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Development of Neutral and Nearly Neutral Theories

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A short history of the major features of neutral theories of molecular evolution is presented. Emphasis is placed on the nearly neutral theory, as this version of the neutral theory has explained the widest range of phenomena. The shift of interest from protein to DNA evolution is chronicled, leading to the modern view that silent and replacement substitutions are responding to different evolutionary forces. However, the exact nature and magnitude of these forces remains controversial, as all current theoretical models suffer either from assumptions that are not quite realistic or from an inability to account readily for all phenomena. Although the gathering of sequence data has been the main effort of contemporary population genetics, further exploration of theoretical models of molecular evolution would provide a more coherent framework for data analysis.

In this paper, we provide a short history of the development of neutral theories of molecular evolution, with emphasis on the early contributions of Motoo Kimura and on the subsequent development of the nearly neutral model, which is the version of the neutral theory that has been able to explain the widest range of phenomena.

THE 1960S

The hypothesis that most molecular polymorphism and substitutions are due to neutral mutations and genetic drift—the neutral theory—was first proposed in the 1960s. Although there were previous suggestions that much of molecular evolution is neutral (Sueoka, 1962; Freese, 1962), Kimura first

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combined population genetics theory with molecular evolution data to arrive at a theory using genetic drift as the main force changing allele frequencies. In 1965, an influential paper by Zuckerkandl and Pauling (1965) appeared which showed, among other things, that hemoglobins are evolving at a steady rate of 1.4×10^{-7} amino acid substitutions per year. Kimura (1968a) realized the general significance of this finding and used the rate to estimate that, in the mammal genome, "one nucleotide pair has been substituted in the population roughly every 2 yr." He claimed that if these substitutions were caused by natural selection, the rate of substitution was too large to be compatible with Haldane's (1957) upper limit based on the cost of natural selection. Therefore, he proposed that many of the substituted alleles must be neutral. This proposition has provoked much controversy among population geneticists and evolutionists.

Although Kimura's original argument for the neutral theory depended on the concept of the cost of natural selection or the substitutional load (Kimura, 1960), subsequent discussions of the neutral theory became almost independent of genetic load considerations. In 1969, Kimura published a paper on the rate of molecular evolution, in which he argued that the rate of amino acid substitutions of homologous proteins is almost constant (Kimura, 1969). He further argued that protein evolution is uncorrelated with phenotypic evolution. Consequently, proteins in living fossils may be expected to have experienced as many amino acid substitutions as corresponding proteins in more rapidly evolving species. The apparent constancy of the amino acid substitution rate, the molecular clock, was thought to support the neutral theory.

In 1969, King and Jukes published "Non-Darwinian Evolution" (King and Jukes, 1969), a paper that independently proposed that most amino acid substitutions are neutral. The title was somewhat misleading and irritated many evolutionary biologists. As this work was a collaboration between a biochemist (Jukes) and a population geneticist (King), it relied more on biochemical arguments than did Kimura's work. In particular, this was the first paper to note the inverse relationship between the importance of a protein or site within a protein and its rate of evolution. The authors suggested that "... proteins, and sites within proteins, differ with regard to the stringency of their requirements." This would later be developed into a Principle of Molecular Evolution that provided support for the neutral theory (Kimura and Ohta, 1974).

Another notable achievement in the 1960s was the infinite-allele model as formulated by Kimura and Crow (1964). One of their results, the expected heterozygosity under the equilibrium between mutation and random drift,

$$\frac{4Nu}{1+4Nu}, \tag{1}$$

where u is the mutation rate and N is the effective population size, has been widely used for interpreting observed polymorphisms at the protein level. Kimura and Crow, along with most population geneticists of that time, were unaware that Malécot (1948) had already derived Eq. (1). Malécot's contributions became known with the translation of his book in 1969. Interestingly, more than two-thirds of the Kimura–Crow paper was devoted to the symmetric overdominance model, even though this paper has mostly been cited for its neutral allele results. In the same year as the original proposal of the neutral theory, Kimura (1968b) expanded the Crow–Kimura analysis to the K-allele neutral model and obtained the equilibrium probability distribution of allele frequencies corresponding to Eq. (1).

