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# Molecular Evolution

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# Chapter 9

# Molecular Evolution

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Molecular evolution emerged as a hybrid discipline in the 1960s. Blending theoretical and experimental traditions from evolutionary genetics, molecular biology, biochemistry, systematics, anthropology, and microbiology, molecular evolution represented a significant reconsideration of several key features of the preceding neo-Darwinian evolutionary synthesis. Where neo-Darwinians articulated a unified understanding of the evolutionary process dominated by selection, by the 1970s most molecular evolutionists recognized that the domain of evolutionary biology was divided into molecular and morphological levels. Where neo-Darwinians advocated variable rates of evolution driven by environmental change, molecular evolutionists advocated a molecular clock that approximated a constant rate of change in proteins and nucleic acids. Where systematics had been based on morphological features, it now had a vast new array of molecular data and the challenge of reconciling sometimes divergent phylogenetic inferences.

The changes introduced by molecular evolution created enormous controversy during the 1960s and 1970s. While these disputes have tended to ease over time, controversy remains one of the persistent features of the history of molecular evolution. As such, molecular evolution provides a very rich history for the analysis of scientific controversy, testing, experimentation, and methodology.

## 1. The Neutral Theory of Molecular Evolution

When molecular evolution emerged as a field in the early 1960s, biochemists, molecular biologists, and some evolutionary biologists began to consider that some changes in proteins and nucleic acids were not selected. The possibility of neutral mutations was widely acknowledged by evolutionary biologists, such as Theodosius Dobzhansky (1955), but the existence of a significant number of neutral mutations was not taken seriously by most evolutionary geneticists.

Attitudes began to change in 1968 when Motoo Kimura argued that many substitutions at the molecular level were not subject to natural selection, but were instead governed by random drift (Kimura, 1968; also see Dietrich, 1994, and Suarez & Barahona, 1996). Using protein sequence data generated by biochemists such as Emile Zuckerkandl

and Emmanuel Margoliash, Kimura and his colleague Tomoko Ohta compared mammalian protein sequences and used the number of detected differences across species to calculate a rate of molecular evolution. Kimura then reasoned that if most mutations were in fact harmful, then the rate of evolution calculated for mammals would create an intolerable genetic load (an accumulation of too many harmful alleles). Since mammals were not extinct or staggering under an enormous genetic load, Kimura concluded that most detected molecular variants were in fact neutral (Kimura, 1968).

Kimura's conclusion and argument were controversial, but the dispute between neutralists and selectionists was guaranteed in 1969 when Tom Jukes and Jack King wrote their neutralist manifesto under the provocative title of "Non-Darwinian Evolution." King and Jukes brought a large variety of evidence to bear in favor of large numbers of neutral mutations (King & Jukes, 1969). By using evidence from the growing field of molecular evolution to support the idea of neutral mutations and the importance of random drift, they spelled out the molecular consequences of the neutral hypothesis more clearly than Kimura had. King and Jukes built their case using phenomena such as synonymous mutations, the Treffors mutator, the relationship between amino acid frequencies and the genetic code, and the growing body of data on specific proteins such as cytochrome c.

Although many biologists were extremely skeptical of the neutral theory, Kimura and his colleague Tomoko Ohta pursued the neutral theory vigorously. 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, 1969). At the same time, Kimura was also calling on his earlier work on stochastic processes in population genetics to forge a solid theoretical foundation for the neutral theory. Kimura's diffusion equation method provided the theoretical framework he needed to 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' long journey to fixation (Kimura & Ohta, 1971).

In 1971 the Sixth Berkeley Symposium on Mathematical Statistics and Probability devoted a session to Darwinian, neo-Darwinian, and non-Darwinian evolution. Selectionist responses to King and Jukes' paper had created a full-blown controversy (Clarke, 1970; Richmond, 1970). Although the positions were becoming well articulated, there had only been a handful of empirical tests proposed. James Crow was charged with giving a review of both sides of the debate to start the conference session. Crow had been Kimura's advisor and remained a close friend and colleague. He was disposed toward the neutral theory, but was more skeptical than Kimura, Ohta, King, or Jukes. Like many others at the time, Crow believed that there was a continuum of fitness values for new mutations ranging from extremely detrimental through neutral to slightly beneficial. The conflict between neutralists and selectionists was thus a matter of the *relative importance* of neutral alleles and random drift relative to selection. In general, the neutralists had two battles to win: they had to prove that neutral alleles exist, and they had to prove that they play a significant role in evolution.

