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SPECIAL FEATURE

On the Occasion of the 25th Anniversary of the Neutral Theory (I)

Introductory comments on major papers by Professor Motoo Kimura

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Professor Motoo Kimura is best known for his neutral, random drift theory of molecular evolution which was proposed 25 years ago. The theory has revolutionized the way we think about molecular evolution. Yet, population geneticists are more impressed by the power, originality, and ingenuity of his research in theoretical population genetics. I believe there are many to be yet learned from his writing. The topics that he has treated during the past 40 years are diverse. Except for a few topics such as those in evolutionary stable strategies and applied quantitative genetics, he has indeed covered most that have been raised in modern evolutionary biology. Because of this diversity, however, it may not be easy to fully appreciate his papers, particularly for students and young researchers. Therefore it is my hope to set the background for his papers and point out their relation to other work and to subsequent developments.

Here, 57 papers are grouped into 18 subjects. This grouping obviously does not follow the chronological order and is neither completely unambiguous nor mutually exclusive. Nevertheless, I have taken this compromise to save space (because the same topic sometimes appears repeatedly in several papers, of course with different favors), but more importantly, to emphasize the prospective value of Professor Kimura's work.

CONTENTS

The numbers in parentheses correspond to the numbered papers in the list of major publications by Motoo Kimura.

I. Random gene frequency drift (**3, 5, 11, 29**)

In the early days when Kimura started to work at the National Institute of Genetics, he presented his preliminary results in the annual report of the Institute. The 1951 annual report contains Kimura's formulas for the moments of the gene frequency distribution under random genetic drift. The drift occurs in every population when gametes unite in no predetermined way in the process of reproduction and plays an important role in shaping the genetic architecture of populations. At that time, he had obtained only the first four eigenvalues but he made a clear distinction between fixed and unfixed probabilities. For instance, if p is the initial allele frequency and t is time in generations, the probability of fixation of this allele for large t was given as

$$p-3p(1-p)\,(1-\frac{1}{2N_e})^t$$

where N_e is the effective population number. Robertson (1952) also gave the exact formulas for the first four moments.

Soon after coming to the University of Wisconsin, Kimura obtained the complete solution by the diffusion approximation method (Kimura **3**). Wright was sufficiently impressed by this work to communicate it to the Proceedings of the National Academy of Sciences USA. Kimura's figures showing the distribution of the unfixed classes are often reproduced in textbooks. Kimura was the first to obtain the complete solution, but it is interesting that the approximation obtained by Malécot in 1944 for the probability density for large t,

$$f(p, x, t)\approx 6p(1-p)e^{-t/2Ne}$$

is the same as the leading term in Kimura's solution (see Nagylaki, 1989a). Most interesting is the rate of steady decay. The rate is $1/(2N_e)$ per generation and after $2N_e$ generations the distribution becomes almost flat, as earlier found by Fisher (1922, 1930) and Wright (1931).

It may be noted that the complete set of eigenvalues $-i(i-1)/(4N_e)$ ($i=1$, 2, ...) of the diffusion equation is the same as that in the coalescent process of Kingman (1982a, b), a death process which describes the family relationships of all the genes at a locus in a randomly mating population. For genealogical processes of neutral genes, see Hudson (1983), Tajima (1983, 1989a), Tavaré (1984) and Watterson (1984) while for those of selected genes, see Takahata (1991) and references therein.

The complete description of gene frequency changes under the joint effects of random sampling drift, selection and other evolutionary forces is often very difficult to obtain. Even for drift and selection alone, there are only a few successful cases. Kimura (**5**) derived the complete solution of the distribution of gene frequency for the case where genic selection and drift interact. The solution is expressed in terms of the Gegenbauer polynomials. The analysis of the smallest eigenvalue that determines the rate of steady decay indicates that selection predominates if $N_e s \geq 1$ where s is the selection coefficient. In the second through the fourth section, Kimura studied the steady distribution of gene frequency due to random fluctuation of selection intensity with and without mutation and random sampling drift. The paper ends up with two notes on cyclic changes in selection intensity and multidimensional diffusion equations. It also includes Malécot's comment on isolation by distance. The main conclusion about fluctuating selection intensity was that the effect is less important in a small population, because selection in any form is ineffective in a relatively monomorphic population. Throughout, it is clear that Kimura was convinced of the stochastic nature of evolutionary processes.

Kimura (**11**) is an extensive review of stochastic processes in genetics. Of five topics treated, the first two are somewhat different from the rest. These are concerned with random assortment of a replicated genetic entity in cell divisions. When there are multiple copies of a genetic element within a cell and if the replicated elements are distributed randomly into the daughter cells, there will be random segregation of these copies. The formulation assumed that each element (chromosome) replicates exactly but the doubled elements are randomly partitioned. This stochastic process is similar to genetic drift caused by random sampling of gametes and is suitable for describing the stochastic process of organelle genes in multicellular organisms (Birky, 1983, 1991; Takahata, 1985) and plasmids in bacteria (Levin and Stewart, 1976). Applying the formulation to *Paramecium* which was considered to have a polyploid macronucleus, Kimura, following the work of Sonneborn, assumed that senescence or aging occurs due to random loss of chromosomes of any one type. In the remaining article, the rate of steady decay of the gene frequency distribution for unfixed classes was provided under the joint effects of sampling drift and selection with an arbitrary degree of dominance (See Chapter 8 in Crow and Kimura 52).

A diffusion equation for describing Brownian motion was invented independent-

ly by A. Einstein and M. von Smoluchowshi. In genetics, Fisher (1922) first investigated random sampling drift (then called the *Hagedoorn effect*) by a diffusion equation similar to that for Brownian motion (Wright, 1931). These preceded Kolmogorov's (1931) rigorous mathematical foundation of diffusion processes. Diffusion theory has played a significant role in developing the modern theory of population genetics. A stochastic process arising from reproduction in a finite population interacting with other evolutionary forces is often very difficult to treat by a Markov chain. As remarked by Ewens (p. 140, 1979), Kimura (**3**, 4, **5**, 7, **11**) based upon diffusion theory heralded a rebirth of the mathematical theory of population genetics.

When Kimura was an assistant in Kihara's laboratory in Kyoto University, he was greatly influenced by Wright's 1931 paper (Kimura 141) and dreamed of the application of the diffusion theory to significant problems in population genetics. He quickly mastered the theory, recognized its usefulness, and solved many difficult stochastic problems by means of diffusion approximations. Kimura (**29**) introduced geneticists to the Kolmogorov forward and backward equations in an elementary way and at the same time demonstrated their power. Diffusion equations in population genetics have singularities at the boundaries which require caution. It is surprising to see his intuition and ability in manipulating such delicate equations, even though Feller's (1951, 1952) pioneer work was by then available (see also Chapter 15 Karlin and Taylor, 1981). We should however mention one error in Kimura (**29**). It occurs in the general formula for obtaining the probability of joint fixation of mutant genes involving two or more loci under epistatic interaction in fitness (see p. 430 in Crow and Kimura 52). Kimura still regrets this, often asking his colleagues if we can get the correct formula or even an approximate answer. So far no one has succeeded (but see Rutledge, 1970 for an approximate solution).