The Ewens sampling distribution (Ewens 1972), has been used to develop tests of agreement between the neutral theory and observed polymorphisms. A mutation whose selection coefficient, s, is much less than the reciprocal of the population size, $s \ll 1/N$, behaves as if it were strictly neutral, $s = 0$. Such mutations were called *nearly neutral* during the 1960s. Later, nearly neutral mutations were defined as ones whose selection coefficients are close to the reciprocal of the population size, $s \approx 1/N$. This evolution of nomenclature must be kept in mind to appreciate fully the change in emphasis in the neutral theory that occurred during the 1970s.

The 1970s

As more protein sequence data became available, it became clear that the rate of protein evolution differs greatly between proteins. Each protein has its own characteristic rate of amino acid substitution, ranging from the fastest, fibrinopeptide at 9×10^{-9} substitutions per site per year, to the slowest, histone IV at 10^{-11} (Dickerson, 1971). The pattern of rate variation echoed the observation of King and Jukes so well that Kimura and Ohta (1974) felt that it should become one of their Principles of Molecular Evolution: "functionally less important molecules or parts of a molecule evolve faster than more important ones." Important parts of proteins were said to be *selectively constrained* because they could not be changed without a fairly severe and detrimental impact on fitness. At this stage, those mutations that fix in populations were thought to be neutral, $s \ll 1/N$, while mutations in constrained regions were rejected by natural selection because of their very deleterious effects, $s \gg 1/N$. Advantageous mutations were thought to be so rare as to make only a negligible contribution to the totality of substitutions.

What about borderline mutations? Do deleterious mutations with $s \approx 1/N$ play an important role in molecular evolution or will they be eliminated like those of larger effect? In the early 1970s Ohta and Kimura

(1971) and Ohta (1972, 1973, 1974) considered this problem and proposed that slightly deleterious borderline mutations might be quite common among amino acid substitutions. If such borderline mutations or nearly neutral mutations constitute a substantial fraction of new mutations, theoretical predictions on the rate and pattern of evolution and polymorphism become different from the neutral prediction. The most notable difference is that there will be a negative correlation between the evolutionary rate and the species population size. For neutral mutations, the evolutionary rate is independent of the population size.

At this time, there were some curious facts about molecular evolution and polymorphisms that appeared to be contrary to predictions of the neutral theory. One of these concerned the generation-time effect. The natural unit of time for the neutral theory is a single generation. As a consequence, creatures with shorter generation times should evolve faster in real (clock) time than those with longer generation times. Proteins do not exhibit a strong generation-time effect, while non-coding DNA does (Kohne, 1970; Laird *et al*., 1969). Clearly, both classes of substitutions cannot be responding to evolutionary forces of the same kind or magnitude. However, if amino acid substitutions are deleterious borderline mutations and non-coding DNA substitutions are neutral, then the different generation-time effects may be explained without having to resort to other forms of natural selection. While it is obvious that non-coding DNA should exhibit a generation-time effect, the argument why protein evolution should not is subtle. Recall that the rate of substitution for borderline mutations is inversely proportional to the population size. In general, large animals tend to have large generation times and small sizes, while small animals have the opposite. Hence, the generation-time effect will be partially canceled by the population-size effect for borderline mutations.

Another curious fact was the upper bound on protein heterozygosities as determined by electrophoresis (Lewontin, 1974). Under the neutral theory, the heterozygosity is expected to increase with the species population size according to Eq. (1). By using the estimate of the neutral mutation rate as determined from molecular evolution studies, it was possible to estimate the effective sizes of populations that would be required for the neutral theory to be true. These estimates tended to fall between about 10^4 and 10^5 (Kimura and Ohta, 1971). Lewontin (1974) was so impressed by the narrowness of this range that he wrote: "... we are required to believe that higher organisms including man, mouse, Drosophila and the horseshoe crab all have population sizes within a factor of 4 of each other. ... The patent absurdity of such a proposition is strong evidence against the neutralist explanation of observed heterozygosity."

Ayala *et al.* (1972), in their discussion of polymorphism within the *Drosophila willistoni* group, argued that the effective size of *Drosophila*

species must be much larger than predicted by the neutral theory. Their subjective feeling was that $N_e > 10^9$ must hold for *D. willistoni* and thus that the average heterozygosity is much lower than the neutral expectation.