Crow's review was sympathetic to Kimura's position and as such answered a number of criticisms and provided several important arguments for the value of the neutral theory. Among the reasons *not* to accept neutralism or non-Darwinian evolution listed by Crow was the idea that "a random theory may discourage a search for other explanations and thus may be intellectually stultifying" (Crow, 1972, p.2) and that neutral changes were not as interesting as adaptive changes. Since adaptive change was a central concern, the neutral theory was not considered relevant. However, Crow notes that the neutral theory leads to a formulation of the important factors in evolution that has both new ideas and quantitative predictions. Moreover, "it is directly concerned with the gene itself, or its immediate products, so that the well-developed theories of population genetics become available. It produces testable theories about the rates of evolution" (Crow, 1972, p.2). Clearly Crow thought that the neutral theory was not intellectually stultifying: it was instead a source of innovation because of its testability and its new integration with molecular biology.

Tapping into the data and techniques of molecular biology was an important source of innovation for population genetics in the early 1970s. For population biology, the 1950s and 60s had been marked by a dispute over the type of genetic variation in natural populations and the forces responsible for maintaining that variation. Extreme positions advocating large amounts of homozygosity and purifying selection (the classical position) or large amounts of heterozygosity and balancing selection (the balance position) divided the community. H. J. Muller and Crow both advocated versions of the classical position, while Theodosius Dobzhansky and many of his students advocated versions of the balance position. By the mid-1960s, however, the controversy had stalemated – traditional experiments using radiation induced mutations were proving to be indecisive and extremely controversial (Beatty, 1987a; Lewontin, 1991). As Richard Lewontin, a student of Dobzhansky's, puts it, "population genetics seemed doomed to a perpetual struggle between alternative interpretations of great masses of inevitably ambiguous data" (Lewontin, 1991, p.658). What was needed was a way of breaking this deadlock. In 1964, Richard Lewontin thought he had found it in Jack Hubby's work using electrophoresis. Electrophoresis is a biochemical technique for separating proteins by charge and size. When applied to proteins from *Drosophila*, Hubby and Lewontin detected higher than expected levels of heterozygosity (Hubby & Lewontin, 1966; Lewontin & Hubby, 1966). This level was high enough to tilt the dispute toward the balance position, if only for a short while. Kimura's proposal that much of the variability that Hubby and Lewontin had detected was in fact neutral shifted the conceptual foundations of the classical balance dispute, but the technique of electrophoresis itself shifted the debate in terms of experimental practice (Suarez & Barahona, 1996).

Electrophoresis brought experimental population genetics down to the molecular level. In Lewontin's words, "Here was a technique that could be learned easily by any moderately competent person, that was relatively cheap as compared with most physiological and biochemical methods, that gave instant gratification by revealing before one's eyes the heritable variation in unambiguously scoreable characters, and most important, could be applied to *any* organism whether or not the organism could be genetically manipulated, artificially crossed, or even cultivated in the laboratory greenhouse. It is little wonder that there was a virtual explosion of electrophoretic investiga-

tions" (Lewontin, 1991, p.658). The introduction of electrophoresis to population genetics opened up the possibility of routine experimentation at the molecular level. It was in this context that Crow had advocated the molecularization of population genetics at the 12th International Congress of Genetics in 1968. There he wrote that, "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. This is especially true for alleles that have small selective differences; until recently these have been largely outside the realm of experimental inquiry" (Crow, 1969, pp.106–7). The value of this kind of experimental access and the quantitative predictions that result from it is in part derived from the immediate context of population genetics: quantitative theory in population genetics, according to Crow, has "mainly centered around the individual gene and its rate of replacement" (Crow, 1969, p.107). To population geneticists used to problematic predictions and ambiguous data, molecular biology seemed to offer a way to sharpen both their predictions and data in such a way as to allow decisive tests to be made.

At the Berkeley Symposium, G. L. Stebbins and Richard Lewontin advocated a selectionist position. According to Stebbins and Lewontin, the neutral theory is so permissive that it is weak as a testable hypothesis (Stebbins & Lewontin, 1972, p.35). For instance, the neutral theory in its simplest form predicts that allele frequencies will vary from population to population, but in *Drosophila pseudoobscura* and *willistoni*, widely separate populations show very similar allele frequencies. A migration rate as low as one migrant per generation, however, is enough to account for the similarity. Armed with these assumptions about migration rate, Stebbins and Lewontin charge that no observation could contradict the prediction. Appealing to Karl Popper's philosophy of science, they labeled the neutral theory "'empirically void' because it has no set of potential falsifiers" (Stebbins & Lewontin, 1972, pp.35–6). Despite their arguments, Stebbins and Lewontin do not reject the idea of neutral mutation and the effects of random drift. Instead they see the nature of evolutionary processes as unresolved and even encourage the pursuit of both neutralist and selectionist explanations (Stebbins & Lewontin, 1972, p.40).

