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From Mendel to Molecules: A Brief History of Evolutionary Genetics

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Biologists have been grappling with selection ever since Darwin. Historians also face a problem of selection—not natural selection, but the selection of which events to include in their narratives. No historical narrative can be complete in the sense of including every event, actor, and idea. Historians must choose which events they will include and which they will not. Writing a survey of the history of evolutionary genetics in such a short space makes this problem of selection especially acute.

A number of different approaches have been taken to the history of evolutionary genetics. Will Provine has suggested that the history of evolutionary biology is one of persistent controversy (Provine 1989; see also Lewontin 1974). Certainly one could write a history of evolutionary genetics in terms of the disputes between, for instance, the Mendelians and Biometricians, Sewall Wright and R. A. Fisher, saltationists and gradualists, the classical and balance approaches, and neutralists and selectionists (Provine 1986, 1990; Beatty 1987b; Dietrich 1994, 1995, 1998; Smocovitis 1996; Skipper 2002). Such an antagonistic view of evolutionary genetics complements histories emphasizing the great collaborations that have also characterized the history of the subject, such as those between Theodosius Dobzhansky and Sewall Wright, E. B. Ford and R. A. Fisher, or indeed those within any of the many laboratory groups working in the twentieth century (Provine 1986). More institutionally minded historians have emphasized the rise of societies, journals, and funding sources (Smocovitis 1996; Cain 1993). At the same time, others have documented the development of theoretical and experimental tools and techniques, such as the use of chromosomal inversions, electrophoresis, sequence data, population cages, computer simulations, and the vast array of evolutionary models and concepts (Lewontin 1981, 1991; Kohler 1991; Powell 1994; Gayon & Veuille 2001).

In this brief history, I will focus on the major controversies that have marked the history of evolutionary genetics in the twentieth century with special emphasis on the nature of genetic variability and the evolutionary processes acting upon this variability. This approach captures key developments in evolutionary genetics such as the resolution of the conflict between Mendelism and Darwinism and the continuing impact of molecular biology and molecular techniques.

MENDELIANS, DARWINIANS, AND THE ORIGINS OF EVOLUTIONARY GENETICS

The study of evolution and heredity have been intertwined since at least Gregor Mendel's and Charles Darwin's separate efforts to make sense of the origins of varieties and the stability of species. Mendel's experiments with many different species sought to explore the idea that new stable varieties could be created through hybridization (Olby 1979). His famous series of experiments with the garden pea quantified the instability of his hybrid crosses as it documented their hereditary patterns. Darwin's much less quantitative approach to hereditary stability or continuity across generations put much greater emphasis on processes of evolutionary change and

the problem of the origin of heritable variation. The differences between Mendel and Darwin were exaggerated after the rediscovery of Mendel's work in 1900 by Carl Correns, Hugo De Vries, and Erich von Tschermak. At this time, Darwinian evolution was criticized as insufficient for the production of new species (Bowler 1983). Evolution was widely acknowledged, but the processes of evolution remained in dispute. Hugo De Vries, for instance, articulated his Mutation Theory as a saltationist alternative to Darwinism during this period. Even Darwin's early defenders expressed concern about Darwin's account of the power of natural selection (Provine 1971).

Darwin acknowledged two forms of variation: continuous or blending variations and "sports" or monstrosities. Although he admitted that his knowledge of variation was insufficient, Darwin thought that continuous variations were the source of heritable variation for natural selection. "Sports" were larger, structural deviations, which Darwin thought were too rare and too harmful to be of evolutionary significance. Fleming Jenkin's criticisms of his views in the *Origin of Species* caused Darwin to take the idea of "sports" or discontinuous variation more seriously. Although "Darwin's bulldog," T. H. Huxley, advocated discontinuous variation, advocacy of this view is often associated with the early Mendelians, Hugo De Vries and William Bateson (Provine 1971; Kim 1994).

Darwin developed his own theory of blending inheritance as a physiological theory called "pangenesis." Like other material theories of heredity that would follow Darwin's in the late nineteenth century, Darwin postulated hereditary particles, pangenes, which corresponded to different body parts and were collected and transmitted via the gametes. While Darwin's cousin, Francis Galton, helped to refute this theory, he supported blending inheritance by developing statistical tools for precisely describing the similarities between characters. Using correlation and regression, Galton reconsidered heredity from a statistical point of view. Because he understood characters to be continuous, Galton believed that their distribution was best described by a normal distribution. The effects of selection were reconsidered in terms of effects on population means and variances. Selection could shift the mean of a population over a number of generations to create a new characteristic population mean. The relationship between parent and offspring was presented in terms of a law of ancestral heredity where a particular character of an offspring can be determined from the diminishing contribution of its ancestors (Provine 1971; Kim 1994). Galton's *Natural Inheritance* (1889) inspired Karl Pearson and W. F. R. Weldon to develop a statistical approach to biology and evolution that they called biometrics. Within the biometrical tradition, Weldon and others applied statistical methods to support gradual Darwinian evolution by natural selection. Weldon himself collected statistical evidence from crab carapaces, which he thought demonstrated the effect of selection in reducing population variability as well as the size of the carapace front. These and other efforts convinced the Biometricians that statistical methods were essential for understanding evolution and heredity.