The upper limit may be accounted for by the nearly neutral theory because the deleterious effects of some electromorphs prevents their increase (Ohta, 1974). Additional support for this view comes from the excess of rare alleles over the neutral expectation sometimes seen in *Drosophila* species (Ohta, 1974). There is little power in such tests, so it is not surprising that the statistical methods developed by Ewens (1972) and by Watterson (1978) could not reject the neutral model for many cases that they examined.

An alternative explanation for low heterozygosities is hitchhiking of neutral loci on portions of the chromosome experiencing direction selection (Maynard Smith and Haigh, 1974; Aquadro, 1992). Interest in hitchhiking has revived recently due to the reduction in silent variation seen in regions of low recombination of *Drosophila* chromosomes (Langley, 1990). Similarly, population size fluctuations may lower average heterozygosities either due to a lowering of the effective population size by relatively rapid fluctuations in size or due to severe bottlenecks, whose effects may last for hundreds of thousands of years (Nei *et al.*, 1975). Both factors may keep populations out of equilibrium in such a way as to make rare alleles appear more frequent than expected under the equilibrium neutral model.

With the advent of DNA sequencing techniques in the late 1970s, comparative studies began their steady shift from amino acid to DNA sequences. Kimura (1977) and Jukes (1978) used the new DNA sequence data to show that synonymous substitutions within coding regions are more rapid than amino acid altering (nonsynonymous) substitutions. This was yet another case of the Principle of Molecular Evolution that less important regions of a sequence evolve faster than more important parts. As such, it was viewed as supportive of the neutral theory.

The 1980s

In this period, DNA sequencing data accumulated rapidly. As a result, there was a flood of comparative studies of DNA sequences. Pseudogenes were shown to evolve rapidly, thus providing further support for the neutral theory (Li *et al.*, 1981; Miyata and Yasunaga, 1981). In the early 1980s, the original neutral theory was persuasive. Substitutions in noncoding DNA and silent substitutions in coding regions are neutral ($s \ll 1/N$), amino acid substitutions are deleterious or nearly neutral ($s \approx 1/N$), and advantageous substitutions make up a minor fraction of all substitutions.

A challenging topic in the 1980s was the problem of silent substitutions. Codon bias was thought to be inconsistent with the neutral allele theory. As a result of Ikemura's (1981) demonstration that codon bias was correlated with transfer RNA abundances in cells, Kimura (1981) proposed an optimum model. In this model, mutants are nearly neutral and their substitutions are due to genetic drift. The rate of substitution is slightly reduced compared to that of completely neutral mutants, but codon usage may be highly biased at equilibrium (Kimura, 1981; Li, 1987, Bulmer, 1991).

A large amount of additional data on codon usage bias have been collected, which showed that there are species-specific patterns (Grantham *et al*., 1980, 1981). In addition, it became apparent that codon usage bias is particularly prominent for highly expressed genes, presumably because of increased selection for efficient translation (Ikemura, 1981; Grantham *et al*., 1981; Sharp and Li, 1987). In most of these discussions, little thought was given to comparisons of silent and replacement substitutions, so they provided no real support for the suggestion that most amino acid substitutions are deleterious.

Studies on DNA polymorphisms, however, did provide evidence that the simple neutral theory is not applicable to all data. The observed heterozygosity at the DNA level—non-coding or silent—was found to differ between species even if the heterozygosity at the protein level was almost the same (Aquadro *et al*., 1988). A simple explanation is that the variation in silent heterozygosity is due to population size differences. This explanation would be compelling if it could be shown that those species with less silent variation had, on average, higher rates of amino acid substitutions. To our knowledge, the data needed for this test are not, available.

Comparative studies of DNA sequences have provided new opportunities for the reconstruction of molecular phylogenies. As DNA sequences accumulate evolutionary changes more steadily than morphological characters, phylogenetic relationships may be more easily estimated. Studies in molecular phylogeny increased markedly during the 1980s. The neutral model was thought to offer a theoretical basis for the relatively steady evolution of DNA sequences (Nei, 1987; Li and Graur, 1991). Notable progress may be found in the estimation of very ancient relationships such as the divergence of phyla and kingdoms (Woese, 1991), and of quite recent ones such as the branching order of the great apes (Wilson, 1985).

