The Origins of the Neutral Theory of Molecular Evolution

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INTRODUCTION

Molecular biology has had a profound impact on the nature of the biological sciences in the twentieth century. Molecular techniques are found now in virtually every area of biology. To reduce the impact of molecular biology to the dissemination of its technologies, however, is to sell it short: it has also dramatically altered researchers' attitudes toward the question which problems or problem areas are of fundamental importance. This shift in attitude is nowhere more evident than in hybrid fields such as molecular evolution.

When the first major conferences on molecular evolution were held in 1964,[1] the vast majority of evolutionary biologists saw the world through the lens of panselectionism – natural selection was accepted as the dominant and most important mechanism of biological evolution.[2] This panselectionism was a product of the evolutionary synthesis in the 1930s and 1940s and the related effort to demonstrate the central role of evolution within the biological sciences. Architects of the evolutionary synthesis, such as George G. Simpson and Ernst Mayr, attended these early conferences on molecular evolution and actively promoted the power of natural selection.[3] For Mayr and Simpson, molecular biology was threat-

1. I am referring to the Conference on Evolving Genes and Proteins held at the Institute of Microbiology at Rutgers on September 17–18, 1964, and the Colloquium on the Evolution of Blood Proteins in Bruges, Belgium, during the summer of 1964.

2. Stephen Jay Gould, "The Hardening of the Modern Synthesis," in *Dimensions of Darwinism*, ed. Marjorie Grene (Cambridge: Cambridge University Press, 1983), p. 75.

3. George G. Simpson's comments from the 1964 conference in Bruges are published as "Organisms and Molecules in Evolution," *Science, 146* (1964), 1535–1538. Ernst Mayr's comments from the 1964 conference at Rutgers are published in the discussion sections of *Evolving Genes and Proteins*, ed. Vernon Bryson and Henry J. Vogel (New York Academic Press, 1965).

Journal of the History of Biology, vol. 27, no. 1 (Spring 1994), pp. 21–59.

ening to drive a wedge between the organismic and molecular levels that endangered both the unifying power of evolutionary biology and the autonomy of biology from the physical sciences.[4]

Despite their best efforts to ensure that molecular evolution developed in agreement with the traditional viewpoint of organismal evolution, Simpson and Mayr could not enforce panselectionism at the molecular level. In 1968 Motoo Kimura, and later Jack King and Thomas Jukes, proposed what would become known as the neutral theory of molecular evolution – a radical hypothesis that directly challenged the importance of natural selection in molecular evolution.[5] The basis of its challenge was Kimura's proposal that most changes detected at the molecular level were not acted upon by natural selection; they were neutral, and the mechanism of their change was random genetic drift.[6] Although others before him had argued for the presence of neutral variations at the molecular level, none would oppose panselectionism as ardently as Motoo Kimura.[7]

Because the neutral theory claimed that random drift was more significant than natural selection in molecular evolution, it helped drive a wedge between the way evolution was discussed at the organismal and molecular levels.[8] In doing so, it provided a theoretical foundation for the development of molecular evolution as a new field of biological inquiry. The development of the neutral theory, I claim, marks one of the most significant impacts

4. V. B. Smocovitis, "Unifying Biology: The Evolutionary Synthesis and Evolutionary Biology," *J. Hist. Biol., 25* (1992), 58–59.

5. Motoo Kimura, "Evolutionary Rate at the Molecular Level," *Nature, 217* (1968), 624–626; Jack L. King and Thomas Jukes, "Non-Darwinian Evolution," *Science, 164* (1969), 788–798.

6. Kimura, "Evolutionary Rate at the Molecular Level," 624–626. Random drift can be understood as the random fluctuations of allele frequencies from generation to generation; it occurs in all finite populations, but is more extensive in smaller populations. See Douglas Futuyma, *Evolutionary Biology*, 2nd ed. (Sunderland, Mass.: Sinauer Associates, 1986), p. 130.

7. Ernst Freese, "On the Evolution of the Base Composition of DNA," *J. Theoret. Biol., 3* (1962), 82–101; Noburo Sueoka, "On the Genetic Basis of Variation and Heterogeneity of DNA Base Composition," *Proc. Nat. Acad. Sci., 48* (1962), 166–169.

8. William Provine has argued that much of the initial misapprehension about the neutral theory was the result of a failure of many biologists to recognize the distinction between claims about the molecular level and those about the organismic or phenotypic level. Provine stresses the importance of understanding the neutral theory as a theory of molecular evolution. See William Provine, "The Neutral Theory of Molecular Evolution in Historical Perspective," in *Population Biology of Genes and Molecules*, ed. Naoyuki Takahata and James Crow (Tokyo: Baifukan, 1990), pp. 17–31.

of molecular biology on evolutionary biology. Obviously the veracity of my claim depends crucially on how the origins of the neutral theory are reconstructed.

To date the only published detailed history of the neutral theory is that written in 1974 by Richard Lewontin, a participant in the controversies that swirled around the theory's origins.[9] From Lewontin's perspective in *The Genetic Basis of Evolutionary Change*, the neutral theory is merely an extension of an earlier position in evolutionary genetics, the classical position. "Classical position"and "balance position" were the names given to two extremes regarding the nature of genetic variation and the kinds of selective forces acting upon that variation: adherents of the classical position were characterized by their advocacy of homozygosity and purifying selection, while adherents of the balance position were characterized by their advocacy of heterozygosity and balancing selection. Put another way, if you held the classical position you would expect that if you were to pick a typical individual from a sexually reproducing population and examine its genotype, you would find it to be homozygous at most of its loci; if you held the balance position, you would expect that a typical individual would be heterozygous at most of its loci.[10] If you held the classical position, you would expect that selection usually acted in a purifying fashion: it swept out deleterious or harmful alleles. If you held the balance position you would expect natural selection to maintain heterozygous combinations, which are superior to either of the alleles when found in homozygous pairs; this kind of selection is called "balancing selection," and the traits or alleles maintained by balancing selection are called "balanced polymorphisms."

Lewontin's claim is that the neutral theory of molecular evolution is actually a continuation of the classical position; so much so that it ought to be called the "neo-classical" theory.[11] A major factor in the transformation of the classical position into the neutralist position, according to Lewontin, was the outcome of a series of experiments he published with Jack Hubby in 1966, in which they found a higher-than-expected level of heterozygosity in a survey of 18 loci of the fruit fly *Drosophila pseudoobscura*. It appeared to Lewontin at the time that the dispute between the

9. Richard Lewontin, *The Genetic Basis of Evolutionary Change* (New York: Columbia University Press, 1974).

10. Ibid., p. 23. The locus of a gene refers to its normal position on the chromosome. Different forms of the same gene are called alleles. So any one of a number of alleles could be found at a given locus.

11. Ibid., p. 198.

classical and balance positions had been resolved by this evidence in favor of the balance position.[12]

It soon became evident, however, that the controversy was not resolved; instead, according to Lewontin, it was transformed. The neutral theory took the place of the classical theory and brought it up-to-date.[13] It did this by proposing a large number of neutral or nearly neutral mutants in order to account for the high levels of heterozygosity found in Lewontin and Hubby's surveys of proteins: natural selection still operates, but the neutralists are held to assert that "it is almost always purifying."[14] The result is that most selected loci are considered to be homozygous, while heterozygous loci are explained not in terms of balancing selection, but in terms of neutral polymorphisms.

Based on his account of the origins of the neutral theory, Lewontin argues that the terms "neutral mutation theory" and "neutralists obscure both the logic of the position and the historical continuity of this theory with the classical position."[15] Accordingly, he renames the neutral theory the "neo-classical theory." I will call this thesis about the historical continuity of the classical and neutral theories Lewontin's Historical Thesis.

Lewontin's 1974 account is an expert review of the controversies, but it is the account of a participant and reflects his particular involvement. As such, Lewontin's Historical Thesis frames the problem of the origins of the neutral theory in terms of the problematic facing evolutionary geneticists before the neutral theory – that is, in terms of problems concerning the nature of genetic variation and natural selection. I will argue that the origins of the neutral theory are better framed in terms of *both* the problematic of evolutionary genetics *and* the newly developing problematic of molecular evolution. The problems and findings of molecular evolution, I claim, are essential features of the neutral theory that cannot be traced to the classical/balance controversy.

Evaluating and expanding Lewontin's account is crucial if we are to grasp the impact of molecular biology on evolutionary biology in the case of the neutral theory. The more far-reaching account of the origins of the neutral theory presented below will demonstrate that the development of the theory helped formulate a set of concerns characteristic of the study of evolution at the molecular level, not the organismal level. Advocates of the neutral

12. Ibid., p. 113.
13. Ibid., p. 198.
14. Ibid.
15. Ibid., p. 197.

theory articulated a new problematic for population geneticists and evolutionary biologists – a problematic grounded in molecular evolution.

I will begin by reviewing the history of the classical/balance controversy and will then turn to the history of molecular evolution and the integration of different lines of research during the development of the neutral theory.

GENETIC LOADS AND THE CLASSICAL/ BALANCE CONTROVERSY

The classical and balance positions were not labeled until 1955, although versions of them were present much earlier. The roots of the classical position are evident in 1950 in two important extensions of a biological argument made by J. B. S. Haldane in 1937: one presented by James Crow, and the other by Herman J. Muller.

In 1937 J. B. S. Haldane published what may appear to be a paradoxical result. He was interested in the effect that deleterious (harmful) mutants had on the fitness of a population at equilibrium. What he found, surprisingly, was that the effect on fitness did not depend on the harmfulness of the mutant: it was strictly a matter of the mutation rate and the dominance of the mutant.[16] Very harmful and slightly harmful mutants will have roughly the same effect on average population fitness, because slightly deleterious mutants will persist longer and so affect a greater number of individuals than a strongly deleterious mutant, which is quickly eliminated. The determining factors in average fitness, then, are how often new mutants are produced, and how they express themselves in heterozygous combinations.

In 1950, James Crow used Haldane's results to try to adjudicate a long-standing debate concerning the causes of hybrid vigor, or "heterosis." The occasion for this effort was a well-attended conference on heterosis sponsored by Iowa State College (now Iowa State University).[17] Heterosis refers to the improved fitness of a hybrid relative to its parental strains. Two theories of heterosis prevailed in 1950: the overdominance hypothesis, and the dominance hypothesis.

16. J. B. S. Haldane, "The Effect of Variation on Fitness," *Amer. Nat., 71* (1937), 337–349.
17. The conference proceedings were not published until 1952, when they appeared as *Heterosis*, ed. J. Gowen (New York: Hafner, 1952). In fact, Crow's results had been presented earlier; see James F. Crows, "Alternative Hypotheses of Hybrid Vigor," *Genetics, 33* (1948), 447–487.