Concerns about testing continued to haunt the neutralist–selectionist controversy for the next decade. While the popularity of electrophoresis meant that plenty of new data on genetic variability was being produced, devising the statistical tests that relied on that data was difficult. Warren Ewens, for instance, created a test for neutrality derived from his sampling formula (Ewens, 1972). When this test was applied to electrophoretic data, however, it did not have sufficient statistical power to distinguish drift from selection. In 1977, Geoff Waterson refined Ewens' test, but could not eliminate the problems with low statistical power (Watterson, 1977; Lewontin, 1991). The results of other tests were similarly indecisive or actively disputed. Francisco Ayala's group, for instance, tested neutralists' predictions about heterozygosity with data on the electrophoretic variability detected in natural populations of *Drosophila*. Ayala and his coworkers predicted that the distribution of heterozygous loci should cluster around a value of 0.177. The observed distribution, however, was fairly even except that it had many loci with very little heterozygosity. Ayala argued that the detected excess of rare alleles was evidence against the neutral theory (Ayala et al., 1974, p.378). Jack King

responded by questioning the assumptions of the model that Ayala had used; the infinite alleles model, King asserted, was the source for the rare alleles discrepancy. Moreover, King noted that the predictions generated with an infinite alleles model should not be compared to data from electrophoresis, since the differences detected by electrophoresis did not necessarily correspond to allelic differences (King, 1976). As a result, Ayala and his coworkers adapted their tests to use the charge ladder model of mutation that was designed for electrophoretic data. The excess of rare alleles remained, however.

As Ayala's results were debated and refined, Tomoko Ohta articulated the Nearly Neutral Theory that proposed a larger proportion of slightly deleterious mutants that while selected were so weakly selected that they acted as if they were neutral (Ohta, 1973, 1992; Ohta & Gillespie, 1996). One of the chief benefits of the Nearly Neutral Model was that it could accommodate the large number of rare alleles observed by Ayala. At the same time, Masatoshi Nei looked to population dynamics such as the possibility of population bottlenecks as a means of explaining the excess of rare alleles (Nei, 2005). In the end, Ayala's test was very influential and created the impetus for significant revisions of the neutralist position, but Ayala's tests did not settle the controversy. Instead, the results of Ayala's and other tests led the neutralists to put more stock in the molecular clock as a source of supporting evidence.

## 2. The Molecular Clock

The idea that the rate of change in biological molecules was constant over time was christened the molecular clock in 1965 by Emile Zuckerkandl and Linus Pauling (Zuckerkandl & Pauling, 1965; Morgan, 1998). Zuckerkandl and Pauling based this claim on their comparison of similarities and differences in the amino acid sequences of hemoglobins from different species. When different hemoglobins were compared, the number of differences seemed to be proportional to the length of time that the species in question had been separated evolutionarily. Zuckerkandl and Pauling were interested in using molecular characteristics to infer evolutionary relationships and immediately saw the value of the molecular clock for not only inferring relationships, but the times of divergence.

The molecular clock was not perfect, however. Like clocks based on radioactive decay, the molecular clock was stochastic. Differences did not emerge at a perfectly constant rate. The constancy of the clock was instead an average of sometimes highly variable substitution events. Thus, from its beginnings, the clock was understood to have some variability in its rate. For the clock's many critics, however, one of the key questions at hand revolved around how much variability the clock could have and still remain a clock.