This period also saw a resurgence of arguments against the neutral theory. The strongest evidence against the simple neutral theory for protein evolution came from taking a closer look at the variability in rates of amino acid substitutions. It had been known since the early 1970s that the

ratio of the variance to the mean number of substitutions on a lineage, $R(t)$, was about 2.5, significantly greater than the value of 1 predicted by the simple neutral theory (Ohta and Kimura, 1971; Langley and Fitch, 1974; Gillespie and Langley, 1979). This excess was thought to be biologically unimportant. However, on careful examination of the statistics underlying the estimation of $R(t)$, it became apparent that even a slight increase of the estimated value of $R(t)$ could indicate that molecular evolution is an erratic process, may even be episodic, with bursts of substitutions separating periods of quiescence (Gillespie, 1984a). Later work with more loci showed that the average value of R for proteins is much higher, $R(t) \approx 7$ (Gillespie, 1989). By contrast, the value of $R(t)$ for silent substitutions is closer to 1. The higher rate of substitution at silent sites requires a greater reliance on corrections for multiple substitutions, which tend to bias the estimate of $R(t)$ upward (Bulmer, 1989). Consequently, it cannot be said with confidence that $R(t)$ is greater than 1 for silent substitutions.

Both the simple neutral theory and the nearly neutral theory were incompatible with large values of $R(t)$. Takahata (1987) proposed a variant of the simple neutral model, the *fluctuating neutral space model*, which assumes that the neutral mutation rate changes with each neutral substitution. His model can easily account for the observed values of $R(t)$, although it does exhibit an awkward sensitivity to one of its parameters. The nearly neutral model could account for the high values of $R(t)$ if it is assumed that population sizes fluctuate through time. However, the time scale of these fluctuations must be commensurate with that of molecular evolution. Thus, the population size would have to be large for millions of generations, then small for millions of generations, and so forth, a demographic pattern that appears to be quite implausible (Takahata, 1988; Gillespie, 1988).

A simple test of the neutral theory was made possible as DNA sequence data became available for the same locus sampled from different individuals from the same species and one individual from a closely related species. If the theory is correct, then regions of the gene that are more polymorphic because of a higher mutation rate should also exhibit more fixations across the two species. Hudson *et al.* (1987) devised a test based on this idea and applied it to the alcohol dehydrogenase (ADH) locus in *Drosophila melanogaster*, which was shown to be incompatible with the simple neutral model. However, as this was only one locus, and as the ADH locus was known for other reasons to be a likely target for strong selection, this one result had little impact on the general applicability of the neutral model.

As the nearly neutral theory gained momentum, it also attracted careful examination of its assumptions (Gillespie, 1987). The assumption that the vast majority of all borderline mutations are deleterious seemed particularly

non biological, even though it is known that most mutations of measurable effect are deleterious. Fisher (1958) argued, for example, that as selection coefficients approach zero, the fraction of mutations that are deleterious should approach one-half. The assumption that selection coefficients are constant for millions of generations was also questioned. The fitness of a genotype is a measure of its success in the natural environment, which is always changing. That selection coefficients should be both very small ($s \approx 1/N$) and constant was considered a severe assumption. If it is weakened, the dynamics of deleterious mutations change dramatically.

By the close of the 1980s, the nearly neutral theory was both stronger, because it could account for most of the escalating observations of molecular evolution and polymorphism, and weaker, because of the problem with $R(t)$ and some questionable assumptions. These threads have continued into the 1990s.

The 1990s

The rapid accumulation of DNA sequence data has continued in the 1990s. Using these data, more progress has been made in contrasting the dynamics of synonymous and nonsynonymous substitutions. In general, nonsynonymous substitutions are thought to be more heavily influenced by selection than are synonymous substitutions. Therefore, the generation-time effect should be more emphatic for synonymous than nonsynonymous substitutions, as the former more faithfully reflects the mutation rate. DNA sequence analysis has verified this prediction (Li *et al.*, 1987; Ohta, 1993, 1995). The absence of the generation-time effect in nonsynonymous substitutions may be due to the cancellation of the generation-time effect by the population-size effect if most substitutions are deleterious and the function of the protein has been conserved for a long time. On the other hand, an acceleration of nonsynonymous substitutions is often observed in genes that acquire a new function (Ohta, 1994).

Theoretical modelling of near neutrality has come to a turning point in the 1990s. Previous studies were based on the shift model, under which selection coefficients are chosen at random from some probability distribution, and the mean fitness of the population shifts back when a mutation fixes. This is done, in part, to meet the assumption that all mutations are deleterious.