William Bateson had also been impressed with Galton's work, but was not convinced that statistical methods were the best tools or that either evolution or heredity should be understood as continuous or blending. In 1894, Bateson argued in his book, *Materials for the Study of Variation with Special Regard to Discontinuity in the Origin of Species*, that discontinuous variations were common and saltational evolution of new species was probably the norm. The dispute between Bateson and the Biometricians began with Weldon's hostile review of his book. It was transformed into the Mendelian–Biometrician controversy when Bateson read Mendel's paper in 1900. Bateson translated Mendel's paper into English and immediately began championing it as the key to heredity and evolution. As a result, Weldon and Pearson would debate the significance of Mendel's paper vociferously over the next 10 years.

The dispute between the Mendelians and Biometricians was at once about genetic variation (continuous vs. discontinuous) and evolutionary change (gradual vs. saltational) as well as the appropriateness of statistical methods, and was overlaid with a struggle for authority and position within English biology. During the course of this dispute, the Biometricians and Mendelians drew on extended networks of biologists, and historian Kyung-Man Kim argues that the controversy was resolved by members of this extended network, not by the principal antagonists who remained strongly polarized (Kim 1994). A. D. Darbishire, for instance, set out to refute Mendelism with a set of experiments on albino and waltzing mice. Following Galton, Darbishire

reasoned that as the proportion of albino mice forming the parental and grandparental generations increased so should the percentage of albino offspring (Darbishire 1904). Darbishire's evidence in 1904 seemed to support exactly this interpretation until both William Castle and William Bateson wrote devastating critiques reinterpreting Darbishire's results in Mendelian terms (Castle 1905; Kim 1994). Darbishire himself was convinced when he tested his hybrids and realized that some of the mice that produced only albino offspring did so because they were dominant. In this case, statistical analysis of external appearance was not a reliable guide to genetic constitution. Darbishire's defection infuriated Pearson, but this was one of several conversions (Kim 1994).

More biologists joined the Mendelians after Wilhelm Johannsen introduced his pure line approach. Beginning in 1901, Johannsen sought to test whether selection could change the mean of a population's character distribution. Using a continuous distribution of bean size and weight, Johannsen selected for large, medium, and small beans. He discovered that after many generations of selection he could isolate a number of pure lines from the original distribution. Pure lines had stable characters and selection no longer had an effect on their individual means. Selection had made a difference in the original population because it was selecting among different pure lines, not because it was selecting within a pure line. Johannsen's distinction between the distribution of a character (phenotype) and the underlying pure line (genotype) was essential for resolving the Mendelian–Biometrician controversy. As early as 1904, English mathematician G. Udny Yule recognized this as a way to reconcile the biometrical description of phenotypes with Mendelian descriptions of genotypes. This route to reconciliation was reinforced with evidence for multiple factors, which allowed Mendelians to explain a continuous character distribution as the result of the interaction of many genes, each of small effect. By 1910, these developments had begun to significantly depolarize this controversy as many biologist recognized the compatibility of the Mendelian and biometrical approaches (Kim 1994).[1]

[1]Historical interpretations of this controversy have themselves been the subject of controversy concerning the relative roles of evidence and social factors in the course of the dispute. See Kim (1994).

THE DEVELOPMENT OF POPULATION GENETICS

Regardless of the outcome of the Mendelian–Biometrician controversy, the use of statistical methods formalized a population approach to evolution in the early twentieth century. At a time when even the basic language of genetics had yet to be standardized, it is not surprising that different approaches to the mathematical description of evolution would also arise. The rise of mathematical population genetics is usually associated with the work of three founders: Sewall Wright, R. A. Fisher, and J. B. S. Haldane. Their work set the foundations for population genetics, as each attempted to formally reconcile Mendelism and Darwinism (Provine 1971).

Wright was an American biologist trained in genetics at Harvard University by William Castle. His early interest in mammalian genetics led him to create the method of path analysis as a staff scientist at the US Department of Agriculture. By 1921, he had developed his method of path coefficients to describe the effects of inbreeding, assortative mating, and selection. When he joined the faculty of the University of Chicago in 1925, Wright shifted his thoughts from guinea pig colonies and cattle herds to evolving natural populations. By 1931, he had articulated his shifting balance theory of evolution in his now classic paper "Evolution in Mendelian Populations" (Wright 1931; Provine 1986).