A heterozygote is called "overdominant" when its expression exceeds that of either of its alleles when they are found in homozygous pairs; if a heterozygote is superior in fitness to its corresponding homozygotes, for instance, it is considered overdominant for fitness. In the heterosis debates, proponents of the *overdominance hypothesis* held that heterosis is explained in terms of the general superiority of the heterozygotic hybrid over the homozygotic parent strains. By the time of Crow's essay, several genes had been discovered that had heterotic effects and were used as support for the overdominance hypothesis.[18]

The *dominance hypothesis* explained hybrid vigor not in terms of the effect of the heterozygote, but in terms of the effects of the dominant allele in the heterozygous pair. The thinking behind the dominance hypothesis was that as lines became inbred, they started collecting homozygous pairs of deleterious recessive alleles. When two inbred lines were crossed, the deleterious recessives of one line could become paired with beneficial dominant alleles of the other line at the same locus; the dominant alleles would mask the effect of the deleterious recessives, with a resulting boost in vigor.

Using Haldane's 1937 results, Crow was able to show that under the dominance hypothesis, there was a maximum average improvement of vigor, while under the overdominance hypothesis, much larger changes were allowed. In the dominance case, the maximum average improvement of vigor is the increase in selective advantage that would take place if all the deleterious recessive alleles were replaced by beneficial dominant alleles. Haldane had shown that the effect of a deleterious gene on the average fitness of a population was equal to the mutation rate of that gene. So, the effect of all the deleterious recessive alleles on the average fitness of a population is the sum of the individual mutation rates; this corresponds to the maximum average improvement in vigor. Crow calculated that for the dominance case the maximum average improvement in vigor was about 5 percent.[19]

The overdominance case does not have the same limit on average increase in vigor: the loss in fitness of the population is proportional, not to the mutation rate, but to the magnitude of the selective disadvantage associated with the homozygotes contributing to the overdominant heterozygote. Roughly speaking, the greater the disadvantage created by the homozygotes, the greater the average

18. James F. Crow, "Dominance and Overdominance in Heterosis," in Gowen, *Heterosis*, p. 286.

19. Ibid., p. 289. Crow also places a number of restrictions on this conclusion, such as the additivity of gene effects.

improvement in vigor that could be ascribed to the overdominant heterozygote when the homozygous lines are crossed.[20] Given the much greater effect that overdominant loci have on population fitness, Crow reasoned that "if such loci are at all frequent they must be important. The question is: how frequent are they?"[21] This is one way of asking the central question of the classical/balance controversy.

In 1950, Crow thought that the dominance and overdominance hypotheses were neither mutually exclusive nor exhaustive. Some combination of the two hypotheses, he thought, was needed to explain the observed deleterious effects of inbreeding, the subsequent recovery when inbred lines were crossed, high population variance, and observations of hybrid vigor substantially greater than the population equilibrium. Overdominant loci made explaining genetic variance easy. Just as overdominant loci have a greater effect on fitness, they also have a much greater effect on the genetic variance of a population. In fact, Crow argued that there do not have to be many overdominant loci for them "to be the most important factor in the genetic variance of a population."[22] In retrospect, Crow writes that this division of roles among dominant and overdominant loci was widely accepted by quantitative geneticists at the time.[23] What had yet to be determined was the relative number of overdominant loci.

While James Crow was putting Haldane's results to good use in the heterosis debates, Herman J. Muller had independently come to the same conclusions as had Haldane in 1937, but with much more dramatic effect. Muller's "Our Load of Mutations," published in 1950, was aimed at assessing the degree of human impairment caused by mutation. Where Crow had talked about selective disadvantage, however, Muller talked about genetic deaths. Muller argued that in a constant-sized population (and with a simple correction for a growing population) each mutation, whatever its degree of harm, leads to one "genetic death" – that is, to one individual who either dies before reproduction or fails to reproduce. Thus, in terms of reduced viability and fertility of the population, the impact of mutation is determined solely by the total mutation

20. Ibid., p. 291. If the selective disadvantages of the homozygotes are equal to s and t, the average reduction in selective disadvantage due to both homozygotes (and so the average improvement if replaced by the overdominant heterozygote) is $st/(s + t)$.

21. Ibid., p. 291.

22. Ibid., p. 292.

23. James Crow, "Muller, Dobzhansky, and Overdominance," *J. Hist. Biol.*, *20* (1987), 357.

rate, and knowledge of individual mutations is not needed. This was (and is) the only method for assessing the total effect of mutation on the population. Muller called the impact of these genetic deaths the "mutation load" or the "genetic load."[24]

Muller did not stop after proposing the concept of genetic load; he went on to trace out the ill effects of deleterious mutations in humans in terms of this new concept. In effect, he provided a new way of articulating eugenic concerns.[25] An orientation toward the application of science for the betterment of humanity characterized Muller's views. Because he had pioneered much of the early work on the effects of X-ray radiation on the *Drosophila* genome, the damaging effects of radiation on genetic material were of major concern, especially in the wake of the use of atomic weapons in World War II. So, naturally, when he discusses dangerous load-increasing factors threatening human populations, radiation is prominent. In his words,

> The use of ionizing radiation and of radioactive materials is increasing and promises to continue increasing to such an extent, both in medical treatment and diagnosis, and in commerce and industry, even without considering military affairs in this connection, that unless more caution is exercised than at present the majority of the population may in each successive generation have its gonads exposed to enough radiation to raise the mutation rate by a significant amount, such as 25% or 50%. . . . only a 25% rise in mutation rate for one generation would, in a population of 100,000,000 per generation whose usual spontaneous rate was only 1 mutant gene in 10 germ cells, cause the eventual "genetic death" of 5,000,000 individuals, scattered through scores of generations.[26]

These kinds of considerations led Muller to urge the necessity of some form of restriction on the uses of radioactive materials.

When Muller published "Our Load of Mutations" in 1950, he was a senior professor at Indiana University and had garnered a Nobel Prize for his work on the effects of X-rays. Crow was much more junior than Muller, having received his Ph.D. in 1941 at the

24. Herman J. Muller, "Our Load of Mutations," *Amer. J. Human Genet., 2* (1950), 111–176. An excellent account of Muller's scientific work is given in E. A. Carlson, *Genes, Radiation, and Society: The Life and Work of H. J. Muller* (Ithaca, N.Y.: Cornell University Press, 1981).

25. See Diane Paul, " "Our Load of Mutations" Revisited," *J. Hist. Biol., 20* (1987), 328.

26. Muller, "Our Load of Mutations," (above, n. 24), p. 172.

University of Texas. Although he was not Muller's student, they became close collaborators during the 1950s. During this same time period another junior and senior pair of biologists, Bruce Wallace and Theodosius Dobzhansky, were developing what would become the balance position. Dobzhansky had emigrated from Russia in 1927 to study genetics with T. H. Morgan. By 1950, he was a senior professor at Columbia and widely regarded as one of the world's top geneticists.[27] Bruce Wallace had been one of Dobzhansky's students until 1949, when he received his Ph.D.; in 1950, he was a geneticist at the Biological Laboratory at Cold Spring Harbor, New York.

Wallace's research at Cold Spring Harbor concerned the genetic effects of radiation on populations. In collaboration with J. C. King, he had been subjecting populations of the fruit fly *Drosophila melanogaster* to different levels of radiation. The purpose of the study was to try to come to a better understanding of the effects of radiation on populations, and of the evolutionary implications of those effects. After a number of generations, samples indicated that the population that had received an acute amount of radiation (7000 R, X-ray) had the highest average frequency of wild-type flies. Moreover, this acutely radiated population had a higher estimated adaptive value than the population receiving no radiation. Adaptive values were intended to be a way of measuring the effects of deleterious alleles relative to an ideal adaptive value of 1. In Wallace and King's study, the population receiving no radiation was given an adaptive value of 1.0, and the population receiving an acute amount of radiation had an adaptive value of 1.04. Populations receiving lower-level chronic amounts of radiation (5.1 R/hr., gamma) had adaptive values of 0.92 and 0.95. From these data, Wallace and King concluded that the higher adaptive value of the acutely irradiated population "could exist not merely *in spite of* but *because of* the original treatment."[28] The reasoning behind this assertion supposes that the radiation-induced mutations were

27. Excellent accounts of the development of Dobzhansky's thought are given in John Beatty, "Dobzhansky and Drift: Facts, Values, and Chance in Evolutionary Biology," in *The Probabilistic Revolution*, vol. II, ed. L. Kruger, G. Gigerenzer, and M. Morgan (Cambridge, Mass.: MIT Press, 1987), 271–311; idem, "Weighing the Risks: Stalemate in the Classical/Balance Controversy," *J. Hist. Biol., 20* (1987), 289–319; and Richard Lewontin, "Introduction: The Scientific Work of Theodosius Dobzhansky," in *Dobzhansky's Genetics of Natural Populations I–XLIII*, ed. R. C. Lewontin, J. A. Moore, W. B. Provine, and B. Wallace (New York: Columbia University Press, 1981), pp. 93–115.

28. Bruce Wallace and J. C. King, "Genetic Changes in Populations under Irradiation," *Amer. Nat., 85* (1951), 221 (emphasis in original).

incorporated into heterotic gene combinations that were then selected for and became established at an extremely rapid rate.

Wallace and King's results were not meant to be conclusive, but were meant to invite further research. Wallace continued his stocks for about three more years, and the acutely irradiated population still had an adaptive value of 1.03.[29] Indeed, Wallace went on to a more refined set of radiation experiments whose results became a focal point of the classical/balanced controversy in the early 1960s and which will be discussed below. Wallace and King's results, together with Muller's comments on genetic loads, ensured that overdominance and radiation would be linked.

The connection of radiation to overdominance raised the stakes concerning its evolutionary importance considerably. Muller had been expressing his fear of the damaging effects of radiation on genes and future generations throughout the 1940s, but in the mid-1950s his concern and the public's concern intensified.[30] By 1954, "fallout" has become a household word in the United States; that year, fallout from above-ground atomic testing in Nevada fell from Utah to New Jersey, fallout from a Soviet nuclear test circled the globe, and United States tests in the Marshall Islands produced fallout that fell on a Japanese fishing boat, the *Lucky Dragon*, causing an international incident.[31] In an effort to assuage public fears, *US News and World Report* ran an article in its March 25, 1955, issue minimizing the effect of atomic tests on future generations. Significantly, they misleadingly summarized Wallace's research as follows:

> AEC TESTS SHOW: Fruit flies, raised for 128 generations in highly radioactive surroundings, did not degenerate, as expected. Instead, they ended up a better race of fruit flies – hardier, more vigorous, more reproductive, with better resistance to disease.[32]

29. Bruce Wallace, "Studies on Irradiated Populations of *Drosophila melanogaster*," J. Genet., 54 (1956), 280–293. Bruce Wallace has presented an account of the history of his work on genetic loads in *Fifty Years of Genetic Load* (Ithaca, N.Y.: Cornell University Press, 1991).