The shift model was replaced with the fixed model, in which the distribution of selection coefficients is assumed to be fixed, independent of the fitness of the parent allele and the mean fitness of the population. The population fitness fluctuates as a result of mutant fixations rather than shifting as with the previous models (Ohta and Tachida, 1990). The fixed model

was originally called the house-of-cards model (Kingman, 1978). Under the fixed model, such average patterns of mutant substitutions as the population-size effect are not very different from that under the shift model (Ohta and Tachida, 1990; Tachida, 1991). However, the substitution rate fluctuates in the fixed model because the effects of each substitution remain to affect subsequent substitutions by changing the mean fitness of the population. In other words, substitutions are interrelated. As a consequence, the variance in the rate becomes larger than the simple Poisson model predicts, and the substitution process becomes episodic (Gillespie, 1994a; Iwasa, 1993).

Of the substitutions that actually fix under the fixed model, one-half are deleterious and one-half are advantageous (Gillespie, 1994a). Thus, the nearly neutral model is no longer a model with only deleterious mutant substitutions, but rather one with a mixture of substitutions of positive and negative effects. The explanation for the lower rate of substitution of proteins takes on a slightly different form. Rather than being due to the difficulty of fixing deleterious alleles, it is because of the lower mutation rate to nearly neutral alleles that occurs as the population evolves to the tail of the distribution of selection coefficients. Thus, the principle of molecular evolution, that is more important regions evolve more slowly, remains one that is readily explained by the nearly neutral model, even though "nearly neutral" no longer implies deleterious, as it did in the past.

A significant problem with the fixed model for molecular evolution is the sensitivity of the rate of substitution to the parameter $\alpha = 2N\sigma_s$. If $\alpha < 0.2$, the model is indistinguishable from the simple neutral model. If $\alpha > 4$, substitutions cease to occur (Tachida, 1991; Gillespie, 1994b). Why should nature conspire to have the value of α fall within such a narrow window for most creatures? One possibility is that selection coefficients vary spatially and that the law of large numbers makes the average σ_s closer to zero for species spread over wider geographic areas (Ohta and Tachida, 1990). This could lead to a negative correlation between σ_s and N. Another possibility is that hitchhiking may keep the effective size at nearly neutral loci fairly constant because the flux of selected substitutions in larger populations will be larger. Such considerations may explain why there is less variation between species in α than might be expected, but do not help in setting the magnitude of α. Clearly, more thought must be given to this very difficult but important issue.

As the fixed model is better able to account for high values of $R(t)$ than the shift models, a study to reexamine the estimates of $R(t)$ was undertaken for a large number of mammalian genes (Ohta, 1995). It was found that both a systematic bias in the rate among lineages and the episodic type of rate variation contribute to the inflation of $R(t)$, but that the former is more significant for synonymous substitutions and the latter for

nonsynonymous substitutions. This result was obtained by examining the regression of $R(t)$ on the number of substitutions per gene. After lineage effects were removed, the regression coefficient for nonsynonymous substitutions was not significant, in agreement with an episodic pattern of substitutions but not with Takahata's fluctuating neutral space model. By contrast, the regression was significantly positive for synonymous substitutions. One reason for this could be that each lineage has a slightly different rate of substitution, which Ohta called a systematic bias. Part of the regression could also be due to the bias in the estimation of $R(t)$ that becomes more pronounced as the level of saturation increases (Bulmer, 1989). Further work on less saturated sequences might show which factor is more important.

In the 1990s, sequence data from samples within populations have also been accumulating rapidly and have generally been found to fit the neutral patterns when examined with tests such as that developed by Tajima (1989). This agreement may well reflect the lack of power of these tests. Departure from the neutral prediction can be detected more easily once again by separate examination of synonymous and nonsynonymous polymorphisms. Starting from the work of McDonald and Kreitman (1991), many reports on synonymous and nonsynonymous polymorphisms have been published. The test compares the relative numbers of synonymous and nonsynonymous differences within a species with those numbers between closely related species. In some cases, the pattern is in accord with the neutral theory. In other cases, departure from the neutral expectation was statistically significant. Some examples of excess nonsynonymous substitutions between species compared those within species are:

- Alcohol dehydrogenase of *D. melanogaster* and *Drosophila simulans* (McDonald and Kreitman, 1991).
- Glucose-6-phosphate dehydrogenase of the same two species (Eanes *et al.*, 1993).
- The chimeric gene *jingwei* of *Drosophila yakuba* and *Drosophila teissieri* (Long and Langley, 1993).