R. A. Fisher was an English biologist trained at Cambridge in mathematics. Introduced to Mendelism and Biometry at Cambridge, Fisher sought to reconcile the two by understanding the biometrical properties of Mendelian populations. This approach led him to characterize similarities within Mendelian populations in terms of their variance and the contributions to variance from genetic sources, environmental sources, dominance, and gene interactions. Fisher's approach emphasized natural selection acting in very large natural populations. He set out his general theory in his 1930 book, *The Genetical Theory of Natural Selection* (Provine 1971, 1986).

J. B. S. Haldane was also an English biologist with broad interests. He studied mathematics at Oxford before switching to classics and philosophy. Beginning in 1922, Haldane sought to analyze the mathematical consequences of natural selection. Starting from simple Mendelian models using two

alleles at a single locus, Haldane went on to consider selection with self-fertilization, inbreeding, overlapping generations, incomplete dominance, isolation, migration, and fluctuating selection intensities (Provine 1971). Haldane's series of nine papers on selection culminated in his 1932 book, *The Causes of Evolution*. In the appendix to this book, Haldane compares his views to those of Fisher and Wright. While he agrees with elements of both of their views, Haldane differed from Fisher by placing greater emphasis on strong selection of single genes, migration, and epistasis. He sided with Fisher, however, in thinking that Wright put too much emphasis on random genetic drift (Provine 1971; Gillespie, Ch. 5 of this volume).

While Fisher, Wright, and Haldane approached evolution and population genetics from different mathematical perspectives, their disagreements were not about mathematics, but about evolutionary processes and concepts and their representation in different mathematical models. According to Will Provine, Fisher and Wright were engaged in a series of disputes from 1929 until 1962 when Fisher died (Provine 1986, 1992). While they debated many things, the core of their difference lay in their general theories of evolution: Wright's shifting balance theory and Fisher's large population theory. Wright's approach incorporated an array of evolutionary processes and emphasized population subdivision (Goodnight, Ch. 6 of this volume). Fisher argued that natural selection was the dominant process and that large populations were the optimum. These differences were most apparent around the issue of the relative importance of random genetic drift. Although Wright continued to elaborate his views, his early work on the shifting balance theory gave random drift a considerable role in evolution. To counter Wright's view, Fisher and his colleague E. B. Ford studied yearly fluctuations in the gene (allele) frequencies of the moth *Panaxia dominula* from 1941 to 1946. They found that the fluctuations they observed were too great to be accounted for by the action of random genetic drift. Instead, they proposed that the fluctuations were the result of random fluctuations in the strength of natural selection. As this dispute intensified and extended in the 1950s to results on banding patterns in the snail *Cepaea nemoralis*, Wright began to modify his views, limiting the action of random drift to large, but subdivided populations where it could serve as a means for generating novel genotypic combinations (Provine 1986, 1992). The Wright–Fisher debate has resurfaced in recent years with new protagonists (Skipper 2002), but the original debate was especially influential because it occurred just as Neo-Darwinism was being articulated in the evolutionary synthesis (Provine 1992).

THE EVOLUTIONARY SYNTHESIS

The evolutionary synthesis is identified by historians with both the emerging discipline of evolutionary biology and the integration of previously divergent fields such as paleontology, zoology, botany, systematics, and genetics. According to this interpretation, the synthesis refers to a time beginning in the 1930s when a range of arguments were offered to show that different fields relevant to evolution were in fact compatible with each other. These compatibility arguments helped spur on the emergence of evolutionary biology as a field of inquiry—as a new and centrally important discipline (Smocovitis 1996). Compatibility arguments do not necessarily imply that there was widespread agreement on a new synthetic theory of evolution. As Provine and others have argued, there was little agreement about the mechanisms of evolution during the 1930s and 1940s. Instead Provine suggests that we reconsider this period as an evolutionary constriction—"a vast cut-down of the variables considered important to the evolutionary process." According to Provine, "The term 'evolutionary constriction' helps us understand that evolutionists after 1930 might disagree intensely with each other about effective population size, population structure, random genetic drift, levels of heterozygosity, mutation rates, migration rates, etc., but all could agree that these variables were or could be important in evolution in nature, and that purposive forces played no role at all" (Provine 1988).

The foundation for the evolutionary synthesis was communicated in a number of now classic texts: R. A. Fisher's *The Genetical Theory of Natural Selection* (1930), Theodosius Dobzhansky's *Genetics and the Origins of Species* (1937), Julian Huxley's *Evolution: The Modern Synthesis* (1942), Ernst Mayr's *Systematics and the Origin of Species* (1942), G. G. Simpson's *Tempo and Mode in Evolution* (1944), and G. L. Stebbins' *Variation and Evolution in Plants* (1950).

Dobzhansky's work represented the state of the art in animal genetics and population genetics.