30. John Beatty, "Genetics in the Atomic Age: The Atomic Bomb Casualty Commission, 1947–1956," in *The Expansion of American Biology*, ed. Keith Benson, Jane Maienschein, and Ronald Rainger (New Brunswick N.J.: Rutgers University Press, 1991), 296–297.

31. Philip Fradkin, *Fallout* (Tucson: University of Arizona Press, 1989); Beatty, "Genetics in the Atomic Age," p. 295.

32. "The Facts about A-Bomb 'Fall-out,'" *US News and World Report*, March 25, 1955, p. 25. "AEC" refers to the Atomic Energy Commission, which funded Wallace's research. Wallace was not mentioned by name.

For Muller, such uses of Wallace's results raised a serious concern that the issue of the genetic effects of radiation would be prematurely resolved in favor of what Dobzhansky would call the balance position.[33]

By the time Dobzhansky labeled the classical and balance positions, lines were already being drawn with relation to the issue of radiation. If anything, Dobzhansky's codification of the extreme positions exacerbated the situation. They were named at the 1955 meeting of the Cold Spring Harbor Symposium on Quantitative Biology. Dobzhansky's paper for the meeting was meant as an overview of the field, and in it he proposed a continuum of possible positions marked by two extremes, the classical and balance positions. These positions were presented as different possible explanations of the origin of the adaptive norm. An adaptive norm, according to Dobzhansky, was "an array of related genotypes consonant with the demands of the environment."[34]

To the *classical position* Dobzhansky attributed the view that "evolutionary changes consist in the main in gradual substitution and eventual fixation of the more favorable, in place of the less favorable, gene alleles and chromosome structures."[35] If the classical position was correct, most of the genes in most of the individuals should be homozygous. Rarely occurring heterozygotes could have four sources: (1) deleterious mutants that are eventually eliminated by natural selection; (2) adaptively neutral genetic variants; (3) "adaptive polymorphisms maintained by the diversity of the environments which the population inhabits"; (4) rare beneficial mutants which have not replaced all of their less-beneficial alleles.[36] The chief source of the classical position, according to Dobzhansky, was Muller's "Our Load of Mutations."

To the *balance position* Dobzhansky attributed the view that the adaptive norm is an array of heterozygous genotypes.[37] Homozygotes would occur in a small number of individuals, rendering them inferior to the norm in terms of fitness. The key difference between the classical and balance positions lies in the relative numbers of superior heterozygous or overdominant loci expected in natural populations. Dobzhansky himself advocated a

33. Beatty, "Weighing the Risks" (above, n. 27), p. 307.

34. Theodosius Dobzhansky, "A Review of Some Fundamental Concepts and Problems in Population Genetics," *Cold Spr. Harbor Symp. Quant. Biol., 20* (1955), 3.

35. Ibid.

36. Ibid. When Dobzhansky speaks of neutral genetic variants, he is referring to genetic variants linked to phenotypic traits, not molecular traits.

37. Ibid., p. 3.

version of the balance position and used most of his paper at the Cold Spring Harbor Symposium defending it.

As the classical/balance controversy started to heat up in the mid-1950s, it gradually displaced the waning debate over the importance of random drift with regard to phenotypic characters.[38] This earlier debate between Sewall Wright and R. A. Fisher concerned neutral phenotypic traits and the role of random drift in evolution.[39] Fisher and his colleague E. B. Ford had studied yearly fluctuations in the frequency of heterozygotes of the moth *Panaxia dominula* and found that the fluctuations were too great to be accounted for by the action of random genetic drift; instead, they proposed that they were the result of random fluctuations in the strength of natural selection.[40] While Wright was able to correct Fisher and Ford's misunderstanding of his position, the general conclusion against random drift held.[41] In the wake of Wright and Fisher's feud, evolutionary biology underwent what Stephen J. Gould has called the hardening of the synthesis – natural selection became accepted as the dominant force in evolution.[42] The result was panselectionism.

It was at this time of shifting interests and rising concerns about the nature of genetic variation and the action of natural selection that Motoo Kimura broke onto the American genetics scene. Kimura's debut conference paper in the United States was presented at the same Cold Spring Harbor Symposium where Dobzhansky labeled the classical and balance positions. Kimura had been working on a Ph.D. at the University of Wisconsin at Madison under James Crow, with some input from Sewall Wright.[43] His paper at Cold Spring Harbor was a review of the work to date on stochastic processes affecting gene frequencies.[44] It is extremely impressive for both its scope and its mathematical complexity. Crow notes

38. Beatty, "Dobzhansky and Drift" (above, n. 27), p. 299.

39. A detailed account of the debates between Wright and Fisher can be found in William Provine, *Sewall Wright and Evolutionary Biology* (Chicago: Chicago University Press, 1986), esp. chaps., 8, 9, 12.

40. R. A. Fisher and E. B. Ford, "The Spread of a Gene in Natural Conditions in a Colony of the Moth *Panaxia dominula*," *Heredity, 1* (1947), 168.

41. Sewall Wright, "On the Roles of Directed and Random Changes in the Genetics of Populations," *Evolution, 2* (1948), 279–294.

42. Gould, "Hardening of the Modern Synthesis" (above, n. 2).

43. Motoo Kimura, "Genes, Populations, and Molecules: A Memoir," in *Population Genetics and Molecular Evolution*, ed. T. Ohta and K. Aoki (Tokyo: Japan Scientific Societies Press), pp. 459–481.

44. Motoo Kimura, "Stochastic Processes and the Distribution of Gene Frequencies under Natural Selection," *Cold Spr. Harbor Symp. Quant. Biol., 20* (1955), 33–53.

in retrospect that few at the conference understood Kimura's paper, but that afterward Sewall Wright "stood up to say that only those who had tried to solve such problems, as he had, could appreciate the magnitude of Kimura's work."[45]

Unlike Dobzhansky's paper, which set the stage for future controversy, Kimura's paper developed further the mathematical population genetics that had been at issue in the debate between Fisher and Wright. Kimura's early concern with the role of random processes is significant for the history of the neutral theory, because it indicates that he was intimately aware of the controversial nature of claims about the significance of random drift. However, as William Provine has shown, the neutral theory is significantly different from this older debate.[46]

Dobzhansky's labeling of the classical/balance controversy and the subsequent polarization and debate eventually enveloped Kimura during the late 1950s and early 1960s. More specifically, Bruce Wallace's 1958 results on the effects of radiation on populations of *Drosophila* focused the attention of most population gene-ticists on the classical/balance debate; Kimura was no exception.

The experimental results Wallace reported in 1958 were the products of much more careful experimentation and analysis than his earlier experiments reported in 1951 and 1956. This time, he placed his results squarely within the classical/balance controversy. His 1958 paper addresses the following two questions:

(1) To what extent do Mendelian populations deviate from the complete genetic uniformity expected as the final outcome of selection for "normal" homozygous individuals?
(2) What is the reason for this deviation?[47]

More specifically, Wallace was striving for a quantitative measure of how much heterozygote superiority (overdominance) contributes to the failure of a population to achieve homozygosity.

His first step was to set out two alternative models – one based on homozygote superiority (classical position), and the other on heterozygote superiority (balance position). He represented these extremes in the form of a table (see Table 1). Both of the extreme models predict that homozygous individuals will have lower

45. James Crow, "Motoo Kimura: An Appreciation," in Ohta and Aoki, *Population Genetics* (above, n. 43), p. 485.

46. Provine, "Neutral Theory" (above, n. 8), p. 19.

47. Bruce Wallace, "The Average Effect of Radiation-Induced Mutations on Viability in *Drosophila melanogaster*," *Evolution, 12* (1958), 553.

Table 1. Expectations of extreme models of the classic and balance positions.

	Situations predicted by extreme models based on	
	Homozygote superiority	Heterozygote superiority
Selection's goal – the "ideal" genotype	$\frac{ABCDEFGH\ldots\ldots}{ABCDEFGH\ldots\ldots}$	$\frac{A_1B_9C_2D_7E_3F_5G_4\ldots}{A_7B_5C_6D_2E_1F_4G_3\ldots}$
Genotype of an average individual under ordinary conditions	$\frac{ABCDeFGH\ldots\ldots}{aBCDEFGH\ldots\ldots}$	$\frac{A_1B_9C_7D_6E_4F_5G_8\ldots}{A_1B_6C_5D_7E_4F_9G_{23}\ldots}$
Genotype of an individual which is homozygous for a chromosome of the sort commonly found in populations	$\frac{ABCDeFGH\ldots\ldots}{ABCDeFGH\ldots\ldots}$	$\frac{A_1B_9C_7D_6E_4F_5G_8\ldots}{A_1B_9C_7D_6E_4F_5G_8\ldots}$
Genotype of an individual similar to the one above but now with a new mutation (′) in the heterozygous condition	$\frac{ABCDeFGH\ldots\ldots}{Ab'CDeFGH\ldots\ldots}$	$\frac{A_1B_9C_7D_6E_4F_5G_8\ldots}{A_1B'C_7D_6E_4F_5G_8\ldots}$

Source: Bruce Wallace, "The Average Effect of Radiation-Induced Mutations on Viability in *Drosophila melanogaster*," *Evolution, 12* (1958), Fig. 1, p. 536.

viabilities, but for different reasons. The homozygote superiority model explains the lower viability in terms of inevitable deleterious mutations that persist until selection eliminates them; according to the heterozygote superiority model, "by definition the viability of heterozygotes is on average the highest attainable."[48]

But if you made sure that one chromosome was homozygous and used radiation to induce new mutations, what would the effect be on viability? Under the homozygote superiority model, chances are that a "normal" allele would be changed to a deleterious allele and the average viability would decrease. Under the heterozygote

48. Ibid., p. 535.

superiority model, chances are that "new mutations should with some measurable frequency improve the viability of homozygotes when such mutations are present in the heterozygous condition."[49] The result of experiments based on this line of reasoning led Wallace to an admittedly preliminary conclusion that "newly induced mutations tested in the heterozygous condition increase the average viability of otherwise homozygous individuals."[50] Moreover, he also tentatively concluded that "on the average an individual member of the Drosophila population studied is heterozygous for genes at 50% or more of its loci."[51] These results strongly favor the heterozygote superiority model.