II. Fluctuation of selection intensity (**2, 107**)

When there is stochastic (temporal) variation in fitness (Wright, 1948), the variance of selection intensity may be as important as the mean. Kimura (**2**) derived and solved a diffusion equation of gene frequency changes when selection intensity fluctuates randomly from generation to generation (see also Ohta, 1972).

Without other evolutionary forces, complete loss or fixation of an allele does not occur in an infinite population, but rather the distribution eventually becomes concentrated near the boundaries (quasi-loss or quasi-fixation). While the diffusion coefficient of this equation of course includes the variance of selection intensity, the drift coefficient does not. This is inconsistent if we base the derivation on the conventional Wright-Fisher model of selection (Gillespie, 1973; Nei and Yokoyama, 1976; Avery, 1977; Takahata, 1981), for the variance term in the drift coefficient has to be incorporated unless the mean relative fitness is

constant.

As a simple example (Crow, 1972), suppose that the fitness $1+s$ of an allele with frequency x fluctuates randomly with a geometric mean one; thus the locus is neutral over time. Then $\Delta x = sx(1-x)/(1+sx) \approx sx(1-x)(1-sx)$. If the mean of $1+s=1$, $E[\log(1+s)] \approx E[s-s^2/2]=0$. But, $E(s^2)=V_s$, so $E(\Delta x)=V_s x(1-x)(1-2x)/2$. Thus there is a centripetal tendency, with the allele frequency tending to move toward 1/2.

When criticism of the neutral theory revolved around the rather low heterozygosity in natural populations, the role of random fluctuation of selection intensity regained attention (reviewed by Felsenstein, 1976). Kimura's (**107**) paper may not appear to be related to this problem because his whole treatment was deterministic. However, under population number regulation the mean population fitness equilibrates rapidly so that gene frequency change per generation is given by $\Delta x = sx(1-x)$ or similar equations with good approximation instead of $\Delta x = sx(1-x)/(1+sx)$. If we base the diffusion approximation on such an equation, the variance term in the drift coefficient can disappear. The effect of fluctuating selection intensity is then to reduce the extent of variation without increasing the allelic substitution rate. All other diffusion equations for random fluctuation of selection intensity have stabilizing factors in the drift coefficient, such as that above, which act in much the same way that overdominance selection does (Gillespie, 1973, 1986). In these models, the extent of genetic variation and the substitution rate can both be greatly enhanced compared to those expected from Kimura's 1954 diffusion equation (115; Takahata, 1981). These properties render most fluctuating selection models unsatisfactory as a means of accounting for the rough constancy of molecular evolution and rather low heterozygosity.

III. Population structure (**1, 28, 119**)

The study of geographically structured populations was pioneered by Wright (1931) with his island model. He assumed an infinite number of finite panmictic subpopulations, with migrants coming from any one of them with equal probability, regardless of distance. While still at the Genetics Institute in Japan, Kimura presented a model (Kimura **1**) in which the migrants came from adjacent subpopulations. He also permitted occasional long-range migration, so Wright's island model is included as a special case.

Unbeknownst to Kimura, Malécot, writing in obscure French journals, had been studying very similar models (see Nagylaki, 1989a). Both he and Kimura provided for short- and long-range migration in a specified manner.

To study the correlation of genetic variation with geographic distance or isolation by distance (Wright, 1943), the topology or distance between subpopulations must be taken into account and the stepping stone model, together with the continuous version (Malécot, 1948, 1955, 1959; Felsenstein, 1975), has become

widely used for this purpose (see Nagylaki, 1974a and Slatkin, 1985 for reviews). A thorough mathematical analysis of the stepping stone model under neutrality was made by Kimura and Weiss (**28**) and Weiss and Kimura (32). The extent of local differentiation is sensitively dependent on the number of dimensions (Malécot, 1955; Cavalli-Sforza and Conterio, 1960). Kimura and Weiss showed that the decay of genetic correlation with distance is slowest in the one dimensional case and fastest in the three dimensional case, as did Malécot (Nagylaki, 1989a). [For a correction of errors in Weiss and Kimura (32) see Nagylaki, 1974b.]

Originally the stepping stone model assumed infinite arrays of subpopulations, but it has been modified to apply also to a finite number of subpopulations by imposing some restrictions on the boundaries (Malécot, 1951; Maruyama, 1970; Fleming and Su, 1974). An obvious difference between the original and modified models lies in the total number of individuals. The expected homozygosity in the entire population as well as the coefficient of kinship depends not only on the extent of gene flow and the size of each subpopulation but also on the number of subpopulations. High homozygosity and geographic uniformity of genetic polymorphisms, which Bulmer (1973) regarded as inconsistent with the neutral theory, actually become consistent if one assumes the stepping stone model with a finite number of subpopulations and therefore a finite number of total individuals (Maruyama and Kimura 82). Nagylaki (1983) and Crow and Aoki (1984) showed that, when migration is frequent, the finite island and stepping stone models predict rather similar extents of genetic variability within and between subpopulations.

It has repeatedly been asked why genetic variability at a locus in diverse organisms is limited (e.g., Lewontin, 1974): The heterozygosity, H, is less than 0.3 (usually 5–10%) and there is a positive correlation between H and population size (Nei, 1975; Nei and Graur, 1984). If the neutral theory is correct, this means that the global effective population size (N_e) must be much smaller than 10^6 or the reciprocal of mutation rate per locus per generation. Of various possibilities for the small value of N_e, local extinction and recolonization of subpopulations was emphasized by Wright (1940) and studied quantitatively by Slatkin (1977). The global effective size can be infinitely large with restricted gene flow, but it decreases to the number of breeding individuals in the entire population as gene flow becomes high (Nei and Takahata, 1993). When local extinction and recolonization occur, N_e may further decrease to the number of individuals within a subpopulation. Maruyama and Kimura (**119**) showed that when this is frequent N_e is much smaller than the actual number of individuals in the whole population and there is little local differentiation. They applied their theoretical results to the genetic variation observed in *Escherichia coli* and *Drosophila willistoni* populations whose sizes were said to be enormous (e.g., Nei, 1975).

IV. Linkage and recombination (**9, 33, 57, 66**)

Kimura (**9**) first deduced the differential equations for the gametic frequencies at two diallelic loci and used them to confirm quantitatively Fisher's (1930) claim that epistatic interaction will always tend to diminish recombination and therefore tighter linkage is favored by natural selection (Bodmer and Felsenstein, 1967; Chapter 5 in Crow and Kimura 52; Chapter 6 in Kimura and Ohta 60; Chapter 8 in Nagylaki, 1977 and references therein).

Kimura (**33**) coined the term quasi-linkage equilibrium. Under loose linkage and relatively weak epistatic interaction, it represents a state in which the ratio of coupling and repulsion chromosome phases is kept approximately constant. This state is attained in a very short time, generally less than 10 or 20 generations, while the equilibrium values of allele frequencies are attained in a much longer time. Under quasi-linkage equilibrium, the mean fitness change of the population is approximately equal to the genic variance and Fisher's fundamental theorem of natural selection is usually a good approximation. This is because the contribution of linkage disequilibrium almost exactly cancels the epistatic variance.