Trained in the Soviet Union and influenced by the work of Nicolai Vavilov and Iurii Filipchenko, Dobzhansky began his career studying variability in natural populations of *Coccinellidae* and *Drosophila melanogaster*. To further his understanding of genetics, he received funding from the Rockefeller Foundation to join T. H. Morgan's famous Fly Group in 1927 (Provine 1981). At Columbia and later Cal Tech, Dobzhansky excelled at the business of *Drosophila* genetics. First with A. H. Sturtevant and later in collaboration with Sewall Wright, Dobzhansky turned to evolutionary genetics—taking *Drosophila* genetics from the laboratory to the field. Dobzhansky's 1937 book, *Genetics and the Origin of Species,* articulated a program of research for evolutionary genetics. The theoretical underpinnings of Dobzhansky's program were deliberately borrowed from Wright's shifting balance theory. Unlike Wright's papers, however, Dobzhansky's presentation was non-mathematical and served to widely popularize the shifting balance theory (Provine 1981). *Genetics and the Origin of Species,* thus, translated one of the dominant general theories of evolution into a research program for evolutionary genetics.

Dobzhansky's evolutionary program was challenged in 1940 by Richard Goldschmidt's *The Material Basis of Evolution*. Goldschmidt had been Director of the Kaiser Wilhelm Institute of Biology in Berlin before he was forced to emigrate in 1936. Once in the United States, Goldschmidt challenged the gradualist model of evolution promoted by Dobzhanksy and others. According to Goldschmidt, Dobzhanksy had not demonstrated that his view fit the evidence any better than the view that there were bridgeless gaps between species which could only be crossed by either systemic mutations (large rearrangements of chromosomal structure) or mutations in developmentally important genes. Goldschmidt's developmentally oriented, saltationist alternative immediately inspired a hostile reaction by the Neo-Darwinians. Subsequent editions of Dobzhansky's *Genetics and the Origin of Species* devoted many pages to Goldschmidt's refutation, as did later work by Mayr and Simpson. This negative response to Goldschmidt's views bolsters Provine's interpretation of the synthesis as a constriction. In fact, opposition to Goldschmidt's saltationism became a defining feature of Neo-Darwinism (Dietrich 1995).

Ernst Mayr's *Systematics and the Origin of Species* (1942) responded to Goldschmidt's claims, but was modeled on Dobzhanky's *Genetics and the Origin of Species*. Where Dobzhansky synthesized genetics with evolutionary biology, Mayr added concepts of speciation and species. Trained as an ornithologist in Germany under Hans Stresseman, Mayr was the world's expert on bird systematics. Although developed with avian exemplars, Mayr argued for the generality of his Biological Species Concept (Mallet, Species Concepts box, pp. 367–373 of this volume) and model of geographic speciation. If Dobzhansky was the first to set the intellectual agenda for evolutionary genetics, Mayr broadened that agenda. Moreover, Mayr was absolutely central to the effort to institutionalize and support the development of evolutionary biology as a discipline. Together with G. G. Simpson, who articulated the contributions of paleontology for the synthesis, Mayr, Dobzhansky, and other scientists in the Northeastern United States discussed the similarities and differences in their approaches to evolution in the Committee on Common Problems in Genetics and Paleontology, which met from 1943 to 1945 when the Society for the Study of Evolution was founded (Smocovitis 1996; Cain 1993). Because of World War II, Mayr, Simpson, and Dobzhansky were somewhat isolated from biologists in England (Huxley and Fisher) and evolutionary biologists on the West Coast of the United States (Stebbins). This temporary isolation may be one reason why Dobzhansky, Simpson, and Mayr were so influential in the development of Neo-Darwinism, and why Neo-Darwinism seemed particularly focused on animal systems. The considerable effort of Stebbins and others to bring plants into the synthesis is surely also a result of the interesting differences between plant and animal genetics (Smocovitis 1996).

The architects of the evolutionary synthesis played a central role in the promotion of evolutionary biology and especially evolutionary genetics. Dobzhansky's work on the genetics of natural populations, in particular, was hailed as an exemplar of Neo-Darwinism (Mayr 1944; Stern 1944). Significantly, during the 1940s Dobzhansky's own research program narrowed. From 1938 to 1976, Dobzhansky and his collaborators produced a series of 43 influential papers under the title of "The Genetics of Natural Populations" (GNP) (Lewontin 1981). Early work in the GNP series was often conducted in collaboration with Sewall Wright and sought to explore different aspects of the shifting balance theory using data from characteristic chromosomal inversion of different natural populations.

Because Dobzhansky thought that selection had little effect on inversion frequency, his work with Wright concentrated on breeding structures and the impact of random drift. As early as 1943, however, Dobzhansky's attention begins to shift toward selection favoring heterozygotes. By 1950, the GNP series and Dobzhansky's research program began increasingly to address problems of heterosis and balancing selection (Beatty 1987a). This transition from drift to selection is emblematic of the emerging view in the 1950s that natural selection is the predominant process of evolution. Dubbed the "hardening of the synthesis" by Stephen Jay Gould, the constriction characteristic of the synthesis period had produced a type of pan-selectionism that would dominate evolutionary biology into the 1970s (Gould 1983). Focusing on selection to the exclusion of other processes did not guarantee that consensus. Instead, new controversies emerged concerning the form of selection and the availability of genetic variation.