While Muller set a graduate student, Raphael Falk, to try to replicate Wallace's results,[52] Kimura made his own evaluation of the classical/balance controversy based on new data about human populations and the predictions of his own quantitative models of the classical and balance positions. Kimura's paper, "Relative Applicability of the Classical and the Balance Hypotheses to Man. Especially with Respect to Quantitative Characters," appeared in the *Journal of Radiation Research* and was clearly motivated by the dangers of genetic damage as a result of ionizing radiation. Kimura specifically picks out Wallace's 1958 results suggesting beneficial effects of radiation and the balance hypothesis as topics to be treated with utmost caution. If Wallace's results and the balance position were accepted, then, in Kimura's words, "our view on genetic damage of ionizing radiation to human population and eugenic policies in future as well as our view on the nature of mutation should drastically be revised."[53] Specifically, Kimura thought that the acceptance of the balance position implied that (1) "mutations are not random and, in a sense adaptive," (2) the genetic effects of radiation are not harmful and may even be beneficial, and (3) there would be little ground for the eugenics policy of discouraging persons with congenital malformations from reproducing.[54]

49. Ibid., p. 536.
50. Ibid., p. 555.
51. Ibid.
52. Herman Muller and Raphael Falk, "Are Induced Mutations in *Drosophila* Overdominant? I. Experimental Design," *Genetics, 46* (1961), 727–737; Raphael Falk, "Are Induced Mutations in *Drosophila* Overdominant? II. Experimental Results," ibid., 737–757.
53. Motoo Kimura, "Relative Applicability of the Classical and the Balance Hypotheses to Man. Especially with Respect to Quantitative Characters," *J. Rad. Res., 1–2* (1960), 155.
54. Ibid., p. 156.

Kimura presents the classical and balance positions as what he calls a conservative and a homeostatic model, respectively. The homeostatic model is taken from a 1956 paper by Alan Robertson, which in turn was based on I. M. Lerner's *Genetic Homeostasis*.[55] The homeostatic model, according to Kimura, assumes that at each locus a pair of alleles is maintained by heterozygote superiority. This can be represented as follows:

Genotype	A_1A_1	A_1A_2	A_2A_2
Fitness	$1 - s_1$	1	$1 - s_2$

The relative fitness of the homozygotes is, thus, always lower than that of the heterozygote by some degree represented by "*s*." The alleles at each locus act additively on the character in question, such that if A_1A_1 is set at 0 for some trait, the allele A_2 in the heterozygote A_1A_2 causes that trait to change by quantity α, and two copies of the A_2allele cause the trait to change by quantity 2α.[56]

The conservative model, according to Kimura, assumes that alleles such as A_1 and A_2 are maintained by a balance between mutation and selection. A_1 is the normal allele and A_2 is the less-fit mutant. Kimura also assumes that A_1 is not completely dominant over A_2, so A_2 can have enough effect on the heterozygote to make it the case that the genes are nearly additive. This model is conservative because it holds that for a particular environment there is a normal or wild-type allele that is maintained by natural selection.[57]

Kimura used these basic quantitative models to generate a number of points of comparison between the two. For instance, he showed that for some metric trait the per-locus contribution to the total additive genetic variance of the trait will be much larger under the homeostatic model. In his words, "this means that even if the number of overdominant loci is only 4% of the total, they can explain over 90% of the genetic variability in the quantitative character."[58] Using his quantitative models, Kimura also argued

55. Alan Robertson, "The Nature of Quantitative Genetic Variation," in *Heritage from Mendel*, ed. R. A. Brink (Madison: University of Wisconsin Press, 1956), pp. 265–280; I. M. Lerner, *Genetic Homeostasis* (New York: John Wiley, 1954). Lerner's book advocates general heterozygote superiority and was used extensively by Dobzhansky to defend the balance position in his 1955 paper; see Dobzhansky, "Review" (above, n. 34), pp. 3, 6–7.

56. Kimura, "Relative Applicability of the Classical and Balance Hypotheses" (above, n. 53), pp. 156–157.

57. Ibid., p. 157.

58. Ibid., p. 163.

that overdominant loci are in the minority for certain quantitative traits.

Kimura's conclusion, – namely, that overdominant loci are in the minority but may account for most of the genetic variability – is the same conclusion given by Crow at the Heterosis Conference in 1950. In fact, Kimura's review of the classical/balance controversy is remarkable for its strong connection to work done by Crow on overdominance and genetic loads. On this basis it is fair to conclude that by the early 1960s Kimura had become firmly aligned with the classical position, as interpreted by James Crow.

This alliance is further reinforced by Kimura's work on genetic loads. The theory and applicability of genetic loads dominated much of the classical/balance controversy. Like Crow, Kimura had been concerned with developing mathematical models for different kinds of genetic loads. By 1959, this work had developed into what he called the "principle of minimum genetic load." This principle asserts that in the course of evolution, spontaneous mutation rates and the rate of inbreeding depression are adjusted to minimize the total genetic load.[59] Using his earlier work, which linked the rate of inbreeding depression to mutation load,[60] he was able to calculate mutation and substitution loads. This work on loads reveals a growing involvement on Kimura's part in one of the central elements (i.e., genetic load theory) of the classical/balance controversy.

It is significant that Kimura's work on genetic loads also fed into his growing interest in molecular evolution and analyses of genetic information. In particular, his work on the principle of minimum genetic load led him to the conclusion that substitution loads are measures of changes in genetic information. Given his estimate of the substitution load, he calculated that the rate of accumulation of genetic information by natural selection is about 0.29 bits

59. Motoo Kimura, "Genetic Load of a Population and Its Significance in Evolution," *Jap. J. Gent., 35* (1960), 7. Inbreeding, and the resulting expression of deleterious recessives, leads to a decline in mean phenotype known as "inbreeding depression."

60. Motoo Kimura, "Theoretical Basis for the Study of Inbreeding in Man," *Jap. J. Human Genet., 3* (1958), 51–70. A mutation load is the load resulting from new mutations, while the substitution load is what J. B. S. Haldane and others called the "cost of selection." The cost of selection refers to the effect on fitness of the process of substitution of a new mutant. The difference between a mutation load and a substitution load is that the first applies to static properties of a population, while the second applied to dynamic properties of a population. See James Crow, "Genetic Loads and the Cost of Natural Selection," in *Mathematical Topics in Population Genetics*, ed. K. Kojima (Berlin: Springer, 1970), pp. 128–177.

per generation.[61] This rate allowed him to estimate that since the Cambrian epoch, 500 million years ago, 10^8 bits of genetic information have accumulated. Kimura considered the maximum amount of genetic information in man to be on the order of 10^{10} bits; the difference between these estimates is explained by repeats of information or redundancies in the genetic code.

Kimura pursued this idea using the work of Noboru Sueoka on the variation in the base content of DNA.[62] Specifically, he explained the observed heterogeneity of the guanine-cytosine (G-C) content of DNA in terms of repetitions in DNA. This early connection to developments in molecular biology, especially to Sueoka's work, is extremely important – it indicates that Kimura was in touch with developing research in the study of molecular evolution. Moreover, Sueoka's work in 1962 pointed directly to the presence of neutral mutations. Kimura was probably aware of this work and of similar work by Ernst Freese, although he did not pursue Sueoka's and Freese's arguments in print until after it was made a supporting feature of the neutral theory in 1969.[63]

Throughout the 1960s, work continued on the classical and balance positions, but theoretical and experimental work incorporating the advances of molecular biology would soon cause changes that would alter the course of the controversy. The vehicles for these changes were the development of the infinite alleles model by Kimura and Crow, the application of electrophoresis to measure protein variability by J. L. Hubby, Richard Lewontin, and Harry Harris, and the advances made in the study of molecular evolution.

The Infinite Alleles Model

In 1958, while working at the National Institute of Genetics in Mishima, Kimura received a letter from Crow that posed the following question:

> Have you ever considered this problem? Suppose every mutant is to an entirely different allele (or at least is counted this way, so that the only homozygosity is homozygosity by descent). Under such a system with a finite population of size n what is the proportion of homozygous loci at equilibrium? Perhaps you have already solved this, but I am not sure. Some of Josh's work suggests that every mutant is distinguishable from every other one if a careful enough test is made; at least this is true for a large number.[64]

61. Motoo Kimura, "Natural Selection as the Process of Accumulating Genetic Information in Adaptive Evolution," *Genet. Res., 2* (1961), 131.

62. N. Sueoka, "A Statistical Analysis of Deoxyribonucleic Acid Distribution in Density Gradient Centrifugation," *Proc. Nat. Acad. Sci., 45* (1959), 1480–90; idem, "On the Genetic Basis of Variation and Heterogeneity of DNA Base Composition," *Proc. nat. Acad. Sci., 48* (1962), 582–592.

63. Freese, "Evolution of the Base Composition of DNA" (above, n. 7). The work of Sueoka and Freese was used in support of the neutral theory in King and Jukes, "Non-Darwinian Evolution" (above, n. 5), p. 790.

In a letter to Crow dated July 24, 1959, Kimura gave his answer to Crow's question concerning the homozygosity of his proposed population. Kimura's solution was for the case of neutral alleles, alleles with no influence from selective forces. Under these conditions, he found that the probability (F) that an individual is homozygous at a locus is

$$F = 1/(4N_e u + 1),$$

where N_e is the effective population number and u is the mutation rate per gene per generation.[65] Crow was greatly impressed by the simplicity of Kimura's solution, and when Kimura returned to the University of Wisconsin for two years starting in 1961, they returned to the problem. The result was their 1964 publication, "The Number of Alleles That Can Be Maintained in a Finite Population."[66]

Kimura and Crow's infinite alleles model had two key assumptions: (1) that there was a large enough number of alleles such that any change was a change to a new allele, and (2) that mutations can have a range of effects from drastic to neutral.[67] The authors explicitly noted that they did not want to argue for the plausibility of neutral alleles, but they did think it was likely that such alleles could exist.

Kimura and Crow examined some of the population consequences of three different allele systems – namely, "(1) A system of selectivity neutral isoalleles whose frequency in the population is determined by the mutation rate and by random drift. (2) A

64. James Crow, "Twenty-Five Years Ago in Genetics: The Infinite Alleles Model," *Genetics, 121* (1989), 631. According to Crow, "Josh" referred to his colleague Joshua Lederberg, who discovered sexual reproduction in *Escherichia coli* and had been a member of Kimura's doctoral dissertation committee at the University of Wisconsin (ibid., p. 631).

65. Ibid.

66. Motoo Kimura and James Crow, "The Number of Alleles That Can Be Maintained in a Finite Population," *Genetics, 49* (1964), 725–738.

67. The name "infinite alleles model" is misleading, since the model assumes only a very large number of alleles, not an infinite number.

system of mutually heterotic alleles. (3) A mixture of heterotic and harmful mutants."[68] In other words, in each of the three cases being studied, every mutation produced a new allele that was neutral, heterotic (overdominant), or either heterotic or harmful, depending on the case at hand. The results would be systems or sets of only neutral alleles, only heterotic alleles, or a mixture of heterotic and harmful alleles.

In the neutral case, Kimura and Crow showed that the effective number of alleles maintained in a population of effective size N_e and mutation rate u is

$$n = 1/F = 4N_e u + 1.$$

In this situation, if $4N_e \ll 1/u$, then F approaches 1 and "almost all the genes in a population at a given locus will be descended from a single mutant."[69] Conversely, if $4N_e \gg 1/u$, then many alleles will be maintained per locus. In this scenario, as the effective population size (N_e) increases, more individuals should be heterozygous. In fact, this scenario provides an estimate of the maximum number of alleles that can be maintained for a given effective population size.

