THE NEUTRAL THEORY

OF MOLECULAR EVOLUTION

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Preface

This book represents my attempt to convince the scientific world that the main cause of evolutionary change at the molecular level – changes in the genetic material itself – is random fixation of selectively neutral or nearly neutral mutants rather than positive Darwinian selection. This thesis, which I here call the neutral theory of molecular evolution, has caused a great deal of controversy since I proposed it in 1968 to explain some then new findings in evolution and variation at the molecular level. The controversy is not surprising, since evolutionary biology has been dominated for more than half a century by the Darwinian view that organisms become progressively adapted to their environments by accumulating beneficial mutants, and evolutionists naturally expected this principle to extend to the molecular level. The neutral theory is not antagonistic to the cherished view that evolution of form and function is guided by Darwinian selection, but it brings out another facet of the evolutionary process by emphasizing the much greater role of mutation pressure and random drift at the molecular level.

The neutral theory has two roots. One is the stochastic theory of population genetics whose foundation traces back to the pathbreaking work of R.A. Fisher, J.B.S. Haldane and Sewall Wright early in the 1930s, and is mathematical in nature. The other is molecular genetics, which has revolutionized our concept of life and whose impact we are still feeling. In particular, molecular advances have permitted the study of evolution at the most basic level, DNA itself – something not imagined two decades ago. The study of intraspecific genetic variability has been similarly revolutionized by new molecular approaches.

Many of the arguments employed to support or refute the neutral theory are quantitative in nature, and are often meticulous and difficult; a careful

and detailed explanation is necessary. This is one reason why the book has become larger than originally intended. In addition, the past few years have produced an explosion of new molecular knowledge bearing on the theory. Naturally, I have tried to incorporate as much of this as I can.

It brings me satisfaction to observe that the neutral theory has survived over a decade of severe tests of many kinds; I believe the probability of its future survival is high, although refinements and modifications will no doubt be made. It is often said that science develops through cooperation of many people throughout the world, and the subject treated in this book is no exception. Readers will see that scientists from various nations have participated in the enterprise. Their work, even when done to refute the theory, have pointed the way to revisions to make it more realistic. The neutral theory of evolution has had an evolution of its own through the interaction between theoretical analyses and experiments or observations, as in the typical scientific paradigm.

The writing of this book has cost me three years of hard work, but I feel some excitement in the realization that in 1982 we commemorate the hundredth anniversary of the death of Charles Darwin. His theory of evolution by natural selection has been the great unifying principle in biology. As new molecular knowledge unfolds we have a new uncharted territory awaiting exploration. I hope that the neutral theory represents a step forward in our search for molecular understanding of evolution and variability.

Drafts of parts of this book have been read by Nigel Calder, Daniel Hartl, Thomas Jukes, Takashi Miyata, Terumi Mukai, Masatoshi Nei, Tomoko Ohta, William Provine, Jacques Ruffié and Naoyuki Takahata. Their comments were much appreciated. I am particularly grateful to James Crow for numerous criticisms and for helping me over various semantic hurdles. Kenichi Aoki also went carefully through the entire manuscript and made suggestions for improved presentation. I would like to take this opportunity to express my indebtedness to James Crow for his guidance and help and to Tomoko Ohta for her cooperation and constructive criticism. Without their help I would never have been able to develop the theory presented here.

This book is dedicated to the late Professor Taku Komai, who was the pioneer evolutionary geneticist in Japan and to whom I am deeply indebted, particularly for his help and encouragement when I first started as a mathematical geneticist. I should like to think that, were he still alive, he would greet this book with approval and satisfaction.

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Introduction

The neutral theory asserts that the great majority of evolutionary changes at the molecular level, as revealed by comparative studies of protein and DNA sequences, are caused not by Darwinian selection but by random drift of selectively neutral or nearly neutral mutants. The theory does not deny the role of natural selection in determining the course of adaptive evolution, but it assumes that only a minute fraction of DNA changes in evolution are adaptive in nature, while the great majority of phenotypically silent molecular substitutions exert no significant influence on survival and reproduction and drift randomly through the species.

The neutral theory also asserts that most of the intraspecific variability at the molecular level, such as is manifested by protein polymorphism, is essentially neutral, so that most polymorphic alleles are maintained in the species by mutational input and random extinction. In other words, the neutral theory regards protein and DNA polymorphisms as a transient phase of molecular evolution and rejects the notion that the majority of such polymorphisms are adaptive and maintained in the species by some form of balancing selection.