It is interesting to ask to what extent genetic variation at a neutral locus is generated through linkage disequilibrium with selected loci. When selected loci are overdominant, the effect of linkage disequilibrium on a neutral locus is called associated or associative overdominance (Frydenberg, 1963) while when selected loci are subjected to purifying selection by favorable alleles, the effect is called hitch-hiking (Maynard Smith and Haigh, 1974). Ohta and Kimura (**57**) studied associative overdominance, assuming that allele frequencies at selected loci are kept constant but neutral alleles experience random drift. There is little effect of associative overdominance unless the linkage is very tight. A similar conclusion was obtained when two loci are kept polymorphic by strong overdominance selection. This would hold, however, only when the two loci are nearly independent and do not generate linkage disequilibrium. Recent DNA sequence data have revived the problem concerning associative overdominance and hitch-hiking (Kaplan et al., 1989; Langley, 1990; Takahata, 1990; Hedrick et al., 1991). For DNA regions in which recombination is rare, nonrandom association of polymorphic sites must have significant effects on genetic variation in populations. The hitch-hiking effect may be regarded as one of the reasons why the extent of heterozygosity is rather low in various organisms. In *E. coli* populations, hitch-hiking of the whole genome is termed periodic selection (appearance of a clone with a high fitness followed by its rapid expansion) by Atwood et al. (1951) (see also Novick and Szilard, 1950; Selander and Levin, 1980).

Ohta and Kimura (**66**) extended the previous work of Kimura (**46**), incorporating linkage between two neutral sites and concluded that marked linkage disequilibrium can arise due to random drift and tight linkage.

V. Evolutionary advantages of sexual reproduction (**34**)

The evolutionary advantages of sexual reproduction have been extensively discussed since Fisher (1930) and Muller (1914, 1932). If the main difference between sexual and asexual reproduction is in the presence or absence of the Mendelian segregation process occurring more or less independently at loci on a chromosome, the question is equivalent to asking whether or not there are evolutionary advantages of recombination. If offspring only duplicate the parental genotype, it is easy to see that the rate of accumulating beneficial mutations is slower while the rate of accumulating harmful mutations is faster than otherwise. Crow and Kimura (**34**), following Muller's approach, first formulated this problem quantitatively. For subsequent more quantitative studies mainly based on computer simulations, see Felsenstein (1974), Felsenstein and Yokoyama (1976), Maynard Smith (1978, 1989), Haigh (1978), Takahata (1982) and Pamilo et al. (1987).

Crow and Kimura (**34**) further argued that diploidy provides an immediate advantage over haploidy by covering over recessive deleterious alleles. This advantage is only transient, however, since eventually the diploid load will be twice as large; but the diploid state may have become fixed before this time. Kondrashov and Crow (1991) showed that with truncation-like selection there is a window of dominance values, around .25, that permits a permanent advantage of diploidy.

The Muller argument, as developed by Crow and Kimura (**34**), is given less weight now than in the past. Most discussions now center on the advantages of sexuality in keeping up with a changing environment and with reducing the mutation load (for a review, see Crow, 1989a; Michod and Levin, 1989).

VI. Natural selection (**13, 19**)

Fisher's (1930) fundamental theorem of natural selection states that the rate of increase of fitness of a population is equal to the additive genetic variance. He provided extensions to include the effect of crowding and deterioration of the environment (Frank and Slatkin, 1992). Fisher's writing is elegant, but obscure, and many writers have attempted to explain what Fisher "really meant" (Edwards, 1971; Price, 1972; Nagylaki, 1989b, 1991; Ewens, 1989; Crow 1990a). Kimura (**13**) provided an explicit formulation in which the terms are explicitly derived and identified. The number of alleles and loci is arbitrary, as is the mating system, and the fitness coefficients need not be constant. Unfortunately, however, only the single-locus results in Kimura (**13**) are correct (Akin, 1979; Nagylaki, 1989b, 1991). Fisher, although he agreed to publish Kimura's paper, did not regard it as a generalization of his theorem, but rather a more explicit specification.

Section 4 of the paper exemplifies the generality, and some pathological, counter-intuitive cases are impressively demonstrated. The genic variance is consumed quite slowly by natural selection; the population is not likely to exhaust its genic variance unless it is small, the number of loci (involved in a quantitative trait) is small, or selection is very intense (5.10 in Crow and Kimura 52; Chapter 12 in Falconer, 1960). Although this holds for short-term results, as in breeding experiments, the maintenance mechanisms of the heritable variation that is observed for most characters in natural populations are still poorly understood. For recent work on this problem, see Lande (1975, 1977), Hill (1982, 1990), Barton and Turelli (1987, 1989), Weir et al. (1988), Tachida and Cockerham (1990).

Kimura's (**19**) view was that the genetic information (or improbability) of organisms was gained by allelic substitutions owing to natural selection of random mutations. He was a strong neo-Darwinist at that time and related the information content to the substitutional load (Kimura 15). He calculated the total amount of genetic information (G) for the period of 500 million years since the Cambrian epoch. Although necessarily crude because of a number of uncertain assumptions, the estimate was 1% of the maximum that could be stored in the human genome. This was based on the horotelic (standard) rate of substitution (1/300 per generation) of Haldane (1957). Because of the small value of G, Kimura suggested that the genome may be highly redundant (Schmalhausen, 1958) and that parts of a gene rather than the whole are duplicated. We may compute G from the nucleotide substitution rate which has become available since 1960. If the genome experiences one nucleotide substitution every other year (Kimura **38**), G becomes 5% of the maximum while for a more recent estimate of nucleotide substitution (10^{-9} per site per year; Li et al., 1985) it becomes 20%. These amounts of genetic information gained may be consistent with the development of complex organisms during the last 500 million years, but the fact is that a large part of the genome in higher organisms is non-coding and that the genome is redundant not only in subunits of genes but also in functional units as in multigene families (Watson et al., 1987).

VII. Meiotic drive (**20**)

Segregation Distorter (*SD*) in *Drosophila melanogaster* was first discovered by Hiraizumi in 1956 (Sandler and Hiraizumi, 1960; Hiraizumi and Crow, 1960), and *SD* and the *t*-haplotype in *Mus* are the two best known meiotic drive systems. *SD* violates the Mendelian segregation rule by producing an excess of *SD* gametes in heterozygous males (Crow, 1979, 1991 for a review). Because of its transmission advantage, *SD* can spread over a population even though it is generally harmful, at least in homozygotes. The special supplement in *The American Naturalist* (Temin et al., 1991) shows that *SD* is now the best characterized meiotic drive system at the molecular level. Theoretical aspects of *SD* have also

been worked out (e.g., Charlesworth and Hartl, 1978; Hartl, 1980).

Meiotic drive is not restricted to animals but exists in plants as well. One such example in plants is supernumerary (or accessory or **B**) chromosomes in the lily, *Lilium callosum*. Combining experiments and observations with population genetic theory, Kimura and Kayano (**20**) showed that **B** chromosomes are predominantly deleterious (when there are more than two in a cell) and they are kept in the population by preferential segregation in embryo sac mother cells. Meiotic drive is a phenomenon that can be studied at the population, cellular, and molecular levels. It may therefore provide a way to understand the poorly known connection between phenotypic and genotypic changes (Wu and Hammer, 1991).