The controversies over the variability of the molecular clock were compounded by its role in the neutralist–selectionist controversy. Zuckerkandl and Pauling had initially invoked both selection and drift to explain the mechanism of the clock (Morgan, 1998). When Kimura, King, and Jukes began to advocate the neutral theory, they recognized that neutrality provided an elegant explanation for the observed constancy (see Dietrich, 1998, and Morgan, 1998). The neutral theory predicted that for neutral sites or alleles

the rate of mutation would be the same as the rate of substitution. Substitutions were the observed differences between molecules. These detected differences did not represent all of the changes produced by mutation. They represented those changes remaining after selection had eliminated the more harmful mutants and fixed the most beneficial. The rate of substitution for a mutation subject to selection would depend on the factors that normally affected selection processes, such as population size and environment. Selection should produce a highly variable rate of substitution. For a neutral allele or site, however, the process of moving from origination as a mutant to fixation was a process of random drift. The rate of substitution should then depend on the rate at which new mutants are introduced. For neutral changes, if the rate of mutation was approximately constant, then the rate of substitution would be as well.

When neutralists championed their explanation of the molecular clock's constancy, they inherited the problem of also explaining its variability. As soon as differences between molecules began to be compared, researchers noted that different molecules seemed to have different rates of change. Neutralists explained these different clocks in terms of the distribution of selected and neutral sites within each type of molecule. Hemoglobins, for instance, have sites that never change across species. These highly conserved sites were understood to be strongly selected; changing them would render the molecule less functional or non-functional and so were selected against. Other regions in hemoglobins show numerous differences among different species. These variable regions were interpreted as being neutral or weakly selected. A molecule such as histone IV was observed to have a large number of constrained sites and a low rate of substitution, whereas fibrinopepetide A was much less constrained and had a much faster molecular clock (King & Jukes, 1969, p.792). The problem of variability across types of molecules could thus be explained away, but rate variability within a molecule type was another matter. Very early in the history of the molecular clock, speedups and slowdowns were observed for the same molecule. Comparisons of insulin sequences, for instance, revealed that insulins in the guinea pig lineage seemed to have evolved faster than insulins in other mammalian lineages (King & Jukes, 1969; Ohta & Kimura, 1971, p.19). Primates, in contrast, seem to have experienced a slower rate of evolution for some proteins. Even as the neutralists defended the idea that types of molecules possessed intrinsic rates of change, they had to explain these deviations.

In 1971, Tomoko Ohta and Motoo Kimura compared sequence differences from alpha and beta hemoglobins, and for cytochrome c. Ohta and Kimura's statistical analysis of the variability in the rate of substitution for these proteins confirmed that both beta hemoglobin and the cytochrome c had significantly more variability than expected (Ohta & Kimura, 1971, p.21). Ohta and Kimura tried to explain away this high variability in terms of the effects of the influence of the positively selected regions in each molecule. Variability was the result of selection, but need not detract from the overall constancy of the molecule (Ohta & Kimura, 1971, p.23). The problem of variability of rates across lineages was not so easily resolved, however. In 1974, Walter Fitch and Charles Langley produced a new statistical analysis that demonstrated even greater variability (Langley & Fitch, 1974).

Additional evidence of slowdowns and speedups from various lineages produced by Morris Goodman and others reinforced doubts about the clock's constancy (Goodman, Moore, & Matsuda, 1975). Kimura responded by emphasizing the constancy of the

intrinsic rate of each type of molecule. Emphasizing "local fluctuations" was, in his mind, "a classic case of 'not seeing the forest for the trees' " (Kimura, 1983). Selectionists did not share Kimura's vision. Indeed, growing evidence of rate variability fueled selectionist criticisms.

In 1984, John Gillespie proposed an episodic molecular clock with a selectionist mechanism that explained both the constancy and variability evident in the patterns of substitution (Gillespie, 1984, 1991). Neutralists, such as Naoyuki Takahata and Tomoko Ohta, revised their models of the molecular clock in order to explain both the observed constancy and variability (Takahata, 1987). At the same time, Francisco Ayala used sequence comparisons for molecules such as superoxide dismutase (SOD) to demonstrate that the clock was erratic and unreliable (Ayala, 1986). The variability of rates across genera and families continued to render other molecules useless as clocks and reinforced Ayala's calls for skepticism of the clock as evidence in support of neutrality (Ayala, 1997, 1999, 2000).

## 3. The Neutral Null Model

The availability of DNA sequence data in the mid-1980s transformed the neutralist–selectionist controversy. While electrophoresis allowed evolutionary biologists access to variability at the molecular level, its resolution was limited. DNA sequencing promised direct access to genetic variability. Indeed, as DNA sequences became available, new tests of neutrality and selection made it possible to distinguish drift from selection.