GENETIC VARIABILITY AND THE CLASSICAL–BALANCE CONTROVERSY

In the 1950 and 1960s, Dobzhansky's research on balanced polymorphisms fueled a major controversy in evolutionary genetics concerning the genetic variability of natural populations, the nature of selection, and the genetic effects of atomic radiation. In 1955 at the meeting of the Cold Spring Harbor Symposium on Quantitative Biology, Dobzhansky articulated two diametrically opposed positions on these issues: the classical position and the balance position. The classical position, according to Dobzhansky, held 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." Most loci, according to the classical position, should be homozygous. Heterozygotes were rare and had four possible sources: (1) deleterious mutations that are eventually eliminated by selection, (2) adaptively neutral mutations, (3) "adaptive polymorphisms maintained by the diversity of the environments which the population inhabits," and (4) rare beneficial mutants which are on their way toward fixation (Dobzhansky 1955). According to Dobzhansky, the main proponent of the classical position was H. J. Muller. The balance position, according to Dobzhansky, held that most loci should be heterozygous. Homozygotes would still occur, but they would not be as advantageous as overdominant heterozygous combinations. In terms of genetic variation, the issue at stake between the classical and balance positions was the relative number and importance of heterozygous superior or overdominant loci. Dobzhansky cast himself as the primary advocate of the balance position.

Muller never agreed with Dobzhansky's characterization of the classical and balance positions, but he had articulated something close to the classical position. An original member of Morgan's Fly Group, Muller was a world leader in genetics having won a Nobel Prize in 1948 for his research on the production of mutations with X-rays. In 1950, he published "Our Load of Mutations," which provided a new way to assess the genetic damage created by mutation. Accepting the premise that the vast majority of mutations are harmful to some degree, Muller argued that in a population of constant size, each mutation leads to one "genetic death"—to one individual that fails to reproduce. The number of deleterious alleles possessed by an individual represented that individual's deviation from a genetic ideal—that person's genetic load. Because he had pioneered much of the early work on the genetic effects of radiation, Muller was adamant about the genetic loads that exposure to radiation could produce. This concern reflected the damaging effects of radiation on genetic material and was motivated by the recent use of atomic weapons in World War II and was heightened by the ongoing Cold War arms race and testing programs. Thus, it was natural that, when Muller discussed factors that would increase genetic loads and put human populations at risk, radiation was prominent (Beatty 1987b).

Muller's radiation fears were exacerbated by a series of ambiguous results from irradiation experiments conducted in the 1950s and 1960s. Bruce Wallace, a student of Dobzhansky's, had been collaborating with J. C. King to study the effects of radiation exposure in *Drosophila*. Setting a control population as the standard, Wallace and King exposed flies to acute and chronic doses of radiation. If Muller was correct, the radiation should induce deleterious mutations and lower the fitnesses of the treated populations relative to the control population. The flies receiving chronic irradiation did indeed have a lower adaptive value, but the acutely irradiated flies had a higher adaptive value. Interpreting this result in light of the balance position,

Wallace and King argued that improvement of the acutely irradiated population "could exist not merely *in spite of* but *because of* the original treatment" (Wallace & King 1951). Wallace and King's results were meant to invite further research, which they did, but they also invited controversy. Wallace himself continued to refine his radiation experiments, while Muller worked with a graduate student, Raphael Falk, to perform similar experiments. None of these experimental efforts were convincing in the end, in part because it was impossible to pin down the exact effects of the irradiation—it was unclear then that irradiation was producing new overdominant loci. Despite efforts to bring the disputants together to work out their differences, by the 1960s the classical–balance controversy had stalemated (Beatty 1987b).[2]

By linking genetic variability to radiation, the stakes in this controversy had been raised beyond those of an intellectual dispute in evolutionary genetics. Both Muller and Dobzhansky saw themselves as struggling for the future of humankind. Hope of some empirical resolution depended on a way of detecting genetic differences more precisely. The tools for addressing this issue had been developing within biochemistry and molecular biology for a number of years. However, the introduction of molecular tools and data into evolutionary genetics would fundamentally alter the classical–balance controversy rather than settle it (Dietrich 1994; Lewontin 1974).