The word 'neutral' is not used in a strict, literal sense. The emphasis is not on neutrality *per se*, but on mutation and random drift as the main explanatory factors. The mutant genes that are important in molecular evolution and polymorphism are assumed to be nearly enough neutral for chance to play the major role. As the theory has developed, more attention has been given to selective molecular constraints, selection acting in indirect ways (as in tRNA abundance-codon usage relationship), and very weak negative selection acting on genes drifting to fixation. The theory does not, then, assume that selection plays no role; however, it does deny that any appreciable fraction of molecular change is due to positive selection or

that molecular polymorphisms are determined by balanced selective forces.

One possibility would be to rename the theory the 'mutation-random drift theory', but the term 'neutral theory' is already widely used and I think it better not to change horses in midstream. I want the reader to realize that 'neutral theory' is shorthand for 'the theory that at the molecular level evolutionary changes and polymorphisms are mainly due to mutations that are nearly enough neutral with respect to natural selection that their behavior and fate are mainly determined by mutation and random drift'. I also must emphasize that the theory does not deny the occurrence of deleterious mutations. On the contrary, selective constraints imposed by negative selection are a very important part of the neutralist explanation of some important features of molecular evolution, as I shall detail in chapter 7.

Classical evolution theory has demonstrated beyond any doubt that the basic mechanism for adaptive evolution is natural selection acting on variations produced by changes in chromosomes and genes. Such considerations as population size and structure, availability of ecological opportunities, change of environment, life-cycle 'strategies', interaction with other species, and in some situations kin or possibly group selection play a large role in our understanding of the process. This field has been greatly enriched by new molecular understanding, which has revealed totally new and unexpected additional possibilities and constraints. One now has to consider the way the primary sequence of amino acids is converted into a three-dimensional structure, which amino acids are hydrophilic and which hydrophobic, which are on the surface and which are buried inside, which are associated with essential functions and which are not, and other detailed aspects of our increasingly deep knowledge of proteins. There are DNA constraints caused by secondary folding and pairing of RNAs, by the matching of codon usage with tRNA abundance and vice versa, and by the processing of RNA to remove intervening sequences. There is the whole new question of the evolution of the genetic code, made more pertinent by the discovery that mitochondria have a somewhat different and variable code dictionary. There is new understanding of chromosome evolution, made possible by new staining and labeling techniques. There is the likelihood of discovering many more pseudogenes – seemingly functionless analogs of known genes, which have been revealed by cloning and rapid DNA sequencing methods. We have to consider 'selfish DNA', transposons, and other possible mechanisms by which the genome increases and decreases in size and the role of 'junk DNA'. So the study of adaptive evolution remains the exciting subject that

it has been since Darwin, but greatly enriched by new and much deeper levels of understanding brought about by the molecular revolution.

But, in my view, the most surprising possibility arising from molecular studies is that the great preponderance of nucleotide changes over time and of nucleotide variability in populations at any one time are selectively neutral or nearly neutral, so that increases and decreases in the mutant frequencies are due mainly to chance. It is remarkable, I think, that their behavior is calculable from the theory of stochastic processes, a theory which until recently has been regarded as too academic to have actual biological applications. Furthermore, as I shall explain in chapter 7 and elsewhere in this book, many of the newly discovered phenomena enumerated above lend support to the neutral theory, a most dramatic example being the rapid evolutionary change of pseudogenes.

Superimposed on this random change of nucleotide frequencies are the directional, adaptive changes brought about by natural selection in an ever-changing environment and the systematic process of removal of deleterious mutations. These are subjects of traditional, and continuing interest to evolutionists.

The whole picture, however, must include *all* the changes that occur in the DNA, however slight in their phenotypic effects. Just as the mathematical treatment of random processes in physics has contributed to a deeper understanding, the mathematical treatment of random genetic processes can illuminate some previously dark secrets of evolution. Furthermore, we should not overlook the possibility that some of the 'neutral' alleles may become advantageous under an appropriate environmental condition or a different genetic background; thus, neutral mutants have a latent potential for selection. This means that polymorphic molecular mutants, even if selectively neutral under prevailing conditions of a species, can be the raw material for future adaptive evolution. To regard random fixation of neutral mutants as 'evolutionary noise' is inappropriate and misleading. Also, there is the possibility (as I shall show in section 6.7) that extensive neutral evolution occurs under stabilizing phenotypic selection, if a large number of segregating loci (or sites) are involved in a quantitative character. In this case, genes that are substituted by random drift and those that are responsible for phenotypic variability belong to the same class.