VIII. Genetic load (**14, 36, 37**)

In 1960 Kimura was awarded the annual prize from the Genetics Society of Japan for his studies of theoretical population genetics. In his award lecture, he talked about the genetic load and its significance in evolution. In the same year, he presented a mathematical treatment of an old idea that if the mutation rate is too high the species will be crushed under a heavy mutational load; if it is too low the species will not be able to cope with adverse environmental changes (Kimura **14**). Thus between these conflicting demands there must be an optimum mutation rate which may correspond to the spontaneous mutation rate. By minimizing the sum of mutational and substitutional loads (the so-called principle of minimum genetic load), he derived a simple relationship between the mutation rate and the horotelic evolutionary rate by advantageous mutations with arbitrary degrees of dominance. Kimura (**37**) later reexamined the same subject and argued that modifiers which enhance the mutation rate of other loci are selected against whereas those which decrease the mutation rate are favored by natural selection. The present theory of modifiers is concerned with not only the mutation rate (Leigh, 1970; Ishii et al., 1989) but other genetic parameters such as the recombination fraction (Nei, 1969; Feldman, 1972; Eshel and Feldman, 1982; Feldman and Liberman, 1986). Compared to the then assumed mutation rate per gamete per generation ($M \approx 0.04$ in *Drosophila*), however, the principle of minimum genetic load led to a much smaller value of optimum mutation rate. The optimum rate equals the total rate of allelic substitutions per generation (E) that can be attained by positive selection. Being puzzled by this large discrepancy, and later finding a still larger value of M (0.5) from amino acid sequence data (Kimura **38**), he came to the conclusion that $E = M$ does not hold at the molecular level. This might have suggested to him that most substitutions are not driven by natural selection.

The Haldane-Muller principle (Haldane, 1937) states that the mutational load (the reduction in mean population fitness) by deleterious mutations is twice as large as the mutation rate in a randomly mating diploid population with partial

dominance and independence between loci. Some experimental data (Mukai, 1965) suggested, however, that the deleterious effects reinforce each other as the number of mutations increases. Kimura and Maruyama (**36**) formulated the mutational load under various forms of epistasis with and without recombination. Those results may be understood if one notices under what circumstances one genetic death can efficiently eliminate more or fewer deleterious mutations than under the circumstances that Haldane (1937) and Muller (1950) envisioned. Synergistic interaction (reinforcing type) is effective if a large number of deleterious genes involved. Truncation or quasi-truncation selection (Crow and Kimura **110**) is especially effective in removing deleterious genes in clusters, whereas this cannot happen in asexual species. This is one argument for the prevalence of sexual reproduction (Kondrashov, 1984).

IX. Inbreeding systems (**23, 25**)

Wright (1918) first used his path analysis to quantify each cause of the variability of quantitative characters by a method similar to that of partitioning the variance. Later he refined the method and defined the path coefficient as the standard deviation of the effect to be found when all causes are constant except the one in question (Wright, 1920); it is a standardized partial regression coefficient. As described in Chapter 5 in Provine (1986), the method of path coefficients proved to have much wider application than was apparent in Wright's first use of it. One application is to compute the effects of inbreeding in various systems of mating (Wright, 1921, 1922; see also Appendices in Wright, 1950). Haldane and Waddington (1931), Cotterman (1940), Malécot (1948) and Falconer (1960) considered the effect of inbreeding on homozygosity due to the more readily understood concept of identity by descent.

Kimura (**23**) developed a means of handling inbreeding systems in terms of probability. It is based on the concept of identity by descent and therefore is rather similar to Malécot's, but it is applied to identity of state and he derived known results in a simpler way. The method was applied to the so-called *circular mating*: Circular mating was an attempt to find the mating system that can conserve heterozygosity in the long run. By arranging males and females alternately in a circle and allowing mating only between the neighboring individuals, Kimura found that the effective number of individuals in this system is proportional to the square of the actual number. He might have anticipated that this system would become widely adopted in animal breeding practice, particularly for preserving endangered species in captivity (Yamada, 1985). Kimura and Crow (**25**) worked out the system of circular mating in addition to *circular pair mating* suggested by Haldane, *circular subpopulation mating,* and a mixture of circular and circular pair mating suggested by Yamada. Kimura and Crow distinguished two related, but distinct effects of inbreeding (random drift and

decrease of heterozygosity), and compared various mating systems together with the one for maximum avoidance of inbreeding by Wright (1921; see also Wright, 1965). Among these systems, circular mating has the lowest rate of decrease of heterozygosity in the long run, though the rate is highest initially, and has the smallest eventual value of drift variance per generation. Boucher and Cotterman (1990) systematically examined regular systems of mating, finding that when the number of individuals is larger than 4, there are many other systems that have slower rates of approach to genetic uniformity than circular mating.

These treatments were based on neutrality of genes and the concept of identity by descent. As remarked by Nagylaki (1989a), identity by descent is a fundamental concept that was discovered independently by Cotterman (1940) and Malécot (1941), and it lies at the heart of the powerful genealogical approach to population genetics.

X. Evolution of quantitative characters (**31, 109, 110, 125**)

Kimura (**31**), following Crow and Kimura (30), derived a formula for the equilibrium genic variance if mutation and selection are in balance, the distributions of allelic effects at a locus are approximately Gaussian, loci are independent, and there are no pleiotropic effects of the loci. As noted by Lande (1975) and others, an implicit assumption is that the variance associated with new mutations at each locus be small, relative to the variance of the effects of the alleles already segregating. In the opposite situation in which individual mutational effects are large, Turelli (1984) obtained a quite different formula, and Slatkin (1987) developed an approximation that bridges these extremes. Although mutation-selection balance is the most commonly invoked explanation for the maintenance of additive genetic variance, there are other suggestions (Turelli, 1988; Barton and Turelli, 1987, 1989; see also Bulmer, 1980).

In evolutionary quantitative genetics, there are two complementary interests. One is to seek the rate of phenotypic evolution with specified intensities of selection on phenotypes and the other is to consider selection at individual loci under a given intensity of phenotypic selection. The former approach was taken by Lande (1976) for example, to account for observed paleontological changes. The latter is the traditional approach in quantitative genetics, and this was greatly extended by Kimura and Crow (**109**). To explain their extension, let $F(X)$ and $W(X)$ be the frequency and fitness functions of individuals with a quantitative character whose value is X. In order to derive the relationship between the average excess of a gene or the average effect of an allele involved in X, say A, and the selective advantage of a particular allele, say s, it is customarily assumed that $F(X)$, $W(X)$ or both are normally (Gaussian) distributed. For example, it is shown under the assumption of $F(X)$ being Gaussian that truncation selection with $W(X)=W_{\max}$ for $X>C$ and $W(X)=0$ for $X<C$ (C; truncation

point) leads to

$$s = AW_{\max}F(C)/\overline{W}$$

in which $\overline{W}$ is the mean fitness defined by $\int W(X)F(X)\mathrm{d}X$. Kimura and Crow demonstrated that no such assumption on $F(X)$ is needed; the only requirements are that the gene effects on the character be additive between loci, the individual effects be small, and the character distribution be differentiable. But assuming that $F(X)$ is Gaussian, they derived various interesting results. Truncation selection is the most efficient form of directional selection at individual loci. The efficiency depends not only on the magnitude of the phenotypic effect, but on the mean fitness (and in general the shape of the fitness function). This general theoretical framework was used by Cockerham and Burrows (1980) to study strategies for maximizing the truncation selection limit for a quantitative trait in a finite population.