In the case of heterotic alleles and systems of mixed heterotic and harmful alleles, Kimura and Crow constructed an equilibrium model that allowed them to calculate the proportion of homozygous loci, the effective number of alleles, and the segregational load. A segregational load occurs when the most-fit genotype is the heterozygote and Mendelian segregation ensures that in each generation inferior homozygous combinations will be formed: the segregation load is the decrease in the fitness of the population that occurs as the result of the formation of the less-fit homozygotes. As the number of heterozygote superior loci increases, so does the segregation load. What Kimura and Crow's calculations showed, given their admittedly unrealistic assumptions, was that "corresponding to a given value of s, N_e, and u there is a certain [segregation] load required to maintain the alleles in the population," where s is the selection coefficient, N_e is the effective population size, and u is the mutation rate.[70]

Kimura and Crow admit that their calculations do not put a severe limit on the number of segregating loci, but they do cast doubt on Bruce Wallace's 1958 assertion that the average

68. Kimura and Crow, "Number of Alleles" (above, n. 66), p. 725.
69. Ibid., p. 727.
70. Ibid., p. 736.

Drosophila individual from his study is heterozygous for 50% or more of its loci. Their calculations of the minimum segregational load associated with heterozygous loci in *Drosophila* lead them to the opposite conclusion – namely, that "it is more likely that the typical *Drosophila* is homozygous for the majority of its genes, though the segregating minority may still be hundreds of loci."[71] The absolute number of segregating polymorphisms could still be quite large, according to Kimura and Crow, since "in large populations, the possibility of many very nearly neutral, highly mutable multiple isoalleles cannot be ruled out, although there is no experimental evidence for the existence of such systems."[72] Since neutral and near-neutral alleles create no segregation load, there could be a large number of polymorphisms and a tolerable segregation load if many of the alleles were neutral or nearly neutral. In 1983, Kimura stated that he thought the evidence for neutral alleles in nature came two years later with the large amounts of variation revealed by the electrophoretic surveys done by Harris, Hubby, and Lewontin.[73]

So, Kimura and Crow state that they do not want to argue for the plausibility of systems of neutral isoalleles, but neither do they want to rule them out. The question is then whether Kimura and Crow wanted to suggest the neutral case as a possible situation in nature, or whether they were simply using it as a simplifying or tractable mathematical case. Evidence points, I think, to the use of neutral alleles as a mathematically tractable case. The neutral case is used to work out the basic mathematical model, which is then applied to more complicated and more "plausible" cases of alleles that are selected either for or against. It is important to note that the paper's argument is intended to cast doubt on Wallace's assertion of the amount of heterozygosity in *Drosophila*. The shift to the advocacy of the neutral theory and the existence of neutral alleles, then, involves realizing and advocating the fact that the simplest mathematical case may in fact hold in nature. With the advent of the neutral theory, the mathematical treatment of the neutral case first presented in 1964 became much more important than the argument against Wallace, so much so that it now seems to overshadow Kimura and Crow's main conclusion against larger numbers of polymorphisms.[74]

71. Ibid.
72. Ibid.
73. Motoo Kimura, *The Neutral Theory of Molecular Evolution* (Cambridge: Cambridge University Press, 1983), p. 29.
74. Crow, "Twenty-Five Years Ago" (above, n. 64), pp. 631–634.

THE ELECTROPHORETIC REVOLUTION

In 1963, Jack L. Hubby published an article in *Genetics* entitled "Protein differences in *Drosophila*. I. *Drosophila melanogaster*."[75] What is significant about this short article is its account of the application of the technique of electrophoresis to the proteins of the much-studied *Drosophila*. Electrophoresis is a biochemical technique that separates proteins based on their size and net charge. It does so by passing an electric current across a medium such as starch or polyacrylimide gel, so that the proteins are drawn toward the opposite electrode at different rates depending on the amount of electrical attraction and the ease with which they can move through the medium.

Originally Hubby seems to have been interested in the physiological genetics of *Drosophila* and to have seen electrophoresis as a method for detecting differences in proteins. His program changed significantly, however, when he began his collaboration with Richard Lewontin. Lewontin had been following the classical/balance debate closely and had been working on the problem of distinguishing alleles. Two years earlier he had turned down a job offer from the University of Chicago, where Hubby was based, but after hearing of Hubby's work on electrophoresis in 1964, Lewontin decided to move to the University of Chicago explicitly to collaborate with him.[76] Hubby and Lewontin's work was a self-conscious attempt to address the problem of the amount of genetic variation in a population.

Lewontin brought with him a list of criteria for the type of technique needed to resolve how much heterozygosity there was per locus in a population. In his words,

> Any technique that is to give the kind of clear information we need must satisfy all of the following criteria: (1) Phenotypic differences caused by allelic substitutions at *single loci* must be detectable in *single individuals*..(2) Allelic substitutions at one locus must be distinguishable from substitutions at other loci. (3) A substantial proportion of (ideally, all) allelic substitutions must be distinguishable from each other. (4) Loci studied must be an unbiased sample of the genome with respect to the physiological effects and degree of variation.[77]

75. J. L. Hubby, "Protein Differences in *Drosophila*. I. *Drosophila melanogaster*," *Genetics, 48* (1963), 871–879.

76. R. Lewontin, pers. comm., August 13, 1990.

77. According to Lewontin, he had articulated these criteria prior to beginning work with Hubby (pers. comm., August 13, 1990). This quotation is from the first of their joint papers: J. L. Hubby and R. C. Lewontin, "A Molecular Approach to the Study of Genic Heterozygosity in Natural Populations. I. The Number of Alleles at Different Loci in *Drosophila pseudoobscura*," *Genetics, 54* (1966), 578. Their emphasis.

Hubby and Lewontin's work tried to meet these criteria and provide a reliable measure of the amount of heterozygosity found in natural populations. The first of their joint papers sets out the experimental problem and demonstrates the ability of the technique to detect and measure differences in electrophoretic mobility that correspond to allelic variation in different strains of *Drosophila pseudoobscura*; the second joint paper presents the results of their surveys of protein variation as well as the implications of these results for the classical/balance controversy and population genetics.[78]

Lewontin and Hubby's survey of eighteen *Drosophila* loci from a number of different laboratory and natural populations revealed a high degree of polymorphism: seven out of eighteen loci were clearly polymorphic in more than one population. Put in terms familiar to the classical/balance controversy, the average individual in a population was heterozygous for 8% to 15% of its loci in different populations, with an average of 12%. Lewontin and Hubby did not argue that there was one mechanism for explaining this variation, but proposed several alternatives. The possibility of neutral alleles was considered, but complete selective neutrality was ruled out. They reasoned that genetic drift should drive a population to homozygosity. Experiments on different populations of *Drosophila*, however, failed to show local races with the expected high levels of homozygosity. This could be the result of a very small amount of migration, but Lewontin and Hubby still took these results to weight against the neutral theory.[79] They also considered the option of a large number of heterotic alleles to explain the observed electrophoretic variation, but they agreed with Kimura and Crow's conclusion that this would carry with it an intolerable segregation load. The problem of explaining the levels of variation they found was, thus, left for the future.

At the same time that Hubby and Lewontin were doing their electrophoretic surveys in Chicago, Harry Harris and a team of researchers in London were surveying electrophoretic variations

78. Ibid.; R. C. Lewontin and J. L. Hubby, "A Molecular Approach to the Study of Genic Heterozygosity in Natural Populations. II. Amount of Variation and Degree of Heterozygosity in Natural Populations of *Drosophila pseudoobscura*," *Genetics, 54* (1966), 595–609.

79. Lewontin and Hubby, "Molecular Approach . . . II," p. 606.

in human blood proteins. Harris's work was aimed at getting a first approximation of the enzyme variation in human populations. To this end he randomly selected ten human blood proteins and, using starch gel electrophoresis, found "three striking examples of enzyme polymorphism."[80] Harris's survey differs from Hubby and Lewontin's in that it is not directly addressed to population genetics issues. Only at the very end of his article does Harris even mention the idea that differences in these enzymes be a result of selection.

Immediately after Lewontin and Hubby published their findings on variation and genetic loads, three different critiques all pointed out that the high segregation load predicted by Lewontin and Hubby was a result of their model's assignment of a very high fitness to the ideal multiple heterozygote.[81] Because this heterozygote was so fit, it looked as if there was a large gap in fitness values between it and the other genotypes. Lewontin and Hubby's critics showed that these lofty ideal heterozygotes rarely if every occur and thus have a negligible effect on the average selective advantage of individual loci. Instead, these critics proposed truncation selection models, or threshold models that posited an upper threshold for fitness values such that heterozygosity not much above the mean heterozygosity has reached a maximum fitness threshold. The optimum genotype is thus not much higher than the population mean.[82] The smaller fitness differential created in these models lowers the segregation load and allows for more polymorphism.

Truncation selection models seemed like a viable remedy for worries about segregation loads and allowed for selectionist explanations of high levels of heterozygosity. Indeed, these models were pursued by a number of population geneticists and were summarized in 1981 by Christopher Wills.[83] But this kind of model was not pursued by one of its initial proponents, Jack L. King; instead, in collaboration with Thomas Jukes, King became one of the early advocates of the neutral theory of molecular evolution – or, as he and Jukes called it, non-Darwinian evolution.[84] As we shall

80. Harry Harris, "Enzyme Polymorphism in Man," *Proc. Roy. Soc. London*, ser. B, *164* (1966), 298–310.

81. J. King, "Continuously Distributed Factors Affecting Fitness," *Genetics*, *55* (1967), 483–492; R. Milkman, "Heterosis as a Major Cause of Heterozygosity in Nature," ibid., 493–495; J. Sved, T. Reed, and W. Bodmer, "The Number of Balanced Polymorphisms That Can Be Maintained in a Neutral Population," ibid., 469–481.

82. Sved, Reed, and Bodmer, "Number of Balanced Polymorphisms," p. 479.

83. Christopher Wills, *Genetic Variability* (Oxford: Clarendon Press, 1981).

84. King and Jukes, "Non-Darwinian Evolution" (above, n. 5).

see, one of the chief arguments used in favor of the neutral theory was that it could explain Lewontin and Hubby's high levels of variation without accumulating high genetic loads. However, as King's change of interest suggests, more was behind the advocacy of the neutral hypothesis in the late 1960s than just electrophoretic surveys and genetic load concerns.