An introductory caution is needed. *Webster's Third International Dictionary* defines evolution as 'the process by which through a series of changes or steps any living organism or group of organisms has acquired the morphological and physiological characters which distinguish it'. This definition was entirely appropriate for Darwin's time and for the first half of

the twentieth century. (Note that Darwin used the term 'descent with modification' and he stated that species change by 'preservation and accumulation of successive slight favourable variations'.) But, with all the changes that molecular biology has revealed – none of which is visible to the naked eye – a much broader definition is needed. In this book, as in earlier discussions of the neutral theory, I include in the word evolution *all* changes, large and small, visible and invisible, adaptive and nonadaptive. In some cases, evolution may occur even by random fixation of very slightly deleterious mutants, whose selection coefficients are comparable to or only slightly larger than the mutation rates.

The first two chapters of this book will be devoted to the historical development of theories on the mechanism of evolution. This will set the neutral theory of molecular evolution in proper historical perspective. In the latter half of chapter 2, I shall document how the neutral theory came to be proposed. Chapter 3 will serve as a systematic introduction to the theory. Readers who want to learn quickly about the theory are invited to read this chapter, skipping over the first two chapters. However, a quick reading of the latter half of chapter 2 will also help to deepen understanding of the theory. In chapter 4, the main features of evolution at the phenotypic level, as exemplified by the history of vertebrates, will be presented, and these will be contrasted with the characteristics of molecular evolution, with special reference to evolutionary rates. A more general discussion of the main features of molecular evolution will follow in chapter 5.

Now, in order to make proper appraisal of the neutral theory, a correct understanding of natural selection is essential. So, the definition, types and action of natural selection will be reviewed in chapter 6. This chapter will serve as an introduction to classical population genetics theory. Most material in this chapter, except that in the last section, is concerned with deterministic theory. I have encountered with dismay a number of occasions in which natural selection is invoked as a panacea to explain virtually any aspect of evolution and variation. It is easy to invent a selectionist explanation for almost any specific observation; proving it is another story. Such facile explanatory excesses can be avoided by being more quantitative. This is one reason why chapter 6 was written. In chapter 7, I shall show how the neutralist paradigm can cope with diverse observational facts coming from molecular evolutionary studies. In fact, data from the new molecular revolution have recently added supporting evidence for the neutral theory, and this will be discussed extensively in this chapter. I am convinced that no other existing theory can give a better and more consistent explanation of these facts. This chapter together with the

next chapter constitute the core of this book. In chapter 8, I shall present a rather extensive account of the stochastic theory of population genetics and various models that have been devised to treat evolution and variation at the molecular level. For most readers, this chapter, particularly from section 8.3 onward, may be difficult reading. This is inevitable because of the intricate nature of the subject, although I have done my best to present the material as clearly as possible. Readers who are mainly interested in the biological aspects (rather than mathematical properties of various models) may proceed to the next chapter, after finishing the first two sections (sections 8.1 and 8.2). Chapter 9 treats the problem: what is the mechanism by which the genetic variability at the molecular level is maintained? This problem has been regarded by some as the most important problem currently facing population genetics. Here again, I shall show that the neutral theory has not only withstood various tests but has proved useful in explaining levels of heterozygosity under various circumstances (such as haploidy vs. diploidy, monomeric vs. dimeric or tetrameric enzyme loci, etc.). Recently, the neutral theory has gained strength in treating the problem of intraspecific variability at the molecular level, and it is quite possible that this problem has essentially been solved by the theory.

In the last chapter (chapter 10), I shall summarize the whole work and give a few concluding remarks.

I am convinced that the neutral theory, as explained in this book, is correct in its essential details, although it will doubtless be refined in the future as it has been in the past. The final arbiter is time; but new data are appearing so rapidly that the time may be quite short.

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1

From Lamarck to population genetics

At the beginning of the nineteenth century it was almost universally believed that all living beings on earth were immutable divine creations. In bold contrast, the French naturalist, Jean Baptiste Lamarck argued that life could change continuously on a grand scale. Moreover, he provided for the first time an appealing, easily understood process by which such changes could occur. Lamarck published his book entitled *Philosophie Zoologique* in 1809 propounding this idea when he was already sixty-five years old. Apparently the book attracted little attention when it appeared. It is said that, in his old age, he was neglected by his contemporaries, became blind, and died miserably. His fame came only long after his death when evolution became a heated subject following the publication of Darwin's *The Origin of Species*. Some of Darwin's opponents drew from Lamarck a theory called neo-Lamarckism, emphasizing the direct effect of environment as the prime factor for evolution. (For an authoritative account of the nineteenth-century approaches to evolution, readers may refer to Simpson, 1964.)