Crow and Kimura (**110**) quantified the efficiency of truncation selection using a modification, due to Milkman (1978), of the standard linear ranking of character values. The modified model was called a broken-line alternative. The aim of the paper is to reconcile the very high mutation rate in viability polygenes (Mukai, 1964, 1990; Ohnishi, 1977; Simmons and Crow, 1977) with a small mutational load. Truncation selection as well as the broken-line alternative can eliminate deleterious mutants in groups even when their allelic effects are additive so that Haldane-Muller's principle "one mutant-one genetic death" is bypassed. Hence, Crow and Kimura concluded that truncation selection, or something even very roughly approximating it, can be very effective in reducing the mutational load for mutants having minor deleterious effects on viability.

Rank order selection had earlier been suggested as a segregation load-reducing mechanism (King, 1967; Milkman, 1967; Sved et al., 1967). The question as to whether nature ranks and truncates, or does something approximating it, was also raised by Nei (1971, 1975). But not until the work of Milkman (1978) and Crow and Kimura (**110**) was it realized that a very crude approximation to truncation is almost as effective.

Another fundamental question centers on the connection of neutral evolution at the molecular level with adaptive evolution at the phenotypic level. It is possible to extract the effect of natural selection acting on a quantitative character related to Darwinian fitness on allele dynamics at loci which determine the character. Assuming the most prevalent type of phenotypic selection (stabilizing selection) and using the general formula in Kimura and Crow (**109**), Kimura (**125**) demonstrated that allele dynamics become underdominant (Bulmer, 1972). Since the selection coefficient is of the second order of allelic effect, stabilizing phenotypic selection leads to near neutrality at individual loci. This may substantiate the very slightly deleterious hypothesis of Ohta (1973), but an important difference from it is that the population fitness does not drift downward under stabilizing

selection. More significant is the picture of evolution depicted in the paper. That is captured as

> *From time to time, the position of the optimum* (of characters) *shifts due to changes in environment and the species tracks such changes rapidly by altering its mean. But, most of the time, stabilizing selection predominates. Under this selection, neutral evolution (random fixation of alleles by sampling drift) occurs extensively, transforming all genes, including those of living fossils, profoundly at the molecular level.*

This line of quantitative pursuit is still premature, but the extensive neutral evolution under stabilizing selection forms one of the bases of Kimura's recent view of evolution (Kimura 151, 154, 157, 158).

XI. Probability and time of fixation or extinction (**21, 45, 51, 111, 140**)

Fisher, Haldane, Wright and Robertson all recognized the importance of the process in which a new mutant gene introduced in a natural or artificial population increases its frequency by interaction between selection and random genetic drift and eventually becomes fixed in the population. Malécot (1948, 1952) used a method of moment-generating function and generalized Fisher's (1922) result (see also Haldane, 1927) to the case of an arbitrary initial frequency and genic selection. Kimura also presented a number of significant results on this problem since his early days as a population geneticist, and (Kimura **21**) derived a more general formula for the ultimate fixation probability. The formula allowed him to obtain the probability under various forms of natural selection and to unify all the previous results of Fisher, Haldane and Wright (Crow, 1987). This formula has been crucial to the development of the neutral theory.

Kimura and Ohta (**45**) were the first to treat separately the mean time of a mutant gene until fixation and that until loss. Although the unconditional mean time, until either of these alternative events, was studied by Watterson (1962) and Ewens (1963), the distinction is important because only mutants that are fixed in a population can play a key role in evolution. However, like alleles at the major histocompatibility complex loci, balanced polymorphic alleles that can persist for a long time in a population and produce descendants may well be evolutionarily significant (Klein, 1986). A neutral gene, initially represented singly in a population of effective size N_e, takes about $4N_e$ generations to become fixed. This is much longer than the unconditional mean time because loss of the gene is much more frequent and the mean time for this is very short. This topic of fixation time was later extended to various cases by a number of authors. For instance, Nei and Roychoudhury (1973) and Maruyama (1974, 1977) studied effects of selection in detail, noting that the mean fixation time for a favorable mutant (with

no dominance) is the same as that for the corresponding deleterious mutant. This is related to time-reversal properties of stochastic processes, the future being the "mirror-image" of the past (Watterson, 1976; Levikson, 1977; Sawyer, 1977). The standard deviation of the fixation time of a neutral mutant is about $2.15N_e$ (Narain, 1970; Kimura and Ohta 50) and the distribution itself was presented by Kimura (**51**).

When a locus is duplicated, harmful mutations and drift may lead to loss of gene expression at one of the loci. Under the assumption of free recombination, Kimura and King (**111**) dealt with the mean time until loss of gene expression and compared the result with experimental data from tetraploid fish. In 1985, Kimura took up the same subject when linkage between two loci is complete. Under particular interactions between duplicated genes, recombination affects the time greatly; the problem has some resemblance to the advantage or disadvantage of sexuality (Muller, 1932; Crow and Kimura **34**; Maynard Smith, 1968; Felsenstein, 1974; Haigh 1978). Further studies by computer simulations were made by Bailey et al. (1978), Takahata and Maruyama (1979) and Li (1980). Watterson (1983) developed an approximate formula for the mean time of duplicated gene silencing.

Kimura (139, **140**) treated the average fixation time of selected alleles under continuous mutation pressure. It takes a long time for a deleterious mutation to become fixed. The situation is different in the case of duplicated loci or where mutations at two loci are individually deleterious but becomes harmless in combination (compensatory neutral). When linkage between two loci is loose, compensatory neutral mutations are unimportant whereas with tight linkage they have an ample chance to participate in evolution. Compensatory mutations may be found as double substitutions in relatively conserved molecules such as tRNA. Kimura carried out numerical calculations of the formulated diffusion equations as well as Monte Carlo experiments. At this time, since the institutional computer was not suited for multiple users, he set up a personal 15KB computer (Hewlett Packard 9825A) at his home and first used it to write this paper. The last sentence of Kimura (**140**) shows his then stance for the neutral theory;

> *Finally, if the present model of compensatory neutral evolution is realistic, it will not only lend support to Ohta's concept of very slightly deleterious mutations, but also help us to understand some "non-random" evolutionary amino acid substitutions under the framework of the neutral theory.*

However, as shown by Li and Nei (1977), when $4N_e s > 10$ in which s is the selective disadvantage, the fixation time becomes too long to be of any evolutionary importance.

XII. Age of alleles and reversibility (**68, 78, 81, 89**)

Maruyama and Kimura (**68**) discovered a general procedure for finding sojourn times and such quantities as the total number of heterozygotes during the time the allele is moving toward fixation. This is a technical paper showing how to obtain an arbitrary moment of the sojourn time and related quantities in a one dimensional diffusion equation with exit boundaries. Two events of gene frequency change, fixation and extinction, are distinguished.