THE MOLECULARIZATION OF POPULATION GENETICS

Electrophoresis was a biochemical technique that introduced population genetics and evolutionary genetics to experimental work at the molecular level. The electrophoretic revolution in population genetics, however, was only a small part of the molecular biology boom going on in the 1960s. Kimura and Crow had begun to introduce molecular biology into population biology in the assumptions underlying their 1964 proposal of the infinite alleles model. More important, though, is the research into molecular evolution that had been on going throughout the 1960s. The results of the molecular evolutionists provided critical evidence during the development of the neutral theory – evidence that is overlooked by Lewontin's Historical Thesis.

The study of molecular evolution has a relatively long history in the search for the origins of life, where it was primarily concerned with the development of organic molecules from inorganic molecules and of complex molecules from simpler molecules. The growing realization of the importance of deoxyribonucleic acid (DNA) and informational macromolecules in the 1940s and 1950s, however, shifted the study of molecular evolution toward more specific questions regarding the evolution of genes and proteins.

After James Watson and Francis Crick's discovery of the double helical structure of DNA in 1953, molecular biology research focused more than ever on informational macromolecules, such as DNA, RNA, and proteins, and the pathways between them. The structure of DNA suggested a method of replication as well as possible ways of storing genetic information. Although work on the evolution of the genetic code had begun even before the code had been firmly established, when Severo Ochoa and Marshall Nirenberg each worked out the genetic code linking DNA, RNA, and proteins in 1963, the doors were opened wide to questions about the evolution of the genetic code and about what could be inferred about the evolution of genes and proteins.[85]

85. See Christian Anfinsen, *The Molecular Basis of Evolution* (New York: John Wiley, 1959).

These issues concerning the evolution of the genetic code, genes, and informational macromolecules are extremely important in the history of the neutral theory. In particular, the publication of the proceedings of a well-attended symposium on "Evolving Genes and Proteins" (held at the Institute of Microbiology of Rutgers University in September 1964) is quite crucial: papers by Émile Zuckerkandl and Linus Pauling, John Buettner-Janusch and Robert L. Hill, Emanuel Margoliash and Emil L. Smith, and Nathan O. Kaplan provided the evidence concerning rates of molecular evolution that is at the foundation of Kimura's 1968 proposal of the importance of neutral mutations and random drift.[86] These proceedings were also widely incorporated into Thomas Jukes's 1966 book, *Molecules and Evolution*, and into his paper with Jack King advocating the importance of neutral mutations.[87]

The importance of molecular biology for population genetics was recognized by Kimura quite early – as evidenced by his work on genetic information in 1961. The importance of molecular biology was further reinforced by the growing concern over mechanisms of molecular evolution.

After attending one of the first conferences discussing molecular evolution in 1964, G. G. Simpson wrote an important paper regarding organismic and molecular evolution. Simpson was trying to ease the "confrontation of molecular and organismal data" and the idea that molecules, specifically serum proteins and cytochromes, "have evolved by some sort of internal constant-rate mutational process and not in an irregular or specifically adaptive way."[88] In other words, he was trying to ward off the possibility that molecular evolution could be driven by a steady stream of mutations and not by the environmentally driven process of natural selection. Organismal and molecular points of view, especially with regard to constructing phylogenies, according to Simpson, needed to be balanced in order to produce more complete explanations.

Ernst Mayr also attended one of the first molecular evolution conferences, the "Evolving Genes and Proteins" conference, and

86. Emile Zuckerkandl and Linus Pauling, "Evolutionary Divergence and Convergence in Proteins," pp. 97–166; John Buettner-Janusch and Robert L. Hill, "Evolution of Hemoglobin in Primates," pp. 167–181; E. Margoliash and Emil L. Smith, "Structural and Functional Aspects of Cytochrome *c* in Relation to Evolution," pp. 221–242; and Nathan O. Kaplan, "Evolution of Dehydrogenases," pp. 243–277, all in Bryson and Vogel, *Evolving Genes and Proteins* (above, n. 3).

87. Thomas Jukes, *Molecules and Evolution* (New York: Columbia University Press, 1966); King and Jukes, "Non-Darwinian Evolution" (above, n. 5).

88. Simpson, "Organisms and Molecules in Evolution" (above, n. 3), p. 1535.

the discussion transcript shows that he made detailed comments, especially regarding the use of molecular data in taxonomy.[89] The discussion Mayr was involved in built on a paper published by Simpson and expressed similar concerns. The fact that evolutionists of Mayr's and Simpson's stature were addressing these specific ways in which molecular evolution might be different from organismal evolution is significant and marks a growing trend toward taking molecular evolution seriously as an area of scientific inquiry.[90] Kimura's awareness of this trend is evident in his use of the molecular evolution literature in his paper presenting the arguments for what will become the neutral theory.

Motoo Kimura's 1968 discussion of the rate of molecular evolution in *Nature* concludes that "we must recognize the great importance of random genetic drift due to finite population number in forming the genetic structure of biological populations."[91] The importance of random genetic drift follows from Kimura's estimation of a high rate of nucleotide substitution from comparative studies of mammalian hemoglobin.[92] These estimates were made as follows: Kimura has his colleague Tomoko Ohta estimate the rate of amino acid change in mammalian hemoglobin, primate hemoglobin, mammalian and avian cytochrome *c*, and triosephosphate dehydrogenase from rabbits and cattle.[93] He then averaged these for a chain 100 amino acids long, producing a rate of one amino acid substitution every 28×10^6 years.[94] Since each amino acid is coded for by a nucleotide triplet, this estimate for a chain of 100 amino acids can be extended to provide an estimate of the rate of evolution for the entire genome. For a mammalian genome, Kimura estimated that the average time taken for one base-pair replacement is 1.8 years. Such a rate a mutation, however, carries with it an intolerable cost of selection (or, as Kimura called it, substitutional load). In order for the rate of substitution to be within the limits of the substitutional load, Kimura had to assume that "most

89. Mayr, "Discussion of Part III," in Bryson and Vogel, *Evolving Genes and Proteins* (above, n. 3), pp. 197–198.

90. The importance of molecular evolution is noted in a letter from George G. Simpson to Ernst Mayr, January 6, 1964, Simpson Papers, American Philosophical Society Library.

91. Kimura, "Evolutionary Rate" (above, n. 5), p. 626.

92. A nucleotide is a subunit of a nucleic acid such as DNA or RNA. It is composed of a sugar, a phosphate, and a base, such as cytosine, thymine, adanine, guanine, or uracil. Nucleotide substitutions can significantly alter the base composition of a nucleic acid.

93. Motoo Kimura, "Thirty Years of Population Genetics with Dr. Crow," *Jap. J. Genet., 63* (1988), 1–10.

94. Kimura, "Evolutionary Rate" (above, n. 5), p. 625.

mutations produced by nucleotide replacement are almost neutral in natural selection."[95] Using mathematical models for substitutional load and the probability of fixation of neutral alleles, he then showed that neutral alleles have a very low substitutional load, and that the probability of a neutral allele becoming fixed or established in a population is roughly equal to its initial frequency. In Kimura's words, "this means that new alleles may be produced at the same rate per individual as they are substituted in the population in evolution."[96] Since the rate of substitution is roughly one every 1.8 years, neutral alleles must be occurring at a rather high rate in mammals – roughly 0.5 per year per gamete. This high rate of neutral mutations, Kimura notes, is compatible with Hubby and Lewontin's and Harris's results from their electrophoretic surveys of protein polymorphisms.

It is significant that despite citations of the work of Hubby, Lewontin, and Harris, the really crucial evidence for rates of change necessary for Kimura's argument comes from molecular evolutionists, not from electrophoretic surveys. Indeed, the papers of Zuckerkandl and Pauling, Buettner-Janusch and Hill, and Margoliash and Smith provide a wealth of information not only about rates of change, but about functional constraints on molecular changes and neutral substitutions. Zuckerkandl and Pauling recognize "indifferent" substitutions that may, in their words, "spread in a population through random drift."[97] They also discuss the possibility of functionally nearly neutral changes to explain the stability of amino acid sequences, despite the changes necessitated by their proposal of a molecular evolutionary clock.[98] Buettner-Janusch and Hill also discuss the possibility of neutral traits and neutral genes, but they conclude that there is no evidence for them yet from complex organisms. Even if there were biologically equivalent substitutions, they think that "the real question is, how does an effective mutation, which is a relatively rare event, become fixed in a population?"[99] The only mechanism Buettner-Janusch and Hill know to account for fixation is natural selection; with genetic drift, they think the trait is likely to disappear or remain at a very low frequency. Margoliash and Smith express very similar

95. Ibid.
96. Ibid.
97. Zuckerkandl and Pauling, "Evolutionary Divergence" (above, n. 86), p. 109.
98. Ibid., pp. 149–150.
99. Buettner-Janusch and Hill, "Evolution of Hemoglobin" (above, n. 8), p. 178.

concerns in their paper.[100] The stage seems set for someone with knowledge of the behavior of neutral alleles in populations and of their chances of fixation. Kimura's work on random genetic drift was ideally suited to address just these questions; where the molecular evolutionists hesitated, Kimura could step in to provide a solution.

It is especially noteworthy that even Crow seemed to show a similar hesitancy about the power of random drift. If Lewontin's Historical Thesis is sufficient to explain the emergence of the neutral theory, then we would expect Crow and Kimura to have similar reactions to Lewontin and Hubby's results, to construct similar cost-of-selection arguments, and to come to similar conclusions. In this light, Crow's 1967 paper, entitled "The Cost of Evolution and Genetic Loads," is significant. Crow examines data very similar to those that Kimura examined concerning the rates of evolution in hemoglobin, but instead of concluding that the genetic costs would be too high, he concludes that the costs "are consistent with a reasonable amount of natural selection."[101] This difference is a result of Kimura's looking at the rate of nucleotide substitutions in the entire genome and Crow's looking at the rate of nucleotide substitutions in 10,000 loci.[102] Thus, using very similar cost-of-selection arguments, Crow comes to a conclusion opposite to the one that Kimura would publish just a few months later.[103]

Crow comes closer to the position Kimura was developing when he considers high levels of variation found in electrophoretic surveys. He discusses several options to account for these data – high levels of heterozygote superiority, or truncated selection, or large numbers of neutral alleles – and concludes by suggesting that the loci studied by Lewontin and Hubby were "in the main not strongly selected. They may be maintained at intermediate frequencies by mutation pressure or by slight heterozygote advantage."[104] The problem of deciding which forces are maintaining

100. Margoliash and Smith, "Structural and Functional Aspects of Cytochrome c" (above, n. 86), p. 236.
101. James Crow, "The Cost of Evolution and Genetic Loads," in *Haldane and Modern Biology*, ed. K. Dronamraju (Baltimore: Johns Hopkins Press, 1967), p. 172. I am indebted to William Provine for drawing my attention to Crow's cost-of-selection argument.
102. The cost of substitution increases as the number of independent loci increases.
103. In retrospect, Crow argues that he was referring to only coding regions of DNA, while Kimura referred to both coding and noncoding regions; the apparently different results are, therefore, due to the fact that Crow did not make his position clear at the time (James Crow, pers. comm., May 20, 1992).
104. Crow, "Cost of Evolution" (above, n. 101), p. 177.

these weakly selected or nearly neutral alleles becomes one of deciding how easily random drift can drive a neutral mutation to fixation (100% representation in a population). In Crow's words, the problem becomes one of "how great is the fixation tendency of random drift."[105] Here he seems to be leaning toward Kimura's position regarding neutral alleles and random drift, but he also seems skeptical of drift's ability to drive so many alleles to fixation.