Here our main interest in Lamarck is that he proposed, probably for the first time in biology, a general theory to explain how evolution occurred. As is well known, he assumed that the effect of use and disuse, which in animals is induced by their living conditions, is inherited by offspring and this causes a perfecting tendency in evolution. For example, the ancestors of the giraffe tried to reach leaves on high twigs by continuously stretching their legs and necks. In each generation, as this effort was continued, it caused these parts to be a little longer, and this tendency was passed on to their offspring. Through the accumulation of small increments of elongation, the present giraffe finally evolved. Lamarck's theory rests on the assumption that acquired characters caused by use or disuse are inherited.

It is now clear that inheritance of such acquired characters does not

occur, and therefore the Lamarckian theory of evolution is wrong. As remarked by Simpson (1964) it is a pity that such a charming theory is not true.

It was to August Weismann's credit that he openly challenged the Lamarckian hypothesis. Through a series of writings he showed that the hypothesis is both unnecessary and improbable and that the supposed evidence for it is weak. (For the role of Weismann in the history of genetics, readers are invited to consult Sturtevant, 1965.) It is a well-known story that he cut off the tails of mice for twenty-two successive generations but found no decrease in the tail length at the end of that time. The vigorous development of Mendelian genetics in this century, culminating in modern molecular genetics, has shown beyond doubt that there is no evidence for inheritance of acquired characters. Yet there are evolutionists even now who cling to this hypothesis, claiming that the modern theory of evolution based on Mendelian genetics is unsatisfactory. Being almost irresistible it is regularly revived and the discovery of reverse transcriptase has invited a new round of Lamarckian speculations (e.g. Steele, 1979).

Weismann led his school of 'neo-Darwinians' in the fight against neo-Lamarckians; his theoretical writings caused one of the most heated controversies in the early post-Darwinian period, making evolutionary studies in the later part of the nineteenth century highly turbulent. Weismann was also an avid selectionist, advocating the selection theory more forcefully than Darwin; it is said that he 'out-Darwined Darwin' (Simpson, 1949). In fact, Weismann rejected all of Darwin's theory of evolution except natural selection.

Scientific studies of evolution really started with Charles Darwin. He published his book *The Origin of Species* when he was fifty years old (Darwin, 1859), half a century after Lamarck's *Philosophie Zoologique*. With his masterly writing and wide ranging examples, Darwin not only persuaded the world that evolution has actually occurred, but also he showed through his theory of natural selection why adaptive evolution is an inevitable process. *The Origin of Species* has had immeasurable influence not only on biology but also on human thought in general. We cherish Darwin for we owe to him our enlightened view of the nature of living things, including ourselves; our civilization would be pitifully immature without the intellectual revolution led by Darwin, even if we were equally well off economically without it. H.J. Muller (1960), in celebrating the hundredth anniversary of the publication of *The Origin of Species*, remarked that it can justly be considered as the greatest book ever written by one person.

Extrapolating from artificial selection which proved to be most efficient in producing domestic races of plants and animals useful to man, Darwin reasoned that the principle of selection applies equally in nature. Because more individuals of each species are born than can possibly survive, a struggle for existence follows, and any variation, however slight, if in any manner profitable to the individual, will have a better chance of surviving. Then, under 'the strong principle of inheritance', such a variation tends to be propagated. He writes:

> Slow though the process of selection may be, if feeble man can do much by his powers of artificial selection, I can see no limit to the amount of change, to the beauty and infinite complexity of the coadaptations between all organic beings, one with another and with their physical conditions of life, which may be effected in the long course of time by nature's power of selection.

Darwin emphasized the importance of accumulation of small beneficial variations, thereby causing a gradual and continuous process of adaptive evolution.

When Darwin formulated his theory, the mechanism of inheritance and the nature of heritable variations were unknown, and this prevented him from being fully confident of the role of natural selection. In fact, through successive editions of *The Origin*, he gradually weakened his claim that natural selection is the main cause of evolution. He conceded that inheritance of acquired characters also plays an important role in evolution. It is all too easy to forget that a storm of opposition and criticism once raged against the Darwinian view, for it is now so well established as to be regarded as almost sacrosanct.