Fisher (1930) argued that the number of individuals having a gene derived from a single neutral mutation cannot greatly exceed the number of generations since its occurrence. Such a retrospective view is important and useful in understanding the nature of extant genetic variations. Kimura and Ohta (**78**) took it up for the first time and opened up the whole topic of retrospective properties of stochastic processes arising in population genetics (p. 112 Ewens, 1979). With a wealth of DNA sequence data that have been accumulating since 1980, much of the theory has been concerned with the ancestral history of genes in a phylogeny. One such theory, the coalescent process of Kingman (1982a, b), has become widely used to understand DNA variations and test the neutral theory (Hudson, 1983; Hudson et al., 1987; Tajima, 1989b).

An interesting property related to reversibility (identity of prospective and retrospective behaviors) of stochastic process arising in population genetics was pointed out by Maruyama and Kimura (**81**). Although the property was recognized in mathematics (Dynkin, 1965), the application to genetics was new. It is counter-intuitive, but true, that the average time required for an advantageous mutant allele to increase its frequency on the way to fixation is equal to that for the same allele to decrease its frequency on the way to extinction against selection. The reversibility argument was extended by Sawyer (1977) to the case where there is no stationary distribution.

An extension of an earlier work on the age of a mutant allele was made so as to compute any moment of the sum of an arbitrary function of the gene frequency along sample paths between two specified frequencies (Maruyama and Kimura **89**). An application of Nagylaki's formula (1974b) to the moments led them to a set of second order differential and difference equations (see also Maruyama, 1977).

XIII. Intergroup selection (**131**)

Kimura's preference for diffusion models is typified in his 1983 paper (**131**). He incorporated intergroup selection into a diffusion model, in addition to conventional individual selection, mutation, migration and random drift, and obtained the condition for group selection to prevail over counteracting individual selection (Wilson, 1975; Uyenoyama, 1979; Aoki 1982). Unlike ordinary diffusion equations, the equation has killing and creating terms of a sample path. Those killing

and creating terms are respectively proportional to the average frequency of a mutant in the whole population and its frequency in a particular deme. The problem is also related to the third phase of Wright's (1970, 1977) shifting balance theory (Crow et al., 1990; Crow, 1990b for review; Kimura 132 for criticism) in which intergroup selection by differential migration facilitates the establishment of favorable gene combinations in other demes. Shimakura (1985) proved the existence and uniqueness of solutions in Kimura's diffusion.

XIV. Infinite allele, infinite site, and ladder models (**27, 46, 79**)

One may imagine that Kimura would not have proposed the neutral theory without writing the celebrated paper on the infinite allele model—more grammatically, the model of infinitely many alleles, or the infinite-number-of-alleles model (Kimura and Crow **27**). Such a guess seems only partly correct for it is clearly Wright (1931), Haldane (1957) and Muller (1958) that were essential in proposing the neutral theory. Kimura and Crow stated:

> *It is not the purpose of this article to discuss the plausibility of many very nearly neutral, highly mutable multiple isoalleles, or the evidence for and against. Instead, we propose to examine some of the population consequences of such a system if it does exist. The possibility seems great enough to warrant such an inquiry.*

Crow (1989b) recalled how they came to the consideration of such a system of isoalleles. There is no indication of the neutral theory in this paper. In fact, the infinite allele model was applied to not only neutral but also overdominant alleles and the time was yet ripe for the neutral theory. Nevertheless, one can still perceive some link in scientific thought.

The infinite allele model has been extensively used in bringing refinements of the neutral theory. The mathematical refinements culminated in the sampling theory (Ewens, 1972), the line-of-descent process (Griffiths, 1980; Watterson, 1984; Tavare, 1984) and measure-valued diffusions (Ethier and Griffiths, 1984), all of which assume the infinite allele model. It also served to develop useful statistical methods for testing the neutral theory. In particular, the Houston group headed by Nei (1987, Kimura 132 for review) has made significant contributions and supported the neutral theory.

Using a Kolmogorov backward equation, Kimura (**46**) obtained the expected number of heterozygous nucleotide sites in a particular range of allele frequencies. This number, under a steady flux of mutations, is mathematically equivalent to the so-called Green function from which other quantities can be derived. The main interest was the average number of heterozygous sites per individual which under random mating is the same as the number of segregating sites between two

haploid genomes that are randomly sampled from a population (Watterson, 1975). Kimura assumed no linkage among those heterozygous sites (cf. Ohta and Kimura **66**) while Watterson assumed complete linkage. This mutation model proposed in 1969 was called the infinite site model (Kimura 59). Under neutrality, Kimura estimated the average number of heterozygous sites per individual to be 80,000 in human populations.

The theoretical results of the original neutral theory rely much on the infinite allele model (Kimura and Crow **27**) and the infinite site model (Kimura **46**, 59). One argument against the neutral theory was that the extent of electrophoretically detectable polymorphisms is rather low. Since the electrophoretic method does not meet the requirement in the infinite allele model, Ohta and Kimura (**79**) considered the charge state, stepwise mutation or ladder model of electrophoretic mutations. The model received much attention from the mathematical point of view (see Moran, 1975, 1976 for the distribution of allele frequencies; Kingman, 1976, 1977), but the biological consequence does not differ much from that obtained from the infinite allele model (Li, 1976; Takahata 1980; Chapter 8 in Kimura 132). The appropriateness of the infinite allele model was supported experimentally by Fuerst and Ferrell (1980) who used hemoglobin variants (Brown et al., 1981 for criticism).

XV. Molecular evolution (**77**, **113**)

Those who currently study DNA sequences for the study of systematics may be surprized to find how little information was available until quite recently. Kimura and Ohta (**77**) compared four DNA sequences of 5S ribosomal RNA genes from human, yeast and two bacteria, and dated the eukaryote-prokaryote divergence as about 2 billion years ago. Now the number of 5S rRNA sequences alone is more than 620 (Hori and Osawa, 1987) and many other genes have become available (see Woese, 1991 for a review of 16S rRNA). The LASL-GDB (version 78.0, 1993) released from GenBank consists of 133,970 entries, the total number of nucleotides stored being 153,217,979. This is one of a very few papers in which Kimura did not discuss the mechanism of molecular evolution in any detail and simply applied the molecular clock to the phylogenetic analysis. Ironically, the paper may mark the beginning of the present bifurcation between molecular evolution and population genetics (long-term vs. short-term evolution). This may not have been Kimura's intention, but at the deepest level the neutral theory indeed demanded a bifurcation of molecular evolution from phenotypic evolution (Provine, 1990).

As soon as amino acid sequence data were generated around 1960, molecular evolution became popular. With the introduction of DNA technology into evolutionary biology about a decade ago, it soon became clear that the genome has been evolving in a complex manner due to its own mechanisms such as unequal crossing

over, gene conversion, duplicative transposition, and such. Stimulated by these molecular evolutionary mechanisms, many population geneticists have gotten involved in quantifying them. Ohta (1976) was one of the first to quantify unequal crossing over on the basis of population genetics considerations. Kimura and Ohta (**113**) analyzed the correlation between two members of a multigene family subjected to intrachromosomal unequal crossing over and ordinary interchromosomal crossing over. The spread of a new mutant in the family is often called concerted evolution but the extent of homogenization may depend on the distance between members. This homogenization process is stochastic and under some simplified assumptions it resembles the spread of a mutant gene in a randomly mating population (e.g., Ohta, 1980).