DNA sequencing was introduced into evolutionary genetics by Martin Kreitman in 1983 (Kreitman, 1983). As Richard Lewontin's graduate student at Harvard, Kreitman used the sequencing techniques he learned in Walter Gilbert's laboratory to analyze the sequences of alcohol dehydrogenase (ADH) genes in *Drosophila melanogaster*. ADH had a well-known polymorphism for fast- and slow-moving electrophoretic variants. Kreitman's investigation of the DNA sequences of the fast/slow ADH polymorphism revealed many differences between the DNA sequences of eleven different alleles, but only one DNA difference that corresponded to an amino acid difference. This non-synonymous DNA substitution was at the site of the fast–slow protein polymorphism. The striking difference between synonymous changes (which cause no change in amino acid sequence) and non-synonymous changes (which cause a change in amino acid sequence) led Kreitman and his collaborators to devise new statistical tests for selection.

Kimura, King, and Jukes had proposed that synonymous changes, which occur mainly in the third position in the triplet of DNA bases (a codon) that code for an amino acid, should be neutral because they do not lead to changes in amino acid composition. If synonymous changes are neutral, they should evolve at a higher rate than amino acid changes that are more likely to be subject to negative selection (assuming that most amino acid changes would be deleterious). The rate of synonymous changes should only be surpassed if positive selection is accelerating the substitution process by driving nucleotide changes to fixation at a higher rate. Using the rate of synonymous substitutions as a measure of the neutral rate of change, Kimura proposed that

comparisons of synonymous and non-synonymous rates could provide a test for positive selection (Kimura, 1983). Kreitman extended Kimura's idea of comparing synonymous and non-synonymous substitutions by contrasting changes within and between species. The resulting McDonald–Kreitman test compares the ratio of non-synonymous to synonymous changes within a species and between two species. If the sequences are neutral, the ratios should remain the same. If there is positive selection, then non-synonymous changes should have accumulated over time, so there would be more non-synonymous changes between species than within a species. The McDonald–Kreitman test and many other statistical tests that followed allow evolutionary biologists to detect balancing selection, adaptive protein evolution, and population subdivision (McDonald & Kreitman, 1991; Kreitman, 2000). Where earlier statistical tests using electrophoretic data had been stalled by low power, these comparisons using DNA sequence data succeeded in distinguishing the effects of drift and selection.

The success of tests of selection did not tip the balance of the neutralist–selectionist controversy in favor of the selectionists. Instead, it supported an important shift in attitude toward the neutral theory that cast it as the methodological starting place for molecular evolutionary analysis. The neutral theory emerged in a climate of panselectionism – most evolutionary biologists understood natural selection to be the most important factor in biological evolution and as a result assumed that searching for selection and its effects was the method of choice (see Kimura, 1983). Indeed in response to Stephen Jay Gould and Richard Lewontin's famous attack on panselectionism in their "The Spandrals of San Marco and the Panglossian Paradigm," Ernst Mayr argued that biologists should give selectionist explanations priority, because random drift could not be demonstrated (Mayr, 1983). Mayr's confidence in selection was the result of earlier efforts that reinterpreted supposed cases of random drift governing the fate of morphological traits as actually the result of natural selection. As a result, for Mayr and many others, drift became equated with an admission of ignorance of how selection was in fact operating (Beatty, 1987b). Indeed part of the initial hostility toward the neutral theory undoubtedly was a result of its equation with these earlier, discredited attempts (Provine, 1990). Ernst Mayr would have been hard pressed to hold such a stringent denial of drift only a few years later as statistical tests using DNA data became accepted tools in molecular evolution. By the late 1980s, both proponents and critics of the neutral theory recognized that neutrality, not selection, was a useful starting hypothesis when analyzing DNA sequences (Kreitman, 2000; Beatty, 1987b).

The methodological shift toward neutrality as a starting assumption is frequently expressed by referring to the neutral theory as a null hypothesis. In Roger Selander's words, "All our work begins with tests of the null hypothesis that variation in allele frequencies generated by random drift is the primary cause of molecular evolutionary change" (Selander, 1985, p.87). Selander notes that beginning with a neutral null hypothesis does not exclude selection as a possibility or predispose him toward neutrality. He starts with neutrality because he prefers "to begin with the simplest model" because it allows him to determine "a baseline for further analysis and interpretation" (Selander, 1985, p.88).