THE ELECTROPHORETIC REVOLUTION

Electrophoresis had been developed in biochemistry as a means for separating molecules by charge and size. In the early 1960s, geneticist Jack L. Hubby began to adapt electrophoresis for use with *Drosophila*. When Richard Lewontin moved to the University of Chicago to collaborate with him in 1964, Hubby's original program of research changed significantly. Lewontin was a student of Dobzhansky's and had been following the classical–balance debate closely. When Lewontin arrived in Chicago, he had a list of criteria for experimentally resolving 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. (Hubby & Lewontin 1966, p. 578)

Hubby and Lewontin's work tried to meet these criteria and provide a reliable measure of the amount of heterozygosity found in *D. pseudoobscura*. Their survey of 18 loci revealed what they understood to be a high degree of polymorphism; the average heterozygosity was 11.5%. Lewontin and Hubby proposed several alternatives to explain this variation. The possibility of neutral alleles was considered, and ruled out because local populations did not have the high levels of homozygosity predicted if drift were prevalent. They also considered the possibility of a large number of overdominant loci, but recognized that so many heterotic loci would carry with them a large segregational load (Lewontin & Hubby 1966). Almost immediately three different groups proposed truncation selection models to address this problem. It looked as if electrophoresis had provided important evidence in favor of the balance position. This sense of resolution was short-lived, however, as the advocacy of neutral molecular evolution, beginning in 1968, redrew the conceptual landscape.

Apart from the classical–balance controversy, electrophoresis had a tremendous impact upon the experimental practice of evolutionary genetics. From 1966 to 1984, the genetic variability of 1111 species was measured using electrophoresis. This "find 'em and grind' em" approach expanded the scope of evolutionary genetics, drew more people to consider the problem of explaining variability, and demonstrated the power of molecular techniques for evolutionary biology (Lewontin 1991). Electrophoresis was only a part of the molecular biology boom going on in the 1960s, however. After James Watson and Francis Crick discovered the double helical structure of DNA in 1953, molecular biologists and biochemists began to address the evolution of DNA, RNA, and proteins, as well as their coding

[2]See the transcript of the Macy Conference at http://hrst.mit.edu/hrs/evolution/public/archives/macyconference/macy.html

properties and interrelations. In the 1960s and 1970s, the new field of molecular evolution would incorporate new data from electrophoresis, immunological assays, hybridization, and sequencing. In doing so it would transform significant parts of evolutionary genetics (Dietrich 1998).

NON-DARWINIAN EVOLUTION AND THE NEUTRALIST–SELECTIONIST CONTROVERSY

Molecular evolutionary genetics developed in the late 1960s with the spread of experimental techniques, such as electrophoresis, and with theoretical developments that embraced these new molecular data. The most significant theoretical or conceptual developments associated with the molecularization of evolutionary genetics were the introduction of the molecular clock and the advocacy of neutral molecular evolution or, as it was called at the time, Non-Darwinian evolution.

In 1965 Emile Zuckerkandl and Linus Pauling articulated what was later referred to as "the most significant result of research in molecular evolution" (Wilson et al. 1977). After comparing the amino acid sequences of proteins from different lineages, Zuckerkandl and Pauling discovered that the differences in amino acid sequence were "approximately proportional in number to evolutionary time" (Zuckerkandl & Pauling 1965). In other words, the rate of amino acid substitution was approximately constant. Zuckerkandl and Pauling christened this constancy the molecular clock (Morgan 1998; Rodríguez-Trelles et al., Ch. 8 of this volume). The value of the molecular clock for systematics was quickly recognized, but the evolutionary mechanisms underlying the clock's constancy were ambiguous until Motoo Kimura, Jack King, and Thomas Jukes made their case for neutral molecular evolution.

Motoo Kimura was a Japanese biologist who had worked with James Crow and Sewall Wright in the United States on mathematical population genetics. As Crow's student, Kimura was familiar with the classical–balance controversy and was sympathetic to the classical position, as was Crow. The possibility of neutral alleles had been frequently mentioned in the course of the classical–balance controversy, but none of the participants seemed to have taken them seriously as an alternative to a system of alleles under some form of selection. Indeed in 1964, Crow and Kimura developed the infinitely many alleles model which, while it presented a model of mutation for neutral alleles, was primarily aimed at demonstrating the high loads produced by more complex models of overdominant alleles. Kimura later shifted his perspective on neutral alleles from a mathematically tractable case to a description of a biological reality. He did so in response to both the high genetic variability observed by Lewontin and Hubby and an array of biochemical evidence for neutral alleles being presented and discussed at the first conferences on molecular evolution, such as the Evolving Genes and Proteins conference in 1965 where Zuckerkandl and Pauling christened the molecular clock. Indeed Kimura's 1968 argument for neutral molecular evolution is based on data about rates of molecular change presented at the Evolving Genes and Proteins conference, including the hemoglobin data presented by Zuckerkandl and Pauling (Dietrich 1994). Kimura's colleague Tomoko Ohta estimated the rate of amino acid change in mammalian hemoglobin, primate hemoglobin, mammalian and avian cytochrome *c*, and triosephosphate dehydrogenase from rabbits and cattle. Kimura then calculated the rate of evolution for a mammalian genome. Kimura's estimate of 1.8 years for the average time taken for one base pair replacement carried with it an intolerable cost of selection. The only way to avoid this high cost or substitutional load was to postulate that most of the observed substitutions were in fact selectively neutral (Kimura 1968).