By the time of the 12th International Congress of Genetics in 1968, however, Crow's position had become much more clearly aligned with Kimura's. Before going to the Congress in Tokyo, Crow visited Kimura and Ohta in Mishima. He had just reviewed a manuscript written by Jack L. King and Thomas Jukes, advocating the position that "most evolutionary change in proteins may be due to neutral mutations and genetic drift."[106] It was, of course, the major topic of conversation between Crow, Kimura, and Ohta.[107]

Although the bulk of King and Jukes's paper concerned data from molecular evolution (which will be discussed below), they were nevertheless mindful of Kimura's arguments. For instance, they incorporated Kimura's work showing the effectiveness of drift in fixing neutral mutations, but were critical of his estimation of the rate of amino acid change and his argument based on intolerable genetic loads. They estimated the rate of amino acid substitution to be one every fifty years, and took seriously the objections raised against high genetic loads by advocates of truncated models of selection (i.e., J. Sved, T. Reed, W. Bodmer, Jack King, and J. Maynard Smith). Despite their opposition to Kimura's chief argument, King and Jukes strongly advocated the importance of neutral mutations and genetic drift.

They chose the provocative title of "Non-Darwinian Evolution" for their paper, and the name stuck to the hypothesis until the early 1970s when it was redubbed the neutral theory of molecular evolution. According to a letter quoted by William Provine, Kimura was not found of the "non-Darwinian" label and asked King and Jukes to change it to emphasize molecular evolution, instead of evolution in general.[108] King and Jukes had chosen their title with the intention of provoking the evolutionary establishment.[109] Given the atmosphere of civil unrest in the late 1960s at Berkeley, where King and Jukes worked, and elsewhere in the United States, the

105. Ibid.
106. King and Jukes, "Non-Darwinian Evolution" (above, n. 5), p. 788.
107. James Crow, pers. comm., May 20, 1992.
108. Provine, "Neutral Theory" (above, n. 8), p. 28.
109. Letter from Jack King to Theodosius Dobzhansky, June 11, 1970, Dobzhansky Papers, American Philosophical Society Library.

antiauthoritarian tone of "Non-Darwinian Evolution" undoubtedly struck a nerve. Although both reviewers rejected their article, it was published upon appeal and the blasphemous title remained unchanged.[110]

The direct challenge to traditional neo-Darwinian panselectionism from King and Jukes made the emerging neutral theory highly visible. Their paper was made even harder to ignore, however, by the wealth of molecular data that they directly brought to bear on the importance of neutral mutations and random drift.

At the 12th International Congress, Crow's keynote paper called for the increased integration of molecular biology and population genetics. According to Crow, "What molecular biology is now doing so elegantly for population genetics is to provide a greatly improved opportunity to study the actual quantities – the gene frequencies and gene substitutions – to which the theory applies most directly."[111] For him, the key example of the integration of molecular biology and population genetics was the neutral theory just proposed by Kimura and by King and Jukes. Crow had written most of this paper while visiting Kimura and Ohta in Mishima.[112] Significantly, he made sure to note that Kimura and Ohta had worked out the details of the rate of neutral substitution and the time to fixation, which before had been stumbling blocks. In fact, instead of being hesitant, he seemed enthusiastic about the prospects of neutral mutations and especially random drift.

Undoubtedly, one reason Crow became so enthusiastic about the integration of molecular biology and population genetics was the variety of support marshalled by King and Jukes. Much of their paper was drawn from the molecular evolution literature. Jukes was himself a molecular evolutionist. He had attended the "Evolving Genes and Proteins" conference and had published a book on the subject entitled *Molecules and Evolution* in 1966."[113] *Molecules and Evolution* is not a non-Darwinian tract, although Jukes does recognize the existence of neutral mutations at the protein level. When he wanted to develop this idea further, he went to Jack King for help. Before King and Jukes had finished their manuscript, Kimura's paper was published in *Nature*; rather than drop the project, they addressed Kimura's argument and further

110. Thomas Jukes's recollections of these events are found in his "Early Developments of the Neutral Theory," *Perspect. Biol. Med., 34* (1991), 473–485.
111. James Crow, "Molecular Genetics and Population Genetics," *Proceedings of the XII International Congress of Genetics*, vol. III (1969), p. 106.
112. James Crow, pers. comm., May 20, 1992.
113. Jukes, *Molecules and Evolution* (above, n. 87).

buttressed their case with data drawn from the molecular evolution literature.[114]

King and Jukes directed their argument for neutral mutations against G. G. Simpson's and Emil Smith's claims of panselectionism at the molecular level.[115] While they did not agree with Kimura's genetic load argument, they did agree with him regarding the significance of synonymous mutations.[116] They claimed that, "as far as is known, synonymous mutations are truly neutral with respect to natural selection."[117] In addition, they offered significant new evidence for the importance of neutral mutations and random drift.

One of the main features differentiating non-Darwinians from selectionists, according to King and Jukes, is the constancy of the rate of molecular evolution. The non-Darwinian (neutralist) expects a constant rate, because it is independent of population size and the environment. The selectionist rate is not independent of environmental effects and the effects of population size, and so must fluctuate with environmental changes. Constant rates of change in the primary structures of hemoglobins and cytochrome *c* molecules, along with work on primate albumins done by V. Sarich and A. Wilson,[118] seem to support non-Darwinian evolution. As King and Jukes put it, "uniform rates of evolutionary change also lend credence to the proposition that a substantial proportion of evolutionary change at the molecular level is due to the random incorporation of functionally insignificant change."[119]

King and Jukes also used the strong correlation between the

114. Jack King, "This Week's Citation Classic: Non-Darwinian Evolution," *Curr. Contents, 34* (1983), 25.

115. Jukes, "Early Development of the Neutral Theory" (above, n. 110), p. 477. King and Jukes were responding to Simpson, "Organisms and Molecules in Evolution" (above, n. 3), and Emil Smith, "The Evolution of Proteins," *Harvey Lect., 62* (1967), 231–246.

116. Motoo Kimura, "Genetic Variability Maintained in a Finite Population Due to Mutational Production of Neutral and Nearly Neutral Isoalleles," *Genet. Res., 11* (1968), 247–269. Kimura argues for the existence of neutral mutations using data from Tracy M. Sonneborn's article on the degeneracy of the genetic code from the "Evolving Genes and Proteins" conference. Mutations that cause no change in the amino acid sequence are called "synonymous," and are taken by Sonneborn and Kimura to usually have a minimal effect. Sonneborn suggested that 20% or more of all single-base mutations were synonymous. See T. M. Sonneborn, "Degeneracy of the Genetic Code: Extent, Nature, and Genetic Implications," in Bryson and Vogel, *Evolving Genes and Proteins* (above, n. 3), pp. 377–397.

117. King and Jukes, "Non-Darwinian Evolution" (above, n. 5), p. 789.

118. V. Sarich and A. Wilson, "Immunological Time Scale for Hominid Evolution," *Science, 158* (1967), 1200–1202.

119. King and Jukes, "Non-Darwinian Evolution," p. 796.

genetic code and the amino acid composition of proteins as evidence for the non-Darwinian model. Some amino acids, such as serine, are coded for by six possible nucleotide triplets, or codons; other amino acids, such as tryptophan, are coded for by only one codon. If the amino acid composition of a protein is the result of random forces, then serine should occur much more frequently than tryptophan. If each amino acid is selected to be just where it is in a protein, then the number of codons per amino acid should not matter; instead, the chemical properties of the amino acid in relation to the function of the protein should matter, and there should be no strong correlation between amino acid composition and number of codons. Kind and Jukes surveyed 53 proteins and found a 0.89 coefficient of correlation between the observed frequencies and the frequencies expected if amino acid composition is a product of random mutation and drift. They interpreted this as strong evidence for the power of random drift at the molecular level.[120]

Using another comparison between the protein and DNA levels, King and Jukes argued that if DNA evolution includes neutral substitutions, then the third base in a coding triplet of nucleotide bases should change at a faster rate than the first two bases, since there are more synonymous mutations in the third position. A difference in rate between protein and DNA evolution was thus expected, and according to King and Jukes it had been shown to occur.[121]

King and Jukes also presented detailed arguments concerning the evolution of a number of proteins, including cytochrome *c*, hemoglobins, immunoglobins, fibrinopeptide A, and histone IV. In the case of cytochrome *c*, they drew on the work of Margoliash and Smith[122] to argue that there can be substitutions of similar amino acids at a site without disturbing the function of the molecule. Because some regions of cytochrome *c* are essential for its function and more sensitive to change, some amino acid substitutions are taken to be more restricted than others – they show functional constraint. Despite these restricted sites, King and Jukes concluded that the possibilities for amino acid replacements are "extensive, and that many of the existing replacements are neutral."[123] Consideration of these different proteins led them to conclude

120. Ibid., pp. 796–797.

121. Ibid., pp. 789–790.

122. Margoliash and Smith, "Structural and Functional Aspects of Cytochrome *c*" (above, n. 86).

123. King and Jukes, "Non-Darwinian Evolution" (above, n. 5), p. 791.

that most proteins have regions that can accept many amino acid substitutions without significantly changing protein function.

A large amount of the initial response to the neutral theory that appeared in print was focused on the molecular data presented by King and Jukes. Bryan Clarke and Rollin Richmond, for instance, took King and Jukes's arguments as outlined above and proceeded to offer point-by-point counterarguments, so beginning the neutralist/selectionist controversy.[124]

King and Jukes are crucially important for the emergence of the neutral theory because they brought to bear, in a highly visible fashion, the vast resources of the study of molecular evolution, where as Kimura had made rather limited use of some of the same information in his first publications on the significance of neutral mutations. Yet, while King and Jukes did reply to their critics, Kimura quickly became the chief advocate of the power of random drift at the molecular level. He did so by immediately refining the work done on the rate of molecular evolution and on the time it would take a mutant gene to reach fixation in a finite population.[125] Much of this work was done in collaboration with Tomoko Ohta, who had actually surveyed the molecular evolution literature to find the specific rates of protein evolution used by Kimura in his cost-of-selection argument.[126] Together with Ohta, Kimura would build the case for the neutral theory using rate constancy and functional constraint.[127]

Part of the motivation behind Kimura's adoption of some of King and Jukes's arguments was probably the poor reception of his cost-of-selection argument. Kimura's genetic load arguments generated responses from truncated selectionists and even from King and Jukes, as noted above. In fact, arguments against the necessity of neutral alleles to explain large segregational loads became a common litany among selectionists.[128] Consequently, as Kimura's

124. Bryan Clarke, "Darwinian Evolution of Proteins," *Science, 168* (1970), 1009–11; Rollin Richmond, "Non-Darwinian Evolution: A Critique," *Nature, 225* (1970), 1025–28.