However, not every drift hypothesis has the form of a standard null hypothesis. If the standard null hypothesis proposes that there is no difference between two populations, there may be many cases where hypotheses of drift do not conform to a claim of

no difference (Beatty, 1987b). That said, predictions generated by the statistical tests of selection and neutrality using DNA data do resemble no-difference null hypotheses. The methodological shift toward neutrality, however, involves more than its usefulness as a null hypothesis in statistical testing. In his review of methods to detect selection, Kreitman argues that "Kimura's theory of neutrally evolving mutations is the backbone for evolutionary analysis of DNA sequence variation and change" because a "substantial fraction" of the genome is best modeled as selectively neutral, because selective neutrality is a "useful null hypothesis," and because "statistical analysis of (potentially) neutral variation in a gene (or other region of the genome) can be informative about selection acting at linked sites" (Kreitman, 2000, pp.541–2). Kreitman's view accepts both that there is a substantial amount of neutral variation and that the neutral theory is essential for detecting selection at the DNA level.

The acceptance of neutrality as a starting place for molecular evolutionary research might be viewed as an important weakening of panselectionism in evolutionary biology. However, the impact of neutralism can be lessened if the rise of molecular evolution is interpreted as a diversification of the levels of biological phenomena. In other words, molecular techniques introduced information about a new level of biological organization: the molecular level where drift plays a significant role. On this view, panselectionism could be alive and well when it comes to morphological traits, but a non-starter when DNA sequence evolution is considered. Molecular evolutionists helped create the divide between the molecular and morphological levels as a way of culling out space where their research could develop independently of the selectionist agenda of the architects of the neo-Darwinian synthesis (Dietrich, 1998; Aronson, 2002; Hagen, 1999). The same molecular evolutionists also sought to find ways to integrate molecular and morphological evolution. Allan Wilson, for instance, proposed that the constant rate of change at the molecular level and the erratic rate of change at the morphological level might be explained by mutations in regulatory genes that produce relatively large phenotypic changes from small molecular changes (Wilson, Maxson, & Sarich, 1974). In a similar fashion, Tomoko Ohta has turned to evolutionary developmental interpretations of heat shock proteins, like Hsp90, to explain how the accumulation of neutral or nearly neutral changes could act as a capacitor for future morphological evolution (Ohta, 2002, 2003). As more integrative explanations link molecular and morphological evolution, morphological panselectionism will continue to weaken, although it will probably never undergo the kind of shift that grants neutralism primacy.

## 4. Controversy in Molecular Evolution

Controversies are a prominent feature of the history of evolutionary genetics and molecular evolution (Dietrich, 2006). While controversies are by definition disputes extended in time, they need not be disagreements between alternate positions such that resolution would be equated with the triumph of one position over the other. Indeed, controversies in molecular evolution, like those in evolutionary genetics, are "relative significance" disputes (Beatty, 1997). Within its proposed domain of application, the relative significance of a theory is "roughly the proportion of phenomena within its

intended domain that the theory correctly describes" (Beatty, 1997, p.S432). For instance, in the neutralist–selectionist controversy the dispute concerns the relative significance of both selection and drift. Selectionists advocate a strong role for selection, but do not deny the possibility of drift at the molecular level. Neutralists acknowledge an important role for selection, but argue that most detected molecular differences are neutral. In part the dispute is over the proportion of the domain of molecular evolution explained by selection or drift.

Where a binary controversy may proceed through the accumulation of evidence in favor of one position over another or conversely the accumulation of a greater number of anomalies by one position when compared to its rival, relative significance controversies tend to have a different dynamic and pattern of resolution. Controversies such as the classical–balance controversy in evolutionary genetics or the neutralist–selectionist controversy in molecular evolution rapidly polarized into extreme positions early in both disputes. Over time, however, these disputes depolarized, meaning that most of the biologists engaged in the controversy moved from advocating large differences in relative significance to smaller differences or a range of differences. For instance, in 1968, Kimura advocated that most detected molecular differences were neutral (Kimura, 1968), while, in 1973, Christopher Wills asserted that "virtually any change in amino acid composition of any protein molecule produces a molecule of slightly different properties and therefore of slightly different selective value from the original" (Wills, 1973, p.23). By contrast, DNA sequencing and successful statistical testing depolarized the dispute by admitting significant roles for both neutrality and selection, while providing a means to empirically detect selection on a case-by-case basis. Depolarized controversies, such as the neutralist–selectionist controversy today, are not closed or settled. Instead they are characterized by a kind of pluralism – both selection and drift are accepted as probable influences on the evolution of a molecule. As a result, the need to declare a winner in the controversy is fading in the face of explanatory diversification.

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## Further Reading

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