XVI. Nucleotide substitutions (**118, 120, 126**)

When data on nucleotide sequences began to accumulate at an accelerated pace and to be used for comparative studies of molecular evolution, it became clear that there are substitutional biases among four nucleotides. At present, there are a number of statistical models (usually based upon stationary Markov processes) which take such biases into account. This line of statistical study was initiated by Kimura (**118**), who distinguished transitional and transversional (type I and II) differences between two homologous sequences. The proposed model was applied to pseudogenes and to each codon position in relation to the neutral theory.

However, the model cannot account for unequal composition of nucleotides in DNA sequences. To explain this, different substitution rates among four nucleotides other than transitions and transversions are required, and hence Takahata and Kimura (**126**) made one such extension. Kimura (**120**) proposed two other substitution models; the three-substitution-type (3ST) and the two-frequency-class (see Lanave et al., 1984 for a more general model and Chapter 5 in Nei, 1987 for a review). The problem in using these elaborated models is that we must determine various unknown parameters and that some of the formulas are often inapplicable because of sampling errors. More seriously, the actual substitution process is sometimes much more complicated than what is ordinarily assumed by time-homogeneous Markov processes (Uzzell and Corbin, 1971; Holmquist et al., 1983). For instance, substitutions occur in such a way that different lineages evolved toward different base compositions (Li et al., 1985; Li and Tanimura, 1987; Easteal, 1991). In addition, it is very difficult to make an accurate correction for extensive multiple-hit substitutions. If such Markov models need to be reexamined, so does the maximum likelihood method of constructing phylogenetic trees (Felsenstein, 1981; Hasegawa and Kishino, 1989).

XVII. Molecular clock (**48, 58, 147**)

Kimura (**48**) regarded the molecular clock (rough constancy of molecular evolutionary rate) of Zuckerkandl and Pauling (1965) as evidence for the neutral theory (Kimura and Ohta **58**), and this raised a heated debate on the concept and its accuracy. It is wrong, however, to say that molecular clock is expected only under neutrality or that the neutral theory always leads to a molecular clock. The existence of the clock depends strictly on how new mutations, selected or unselected, arise. The usual subsidiary assumption in the neutral theory is that new mutations occur by a Poisson process so that the variance to mean ratio of the number of substitutions must be one (Ohta and Kimura 65; Kimura 132, **147**). If advantageous mutations occur under the same assumption, the Poisson clock also exists as far as a single lineage under a constant environment is concerned.

Kimura (**48**) could not finish this paper without predicting that living fossils would have accumulated amino acid changes at the same rate as rapidly evolving species. Living fossils are morphologically in *status quo* for long time so that they provide an excellent example of the bifurcation between molecular and phenotypic evolution.

To Kimura, the constancy of molecular evolutionary rate always concerns the uniformity over diverse lineages. He originally thought that the neutral theory can account for it only by assuming that mutation rates per year rather than per generation are uniform among lineages. However, he was not certain, and later, instead of asking why this is so (Wilson et al., 1977; Wu and Li, 1985; Britten, 1986 for the so-called generation time effect), Kimura and Ohta (**58**) argued that

> *the species with short generation time tends to have small body size and attain a large population number, while the species which takes many years for one generation tends to have a small population number.*

This was the first inkling of the negative correlation between the generation time and effective population size (N_e).

Once a slightly deleterious mutation arises in a population, the chance for it to contribute to molecular evolution is larger in a smaller population. The supposition on the relationship between N_e and generation time, on the other hand, implies a high per-year rate of slightly deleterious mutations in a large population, provided that the mutation rate is nearly constant *per generation*. It follows that the substitution rate of slightly deleterious mutations may become more or less constant *per year*. This is the argument in Ohta and Kimura (65) and subsequent refinements of the neutral random drift theory along this line were made most extensively by Ohta (1973, 1976, 1977). One may note, however, that there is little quantitative evidence for the assumption of the constant mutation rate per generation. Recently, Kimura has returned to his original explanation that the

mutation rate must be constant per year if the rate of molecular evolution is uniform among diverse lineages (Kimura 157, 158). He reasoned this on the basis that the per-generation constancy is based on traditional mutation studies on visible characters and viability polygenes, and that these may not be applied to neutral or near neutral mutations.

However, it is still not clear why neutral mutations are constant per year rather than per generation (Wilson et al., 1977; Gillespie, 1987). In a special issue of *Journal of Molecular Evolution*—Molecular Evolutionary Clock, Kimura (**147**) called for some experiments to settle this issue. According to the neutral theory, there are two sources responsible for irregular rates of molecular clock; the change in the mutation rate per year and changes in selective constraint (Takahata, 1987 for a quantitative discussion of these possibilities, but see Gillespie, 1984 for an alternative explanation). The largest deviation from the molecular clock was found in the hystricognath rodent insulin gene and was attributed to loss of selective constraint. Graur et al. (1991) suggested, however, that the guinea-pig diverged before the separation of the primates and the artiodactyls from the myomorph rodents (rats and mice) and this revised time scale supported the molecular clock.

XVIII. Neutral theory (**38, 40, 76, 84, 97, 99, 112, 129, 133, 143**)

Kimura's best known contribution is the neutral mutation-random drift theory of molecular evolution, for brevity called the neutral theory. It was first presented in 1968 (Kimura **38**). The theory was rejected uncritically by many evolutionists and accepted, equally uncritically, by a number of molecular biologists. Now, although not without controversy, it is a part of the standard body of information on molecular evolution.

The theory was independently proposed by King and Jukes (1969) under the provocative title, "Non-Darwinian evolution". Written in early 1968, it was at first rejected by *Science*, but later accepted after additional reviews, but not published until 1969. Although the formal publication priority belongs to Kimura, this is clearly an example of an idea being independently thought of by more than one person. Although King and Jukes share with Kimura the credit for proposing the basic idea, further developments have been almost entirely due to Kimura. The early history of the theory has been reviewed by Jukes (1991).

Although Kimura's theory was presented *de novo*, there were nevertheless earlier arguments for neutrality. Darwin himself suggested that some traits might be neutral. Biochemists noted that proteins often function equally well if some amino acids are changed; but one must realize that a change of a magnitude far too small to detect by physiological or chemical methods could still be selected over long time periods. Several people, following the discovery of considerable isozyme polymorphism by Lewontin and Hubby (1966), mentioned the possibility of neutral polymorphisms, but without much conviction and without developing

the idea.

The closest approach to the neutral theory in the pre-Kimura days was by Sueoka (1962) and Freese (1962). Both were concerned that DNA differences among different species of bacteria differed greatly, whereas the amino acid differences much less. Both suggested mutation pressure was responsible for the different base ratios, and that many amino acids were interchangeable. Remarkably, these papers were written before the degeneracy of the code was established. These authors clearly had the germ of the neutral idea, but neither paper had much impact on the biological community. Probably this was because most evolutionists paid little attention to bacteria, and neither author developed the idea further.

It was not until Kimura's forceful advocacy and his presentation of one argument after another in successive papers that the theory made its major impact on evolutionary thinking.