Some examples in which the deviation is the opposite, that is, an excess of intra-specific nonsynonymous polymorphisms, are:

- Mitochondrial NADH dehydrogenase subunit 3 of *Mus musculus* and *Mus spretus* (Nachman *et al.*, 1994).
- Mitochondrial cytochrome b of *D. melanogaster* and *D. simulans* (Ballard and Kreitman, 1994).

A difficulty with these tests is the fact that the synonymous variation is not always neutral due to selection for codon bias, and hence the pattern

of substitutions is not a good standard for comparison with nonsynonymous substitutions. Nevertheless, it is true that synonymous changes accumulate steadily and the nonsynonymous substitutions are more erratic (Gillespie, 1989; Ohta, 1995). The ratio of nonsynonymous to synonymous substitution numbers may fluctuate according to the shifts of interactive systems at the molecular level, as will be discussed later.

Several theories of molecular evolution where natural selection rather than genetic drift is the main force, causing fixations, have been pursued during the 1990s. One of these is called the mutational landscape model (Gillespie, 1984b, 1991), which was proposed to explain the episodic nature of amino acid substitutions. The model pictures molecular evolution as generally stagnated at a local optimum due to the very low mutation rates to sequences that are more than one mutational step away from the locally optimal sequence. An environmental shift is needed to move the population off of the local peak and into a burst of substitutions until it stagnates once again at another local maximum. As environmental changes set the pace of evolution rather than the mutation rate, this model does not exhibit a generation-time effect. Kauffman (1993) called his generalization of the mutational landscape model the *NK* model. N is the number of amino acids in the protein. Each amino acid makes a fitness contribution which depends upon that amino acid and upon K other amino acids. In other words, this is a model of epistatic interaction among $K+1$ amino acids. According to Kauffman, the fitness landscape is very rugged for $K \geqslant 2$. When $N = K$ and both are large, we have the mutational landscape model. If the height of the peaks is much greater than the reciprocal of the population size, then environmental fluctuations are required for continuing evolution. If they are similar to $1/N$, then there will be nearly neutral evolution. This picture may be a modern view of the shifting balance theory (Wright, 1931).

Two other models that depend on fluctuating environments to drive protein evolution have been proposed. The TIM model (Takahata *et al.*, 1975) is similar to the nearly neutral model except that the fitnesses of genotypes change slowly through time. If the rate of change of the environment is relatively fast, the model is mutation limited, exhibits a generation-time effect, and $R(t) < 1$. If the environment changes very slowly, the generation-time effect disappears and $R > 1$ (Gillespie, 1993). The SAS–CFF model is similar to the TIM model except that it has an additional parameter, $B > 1$, that reflects a balancing component to selection which appears with rapid changes in the environment. The SAS–CFF model has been developed to explain patterns of protein evolution mainly when selection is strong and mutation is weak (Gillespie, 1991). In this case, long-term environmental fluctuations are required to elevate $R(t)$, just as for the TIM and mutational landscape models. In fact, one conclusion

from a simulation study of many different population genetics models is that high values of $R(t)$ are difficult to explain without environmental fluctuations that occur on very long time scales (Gillespie, 1994a).

While models based on fluctuating environments can explain the absence of a generation-time effect and large $R(t)$ in protein evolution, they are conspicuously poor at providing explanations for other aspects of protein evolution. They cannot explain why the rate of amino acid substitution per site is so similar to the mutation rate for many proteins. They cannot explain why most proteins evolve slower than pseudogenes. They cannot explain why there is not even more variation in rates of substitution. For example, why have we not found some long lineages on which hemoglobin evolution has stopped? The neutral and nearly neutral theories provide a much better framework for explaining these phenomena.

As this short history demonstrates, population genetics has made remarkable strides in understanding both the phenomenology and the theoretical models of molecular evolution. However, it also demonstrates that we have yet to find a mechanistic theory of molecular evolution that can readily account for all of the phenomenology. Thus, while the 1990s will most likely be a decade dominated by the gathering of data, we would like to call attention to a looming crisis as theoretical investigations lag behind the phenomenology. Perhaps 1990s can also be a decade of joint exploration of all viable models of molecular evolution.

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