125. Motoo Kimura, "The Rate of Molecular Evolution Considered from the Standpoint of Population Genetics," *Proc. Nat. Acad. Sci., 63* (1969), 1181–88; Motoo Kimura and Tomoko Ohta, "The Average Number of Generations until Fixation of a Mutant Gene in a Finite Population," *Genetics, 61* (1969), 763–771.

126. Kimura, "Thirty Years of Population Genetics" (above, n. 93), pp. 1–10.

127. Motoo Kimura and Tomoko Ohta, "On the Rate of Molecular Evolution," *J. Mol. Evol., 1* (1971), 1–17; idem, "Mutation and Evolution at the Molecular Level," *Genetics*, suppl. *73* (1973), 19–35; "On Some Principles Governing Molecular Evolution," *Proc. Nat. Acad. Sci., 71* (1974), 2848–52.

128. See G. Ledyard Stebbins and Richard Lewontin, "Comparative Evolution

genetic load arguments were shown to be problematic, his argument based on constant rates and on King and Jukes's other arguments came to characterize the neutral theory. Kimura's book, *The Neutral Theory of Molecular Evolution*, for instance, discusses genetic loads only briefly and notes that genetic load arguments were used when the neutral theory was initially proposed but remain controversial.[129]

Reflecting on this early period, James Crow writes: "The initial response was generally one of dismay and disbelief. The reactions ranged from skepticism to outright rejection. To some it was utter nonsense." Another response was that neutral changes were simply uninteresting; the proper business of evolutionary biologists was the study of adaptations.[130] Clearly, King, Jukes, and Kimura had provoked the evolutionary establishment in 1968 and 1969 and had sparked a controversy that would move beyond the concerns of the classical and balance positions.

IS THE NEUTRAL THEORY NEO-CLASSICAL?

There is strong support for Lewontin's claim for continuity between the classical position and the neutralist position in terms of the commitment to purifying selection. Kimura advocated Crow's version of the classical position in the 1960s, and the neutral theory shows the influence of the classical position both in its development from genetic load theory and in its opposition to balancing selection in favor of purifying selection. Indeed, in their book *Theoretical Aspects of Population Genetics* (1972), Kimura and Ohta wrote:

> We conclude that the extended form of the classical hypothesis [the neutral theory] can explain the maintenance of the majority of genetic variabilities. That is, the majority of lethals and detrimentals are maintained by the balance between mutation and selection, while the majority of isoalleles are maintained by the balance between mutation and random drift. The balancing

at the Level of Molecules, Organisms, and Populations," in *Proceedings of the Sixth Berkeley Symposium on Mathematical Statistics and Probability*, vol. V, *Darwinian, Neo-Darwinian, and Non-Darwinian Evolution*, ed. L. M. LeCam, J. Neyman, and E. L. Scot (Berkeley: University of California Press, 1972), pp. 23–42; J. Maynard Smith, ""Haldane's Dilemma" and the Rate of Evolution," *Nature, 219* (1968), 1114–16.

129. Kimura, *Neutral Theory* (above, n. 73), pp. 134–135.

130. Crow, "Motoo Kimura" (above, n. 45), p. 1.

selection would probably operate on a small fraction of the total variabilities.[131]

It would be seriously misleading, however, to conclude that this commitment to the classical position and the high levels of variation found by electrophoretic surveys alone forced Kimura to propose the neutral theory. Kimura's argument and King and Jukes's arguments were based on results from molecular evolution, not electrophoretic surveys. The electrophoretic survey data brought the problem of explaining the observed high levels of protein variation and high genetic loads back to center stage, but, as Lewontin and Hubby noted in 1966, there were a number of ways to explain their results. This point was appreciated by Dobzhansky, who in 1970 interpreted the connection between the classical position and the neutralist position in much the same way Lewontin would, but noted significant differences among the neutralists.

In correspondence with King in 1970 and later, Dobzhansky tried to persuade him that his position as a neutralist or non-Darwinian was significantly different from Kimura's or Crow's. In particular, Dobzhansky wanted to persuade King that he was "not constrained to be a non-Darwinian, as others are by their past 'sins.'" It was plain to Dobzhansky "that Kimura, Crow, et al., have embraced the notion of [the] prevalence of neutral mutations since they have given "proof" that polymorphisms cannot be as numerous as they are, and evolution cannot be as fast as it is." Because of his work on truncation selection, King was not in the same predicament, since, in Dobzhansky's words, "you, as well as Sved et al., have shown that the predicament does not exist."[132] Dobzhansky thought that King was playing devil's advocate, whereas Kimura was not.

King's position was significantly different from Kimura's. In his reply to Dobzhansky, King wrote a personal note at the end of his letter stating that he wanted his work on non-Darwinian evolution to be more than just disruptive. According to King, his "ultimate goal would be to bring together Dobzhansky and Ayala with Kimura and Crow" – that is, to bring together the balance and classical positions.[133] King actually pursued this motivation

131. Motoo Kimura and Tomoko Ohta, *Aspects of Theoretical Population Biology* (Princeton: Princeton University Press, 1971), pp. 158–159.

132. Theodosius Dobzhansky to Jack King, June 8, 1970, Dobzhansky Papers, American Philosophical Society Library.

133. Jack King to Theodosius Dobzhansky, June 11, 1970, Dobzhansky Papers, American Philosophical Society Library.

while working on the neutral theory. In an article written with Tomoko Ohta and published in 1975, he explicitly assumed the classical view to construct a model of the equilibrium between mutation and selection in terms of electromorphs.[134] Yet in a letter to Dobzhansky discussing this article, he vacillates and states that he believes that the balance view is essentially correct; he concludes the letter by simply noting that "it is my folly, you know well, to attempt to reconcile the classical and balance views."[135]

Like King's, part of Kimura's motivation in proposing the neutral theory was probably the desire to address issues raised in the classical/balance controversy regarding genetic variation and genetic loads. Jukes's motivation, however, had nothing to do with the classical/balance controversy and everything to do with the power of selection in protein evolution.[136] Kimura and Jukes came to the neutral theory from different directions. The theory that is articulated and labeled the neutral theory in the early 1970s reflects a synthesis of commitments, including Kimura's, King's, Jukes's, Crow's, and Ohta's commitments to the concerns of molecular evolution and the concerns of population genetics.

THE NEUTRAL THEORY AS A THEORY OF MOLECULAR EVOLUTION

Lewontin's *Genetic Basis of Evolutionary Change* has been jokingly called "101 Ways to Save the Classical and Balance Positions."[137] In many ways, Lewontin *is* trying to save this controversy. The question of the nature of genetic variation is the problem that has driven his career.[138] When the neutral theory is viewed in terms of the problem of genetic variability, it is natural to emphasize its connection to the classical theory.[139] Lewontin's account of the historical connections that he sees between the classical and neutral positions reflects his participation in the controversies themselves, as well as what he sees as the central

134. Jack King and Tomoko Ohta, "Polyallelic Mutational Equilibria," *Genetics, 79* (1975), 688.

135. Jack King to Theodosius Dobzhansky, no date [but after 1975], Dobzhansky Papers, American Philosophical Society Library (quoted with permission).

136. Thomas Jukes, pers. comm., July 28, 1992.

137. Beatty, "Weighing the Risks" (above, n. 27), p. 290.

138. Richard Lewontin, pers. comm., August 13, 1992.

139. Lewontin has said that he was only interested in the issue of genetic variation when he wrote his historical overview of the two controversies (pers. comm., August 13, 1992).

concern of evolutionary genetics. What the preceding examination of Lewontin's Historical Thesis makes clear is that the neutral theory addresses traditional concerns in population genetics; additionally, it helps clarify the neutral theory's important role in highlighting and further articulating fundamental questions for molecular evolutionists.

As a theory of molecular evolution, the neutral theory was concerned with the processes governing change in biological macromolecules. It provided an elegant explanation for rate constancy and so provided a mechanism for the molecular clock. The molecular clock, and the issue of rate constancy, has since become a central topic in the molecular evolution literature.[140] The neutral theory also provided an explanation for differences in invariant and variant regions. In fact, in 1974 one of the five principles governing molecular evolution, according to Kimura and Ohta, was that "functionally less important molecules evolve (in terms of mutuant substitutions) faster than the important ones."[141] This issue of functional constraints has also become an important topic for molecular evolutionists.[142]

These concerns about rate constancy and functional constraint grew directly out of the molecular evolution literature and were developed and articulated as key elements of the neutral theory. The neutral theory is, thus, better viewed, not just as the resurrection of the classical position, but indeed as one of the first general theories of molecular evolution. As such, the neutral theory is one of the most significant products of the impact of molecular biology on the rest of the biological sciences.

Acknowledgments

I am indebted to a number of people for their support, commentary, and criticisms. Elisabeth Lloyd, James Griesemer, Richard Burian, B. J. T. Dobbs, Laura Lovett, and the members

140. See A. Wilson, S. Carlson, and T. White, "Biochemical Evolution," *Ann. Rev. Biochem., 46* (1977), 573–639; and the debate between Gillespie, Takahata, and Kimura; John Gillespie, "The Molecular Clock May Be an Episodic Clock," *Proc. Nat. Acad. Sci., 81* (1984), 8009–13; Naoyuki Takahata, "On the Overdispersed Molecular Clock," *Genetics, 116* (1987), 169–179; Motoo Kimura, "Molecular Evolutionary Clock and the Neutral Theory," *J. Mol. Evol., 26* (1987), 24–33.

141. Kimura and Ohta, "On Some Principles Governing Molecular Evolution" (above, n. 127), p. 2848.

142. For criticisms, see John Gillespie, *The Causes of Molecular Evolution* (Oxford: Oxford University Press, 1991), pp. 283–285.

of my dissertation committee at the University of California, San Diego shared their support and suggestions. Motoo Kimura, John Gillespie, Charles Langley, Montgomery Slatkin, Bruce Wallace, and Christopher Wills provided useful commentary on biological matters. Very helpful feedback was provided by members of the audience at the 1991 meeting of the International Society for the History, Philosophy, and Social Studies of Biological Science after a version of this paper was presented. I am especially indebted to Philip Kitcher, who supported this project at its start as a chapter of my dissertation; to John Beatty and William Provine, who shared their invaluable historical insight; and to Richard Lewontin, James Crow, and Thomas Jukes, who engaged in candid and extremely informative discussions of the history of which they were a part.