in *Drosophila melanogaster*," *Genetics*, Vol. 61 (1969), pp. 497–519.

[45] N. W. TIMOFEEFF-RESSOVSKY, "Auslösung von Vitalitätsmutationen durch Röntgenbestrahlung bei *Drosophila melanogaster*," *Nachr. Ges. Wiss. Göttingen, Biol. N. F.*, Vol. 1 (1935), pp. 163–180.

[46] J. WATSON, *Molecular Biology of the Gene*, New York, Benjamin, 1970.

[47] S. WRIGHT, "Evolution in Mendelian populations," *Genetics*, Vol. 16 (1931), pp. 97–159.

[48] ———, "Adaptation and selection," *Genetics, Paleontology and Evolution* (edited by G. L. Jepson, G. G. Simpson, and E. Mayr), Princeton, Princeton University Press, 1949, pp. 365–389.

[49] ———, "The theoretical course of directional selection," *Amer. Natur.*, Vol. 103 (1969), pp. 561–574.

[50] ———, "Random drift and the shifting balance theory of evolution," *Mathematical Topics in Population Biology* (edited by K. Kojima), Berlin-Heidelberg-New York, Springer-Verlag, 1970, pp. 1–31.