Kimura's position was strongly reinforced the next year by Jack King and Tom Jukes who strongly advocated the importance of neutral mutations and genetic drift. Jukes was a biochemist by training and an early 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. Like many other biochemists interested in evolution, Jukes recognized the existence of neutral substitutions, but to develop his views he needed the help of a population geneticist. Jukes sought out Jack King, a young biologist with training in evolutionary genetics. Together they assembled a broad range of evidence from biochemistry and molecular evolution to directly counter G. G. Simpson's and Emil Smith's claims for panselectionism at the molecular level (Dietrich 1994). Under the intentionally provocative title of Non-Darwinian Evolution, they presented a case for neutral molecular evolution that included Kimura's cost of selection argument as well as

arguments based on the significance of synonymous mutations, correlation between the genetic code and the amino acid composition of proteins, higher rates of change at third positions of codons, and overall constancy of the rate of molecular evolution. The response to Kimura, King, and Jukes was immediate and hostile. Bryan Clarke and Rollin Richmond, for instance, offered point by point counterarguments to the evidence presented by King and Jukes, thereby inaugurating the neutralist–selectionist controversy (Clarke 1970; Richmond 1970).

In 1969, Kimura used the constancy of the rate of amino acid substitutions in homologous proteins to argue powerfully for neutral mutations and the importance of random drift in molecular evolution (Kimura 1969b). At the same time, Kimura was also calling on his earlier work on stochastic processes in population genetics (Gillespie, Ch. 5 of this volume) to forge a solid theoretical foundation for the neutral theory. Kimura's diffusion equation method provided the theoretical framework he needed formulate specific models which in turn allowed him to address issues such as the probability and time to fixation of a mutant substitution as well as the rate of mutant substitutions in evolution (Kimura 1970). Working in collaboration with Tomoko Ohta, Kimura also extended the neutral theory to encompass the problem of explaining protein polymorphisms. This was a central concern of population genetics, and Kimura and Ohta were able to show that protein polymorphisms were a phase in mutations' journey to fixation (Kimura & Ohta 1971a).

In 1971 the Sixth Berkeley Symposium on Mathematical Statistics and Probability devoted a session to Darwinian, Neo-Darwinian, and Non-Darwinian evolution. By this time, the debate between the neutralists and selectionists was well under way. Although few tests had been done, there had been quite a bit of talk about the ability of rival hypotheses to explain a wide variety of data and the positions were well articulated. James Crow was charged with giving a review of both sides of the debate to start the conference session. Crow was disposed toward the neutral theory, but was more skeptical than either Kimura or Ohta. As a participant in the classical–balance controversy, Crow had experienced the frustration of trying to find definitive tests for either position; as a result he valued the neutral theory because it offered quantitative predictions that could be tested and seemed to move beyond the classical–balance stalemate (Crow 1972).

At the same symposium, G. L. Stebbins and Richard Lewontin attacked the neutral theory as a testable hypothesis. According to Stebbins and Lewontin, the neutral theory in its simplest form predicts that allele frequencies will vary from population to population, but in *D. pseudoobscura* and *D. willistoni*, widely separate populations show very similar allele frequencies. A migration rate as low as one migrant per generation could account for the similarity. Because assumptions about migration rate could always explain away allele frequency data, Stebbins and Lewontin charged that no observation could contradict the neutral theory's prediction. They even directly appealed to Karl Popper's philosophy of science and labeled the neutral theory "'empirically void' because it has no set of potential falsifiers" (Stebbins & Lewontin 1972). Yet, Stebbins and Lewontin did not reject the idea of neutral mutation and the effects of random drift; instead they claimed that the nature of evolutionary processes was unresolved and encouraged the diverse pursuits of selectionists and neutralists (Stebbins & Lewontin 1972).

Stebbins and Lewontin's concerns about testing the neutral theory would be compounded over the next 10 years. Despite an abundance of data from electrophoretic surveys, using this data to test predictions from the neutral theory was not as straightforward as it had been supposed. Tests proposed by Warren Ewens in 1972 and later refined by Geoff Watterson in 1977 were designed for electrophoretic data, but when applied did not have the statistical power to discriminate between neutrality and selection (Lewontin 1991). The consequence of this and other difficulties with testing the neutral theory was that neutralists put more stock in the molecular clock as evidence in support of neutrality.

In 1971, Tomoko Ohta and Motoo Kimura asserted that the "remarkable constancy of the rate of amino acid substitutions in each protein over a vast period of geologic time constitutes so far the strongest evidence for the theory (Kimura 1968; King and Jukes 1969) that the major cause of molecular evolution is random fixation of selectively neutral or nearly neutral mutations" (Ohta & Kimura 1971). Kimura had shown that for neutral changes the rate of substitution was equivalent to the rate of mutation. Because the rate of mutation was understood to be the result a stochastic process similar to radioactive decay, the rate of substitution could also be understood as constant generated by an underlying stochastic process. The rate of selected

substitutions, however, was subject to changes in selection intensity and population size and so could not be expected to be constant over any long period of time.