Stimulated by Wright's (1966) work and the finding of degeneracy in genetic codes, Kimura (**40**) presented the full treatment of the so-called K allele model for neutral mutations, the infinite allele model being the limit of $K=\infty$. From the complete solution of the transient distribution of gene frequency expressed by a series of hypergeometric functions, he deduced the stationary distribution, the probability of temporal loss and fixation, and the effective and actual number of alleles. For $K=\infty$, the distribution is called the expected number of alleles in a given gene frequency class or the frequency spectrum (Ewens, 1979). The discussion and Appendix I of Kimura's paper argued that neutral or nearly neutral mutations at the DNA level must be more common than previously thought. As a whole, this paper contains a more thorough argument for the neutral theory than his 1968 *Nature* paper.

In those days, Kimura used to check the validity of theoretical formulas by computer simulation. The detailed description of simulation methods in this paper may still be very instructive to students. Nowadays there are those who rely on a computer exclusively. We have to be careful about such results, because of many unexpected errors in programming, too few runs, etc. Even conventional numerical methods for solving a continuum model can create spurious solutions and may lead to nonsense on unconventional problems (Stewart, 1992). The cross-checking of simulation with theoretical results is always preferable, as Kimura did.

If the amino acid substitution rate estimated from protein sequences is extrapolated to the rate in the whole genome, the rate becomes unacceptably high, which is in sharp contrast to the horotelic evolution of Haldane (1957). Muller's (1958) estimate (4×10^9) of the total number of nucleotides per genome was particularly important. He measured the DNA content of human sperm and divided it by the average weight of a nucleotide. Kimura (**38**) came to the conclusion that most mutations produced by nucleotide replacements are almost neutral in natural

selection (see Crow, 1972) and discussed the extent of polymorphism then newly revealed by electrophoresis (Harris, 1966; Hubby and Lewontin, 1966; Lewontin, 1991). The conclusion of this landmark paper is epitomized by Kimura's remark:

> *To emphasize the founder principle but deny the importance of random genetic drift ..., is, ... rather similar to assuming a great flood to explain the formation of deep valleys but rejecting a gradual but long lasting process of erosion by water as insufficient to produce such a result.*

Two figures in Kimura and Ohta (**76**) illustrated some important consequences of the neutral theory. Particularly interesting is the qualitative description about the ancestral relationships of alleles and the number of mutations by which they differ from each other. When Wright read this manuscript, he pointed out that the contrast between the neutral and positive Darwinian theories becomes much sharper in interpreting rapidly evolving molecules: under neutrality, the upper limit of evolutionary rate is set by mutation rate while under positive Darwinian selection there is no such limit. The amino acid sequence analysis of α and β hemoglobins makes clear the points of the paper.

The major features of molecular evolution were enumerated as five principles in Kimura and Ohta (**84**). Three of them are: (1) the constant rate and its usefulness in reconstructing phylogenetic trees, (2) the important role of negative selection or functional constraints (without which the neutral theory cannot account for different evolutionary rates of different molecules or different parts of a molecule), and therefore (3) the conservative nature, in contrast to opportunistic ways of phenotypic or adaptive evolution, and faster rates for substitutions that are less disruptive to the established structures and functions of molecules [see Ohno (1970) and Chapter 5 in Kimura (132) for further discussion]. One example of these principles was soon demonstrated by Kimura (**99**), when mRNA sequence data began to accumulate. It was immediately clear that most changes occur at the third codon positions so that they are largely synonymous. Although such sequence data for evolutionary studies were very limited at that time, he quickly noticed the high synonymous rate even in the most conserved histone gene (Grunstein et al., 1976) which argued for the neutral theory. Kimura (**97**) eloquently reviewed the then status of the neutral theory. It was the dawn of a flood of DNA sequence information. A decade later, he presented to the Royal Society a review paper entitled *DNA and the neutral theory* (Kimura **143**).

Some arguments often raised against the original neutral theory are the constancy of molecular evolutionary rate per year, rather low extents of heterozygosity and of its variance, and an excess of rare variants (Nei, 1975). To overcome these problems, Kimura (**112**) proposed a model of effectively neutral mutations. This model is a modification of Ohta's (1973, 1976) slightly deleterious model, but allows greater possibility of effectively neutral mutations even in a

large population. Both models predict lower levels of heterozygosity and specific relationships between substitution rates and population sizes. Kimura and Takahata (**129**) showed by a new simulation method that under the effectively neutral model the variance of heterozygosity can be smaller than that expected from the original neutral theory (Nei et al., 1976; Fuerst et al., 1977).

A difficulty in the effectively neutral as well as slightly deleterious model is, however, that lowering heterozygosity tends to slow down or even stop the substitution process (Nei, 1975). To account for the constant rate per year, it is also necessary to assume specific relationships between generation time and population size which are, however, necessarily indirect and crude. Kimura reviewed the model in *Scientific American* (Kimura 114), *Molecular Evolution, Protein Polymorphism and the Neutral Theory* which he edited (Kimura 128), and his book *The Neutral Theory of Molecular Evolution* (132). He also briefly mentioned it in Chapter 11 in *Evolution of Genes and Proteins* (Kimura 130 in Nei and Koehn, 1983). But he has never mentioned the effectively neutral model in any detail since 1986. He has returned to the original neutral theory. It seems that his change was based on DNA sequence data which could be better interpreted by two distinct classes of mutations; completely neutral or definitely deleterious. We may also imagine that Kimura recognizes the role of a scientific model in the way that Russell (*Sceptical* Essays see pp. 64–65 in Medawar, 1979), Popper (1975), Wimsatt (1987) and others do (Kimura **38**, 157, 158). As pointed out by Crow (1985), the (original) neutral theory has, in fact, several attributes to be a good scientific theory.

If protein polymorphism is maintained largely by neutral mutation and random extinction of existing alleles, one may wish to know the proportion of neutral mutation rate to the total. Using rare variant as well as polymorphic alleles simultaneously, Kimura (**133**) estimated the average proportion to be 14% (cf. Nei, 1977). The proportion varies from species to species (humans, fruit flies, Japanese monkey, plaice) or between substrate-specific and nonspecific enzymes, but the 14% neutral mutation is in good agreement with an estimate from globin DNA sequence data. Kimura's estimator was shown to be biased and affected much by deleterious alleles and changes in population size (Watterson, 1987; Ewens and Li, 1980). Nevertheless, it was a pivotal question to ask what fraction of the total mutation is neutral.

To answer such a question, DNA sequence rather than allele frequency data provide a more direct means. Kimura (**143**) reviewed several findings at the DNA level in relation to the neutral theory, and stressed the value as a scientific hypothesis. The neutral theory makes various testable predictions and therefore enables us to examine observations in quantitative terms. These include (1) high substitution rates at the synonymous sites in the protein coding regions and at all sites in introns as well as pseudogenes, (2) rough constancy of synonymous rates in various genes, (3) a preponderance of synonymous substitutions over amino acid

replacements even in rapidly evolving RNA viruses, and (4) unequal usage of synonymous codons (e.g., Ikemura, 1985). Kimura (**143**) has become more and more confident of the neutral theory in its simplest form;

> *A certain fraction (presumably a large fraction) of nucleotide sites produce no phenotypic effects at all, and therefore are completely neutral while a certain fraction (presumably a very small fraction) of nucleotide or amino acid substitutions are definitely advantageous for the species in adapting to new environments, and therefore they are subjected to straightforward positive natural selection.*

In this view, there is little or no room for slightly deleterious mutations as being important in molecular evolution.

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