With the rise of Mendelian genetics in this century the way was open to elucidate the mechanism of inheritance and the nature of heritable variations, which Darwin vainly struggled to understand. The dawn of the Mendelian era, however, was stormy. Soon, a bitter conflict arose between the biometricians championed by Karl Pearson and W.F.R. Weldon, and the Mendelians led by William Bateson (see Provine, 1971). Actually, the conflict between these two groups had started before the rediscovery of Mendel's law in 1900. Weldon, who was a biologist, came to the belief, stimulated by Francis Galton, that evolution can best be studied by the statistical method. He made many measurements on animal and plant characters with the aim of estimating the evolutionary rate and intensity of natural selection. Through personal contact with Weldon, Karl Pearson, who was an outstanding applied mathematician, became interested in the

problems of evolution. Although the theory of heredity he formulated was wrong, the statistical methods which Pearson developed, such as the χ^2 method, turned out to be of enormous value for later studies of evolution and variation, as pointed out by Haldane (1957a). Both Weldon and Pearson believed, following Darwin, that evolution occurs gradually by natural selection operating on small differences.

On the other hand, William Bateson, through his studies of variation in plants and animals, came to the definite conclusion that evolution could not occur by natural selection acting on continuous variations, contrary to Darwin's view. Bateson emphasized instead the importance of discontinuous variation.

With the rediscovery of Mendel's laws, the conflict between Mendelians and biometricians was exacerbated. While Bateson was impressed by the importance of Mendel's laws, both Weldon and Pearson vigorously attacked Mendelism. It is said that from the strenuous effort to disprove Mendelism by searching for exceptions in the huge volumes of stud books of race horses, Weldon became exhausted, contracted pneumonia, and died in the prime of his life (see Pearson, 1906). As one who has been honored by the Weldon Memorial Prize, I feel a particular interest in Weldon's life, and would like to add that he made an important discovery concerning the action of natural selection. He studied (Weldon, 1901) snail shells and measured the numbers of turns of the spiral in a given length of axis. By comparing this number in young shells and in the corresponding part of the adult shells, he found that young snails that had too many or too few turns showed a higher mortality than those whose shells were near the average. This is one of the first reports of 'centripetal selection', as pointed out by Haldane (1959).

After Weldon's death, biometricians were in retreat and the victory of Mendelians was soon evident, being supported by overwhelming facts. Many Mendelians at that time doubted that natural selection acting on small continuous variations could be effective in producing evolutionary change as envisaged by Darwin. Rather, they adopted the mutation theory of Hugo de Vries, claiming that a new species arises by mutational leaps rather than by gradual natural selection. The mutation theory, proposed at the beginning of this century, became very popular among biologists and found many adherents at that time.

It is now known that the 'mutations' observed by de Vries in the evening primrose *Oenothera lamarckiana* were probably due to this plant being heterozygous for special multiple chromosome rearrangements. As remarked by Sturtevant (1965), it is ironic that few of those mutations would

now be called mutations. Nevertheless, de Vries' theory, by calling wide attention among scientists to the possibility of mutation as the real cause of genetic variation, opened the way to its eventual confirmation and elucidation by H.J. Muller, to whose great contribution to our understanding of the mechanism of evolution we have to return later.

The first decade of this century witnessed active experiments to settle the issue whether natural selection on continuous characters is effective (as assumed by Darwin) or not. Among such experiments, those made by Wilhelm Johannsen are probably best known. He proposed the pure line theory, showing that selection is ineffective within a pure line. The exciting but confusing atmosphere of this period is well described by Provine (1971).

Gradually, however, it became understood that Mendelism and Darwinism are mutually compatible. Such a change of atmosphere was due to the vigorous development of genetics, of which *Drosophila* genetics was particularly important, revealing that mutational changes can be very small. Eventually, population genetics was developed through the effort to bring about a synthesis of Darwinism and Mendelism by the method of biometry.

The contributions of Hardy (1908) and Weinberg (1908) serve as a convenient starting point to discuss the history of population genetics. They showed that under random mating and Mendelian inheritance, genotypic frequencies at an autosomal locus remain unchanged from generation to generation. Furthermore, if a pair of alleles A and a occur with relative frequencies of p and q (where $p + q = 1$), then the equilibrium genotypic frequencies are

$$p^2AA : 2pqAa : q^2\text{aa}.$$

The finding that genotypic frequencies remain unchanged was significant, for some biometricians at that time mistakenly criticized Mendel's laws by saying that if they were true, a dominant trait (such as brachydactyly in man) would increase in frequency until it reaches 75%. Hardy's paper helped to dispel this misconception. Now that Mendelian inheritance is well established there is no need for us to worry about such a matter.

A part of the findings by Hardy and Weinberg which we can still appreciate is the rule that genotypic (or zygotic) frequencies in a random mating population can be computed by simply multiplying relevant gene frequencies. It is, however, no more than a useful rule. Their findings serve as a convenient starting point for the teaching of population genetics. However, I find it surprising that the Hardy–Weinberg principle is customarily presented with an exaggerated importance attached to its