Whether recognized as a proxy for the neutralist–selectionist debate or not, the molecular clock was the subject of intense debate. For instance, because the molecular clock was a stochastic clock, some variability in its rate was expected. By as early as 1974, however, Walter Fitch and Charles Langley argued that the rate of substitution was not as uniform across different lineages as it ought to be if the neutralist explanation was correct (Langley & Fitch 1974). Morris Goodman and others joined in this line of criticism, adding evidence of slowdowns and speedups from various lineages. In response, Kimura admitted that the rate of molecular evolution was not perfectly uniform, but in his opinion, "emphasizing local fluctuations as evidence against the neutral theory, while neglecting to inquire why the overall rate is intrinsically so regular or constant is picayunish. It is a classic case of 'not seeing the forest for the trees'" (Kimura 1983). Selectionist critics were undeterred. With growing evidence that rate variability was much more pronounced than had been supposed, John Gillespie proposed a selectionist episodic molecular clock that he claimed could explain patterns of substitution better than Kimura's neutralist explanation (Gillespie 1984). To answer Gillespie's claims, neutralists revised their models of substitution to accommodate greater variability. The amount of variability that can be accommodated by the clock concept remains an open question (although see Rodríguez-Trelles et al., Ch. 8 of this volume).

The neutralist–selectionist controversy itself was transformed during the 1980s with the introduction of DNA sequence data. As a graduate student working with Richard Lewontin, Martin Kreitman learned how to sequence DNA in Walter Gilbert's laboratory at Harvard. Kreitman then sequenced ADH genes in *Drosophila* looking for evidence of polymorphisms. Kreitman's detection of polymorphisms in the DNA sequences suggested that there was selection at the ADH locus and that differences between synonymous and non-synonymous sites were significant. Kreitman would develop the analysis of patterns of nucleotide sequence comparisons into the Hudson–Kreitman–Aguadé test and the McDonald–Kreitman test. These statistical tests and others allowed evolutionary geneticists to detect selection at the molecular level (Kreitman 2000). Where earlier tests had been unable to discriminate between neutrality and selection, these tests applied to nucleotide sequence data succeeded.[3]

Accompanying the availability of DNA data was a significant change in attitude toward neutrality. When Kimura proposed the neutral theory in 1968, the dominant attitude of biologists was that natural selection was the only important cause of evolutionary change at any level of organization. This panselectionist attitude informed the early opposition to the possibility of neutral molecular evolution. By the mid-1980s, however, the dominant attitude among evolutionary geneticists using molecular data was that the neutral theory provided the starting place for investigation in the sense of being the accepted null model (Kreitman 2000). Why hypotheses of neutral molecular evolution became accepted as null hypotheses at this time has yet to be investigated by historians, but the rise of neutral null models seems to coincide with increased availability of DNA sequence data, increasing use of molecular clocks in systematics, increasing use of coalescents, and the spread of tests such as the Hudson–Kreitman–Aguadé test.

CONTROVERSY AND THE HISTORY OF EVOLUTIONARY GENETICS

By emphasizing controversy, I have presented one perspective on the history of evolutionary genetics. The controversies of evolutionary genetics highlight the interplay of theory and experiment, the impact of new concepts and results, as well as the power of personality and politics. Controversies, such as those between the Mendelians and Biometricians or Fisher and Wright were often heated and sometimes quite personal. Like all criticism in science, however, controversies also present the possibility of change. The controversies of evolutionary genetics typically began as highly polarized disputes, but the positions in question developed, sometimes radically, sometimes more subtly. These transformations allowed the controversies to depolarize by enabling some participants to disengage, revise their opinions, or change their focus. Whether the future of evolutionary

[3]The history of these tests as well as a discussion of their development and significance by Martin Kreitman and Richard Lewontin are available at http://hrst.mit.edu/hrs/evolution/public/kreitman.html.

genetics is doomed to persistent controversy is hard to say, but controversy has been an unavoidable feature of its past.

SUGGESTIONS FOR FURTHER READING

Provine (1986) provides an excellent overview of the development of evolutionary genetics as it traces the life of Sewall Wright. The earlier debate between the Mendelians and Biometricians is expertly analyzed in Kim (1994). Because it also includes commentaries by other historians of genetics, Kim (1994) provides a useful introduction to the debates among historians, sociologists, and philosophers over scientific controversy. Lewontin et al. (1981) is a collection of Theodosius Dobzhansky's papers in the Genetics of Natural Population series. This very influential set of papers is contextualized by two extensive introductions, one by Provine and the other by Lewontin. The impact of molecular biology on evolutionary genetics and the rise of molecular evolution are examined in Dietrich (1994).

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