Introduction

The neutral theory, formally known as the neutral theory of molecular evolution, was independently proposed by M. Kimura in 1968 (see Kimura 1983, cited under General Overviews, the Neutral Hypothesis of Phenotypic Evolution, and Outstanding Questions; Kimura 1994, cited under General Overviews; and Kimura 1968, cited under Origin of the Theory) and J. L. King and T. H. Jukes in 1969 (see King and Jukes 1969, cited under Origin of the Theory). The chief tenet of the theory is that most genetic differences between species and polymorphisms within species are selectively neutral and result from mutation and genetic drift. This view sharply contrasts that of neo-Darwinists, who maintain that most of these variations are adaptive. While the neutral theory has been controversial since its debut, the neutralist-selectionist debate has been largely healthy in that it greatly stimulated the development of rigorous neutrality tests, and it is now customary to invoke adaptive explanations only when a null hypothesis of neutrality is rejected. The neutral theory has been revised and broadened in a number of ways since the 1990s, including the emphasis of nearly neutral mutations in the nearly neutral theory, expansion to evolutionary explanations of genomic architectures, and application to phenotypic evolution. While these studies are not without contention, the idea that genetic drift, rather than adaptation, could explain origins of nonrandom patterns in biology has had profound impacts on many biologists' view of evolution and the living world. The neutral theory remains the sole paradigm-changing conceptual revolution in evolutionary biology since the maturation of the neo-Darwinism in the 1950s.

General Overviews

There are a few books on the neutral theory, and Kimura 1983 remains the most comprehensive and authoritative book on the topic. Kimura 1994 is an edited volume of Kimura’s original papers on neutral theory and his underlying population genetic work. Crow and Kimura 1970 is a classic textbook of population genetic theory and deals with many fundamental concepts; it is highly useful for understanding the population genetic basis of the neutral theory. Ohta 2009 is a Japanese book on nearly neutral theory, which asserts that most intraspecific and interspecific genetic variations are not strictly neutral but are nearly neutral. Lynch 2007 argues that fixations of slightly deleterious mutations by drift led to the origins of various complex genomic architectures. Nei 2013 argues for the role of mutation and drift in evolution in general. Textbooks of molecular evolution that contain lucid discussions about the neutral theory and related topics include Nei 1987 and Li 1997.

A classic textbook of theoretical population genetics, it provides many theoretical results pertaining to the neutral theory.

In this single most important book of evolutionary biology since the 1960s, the neutral theory is lucidly, comprehensively, and forcefully argued for by its chief advocate.

An edited volume of Kimura’s original papers, many key to the development of the neutral theory.


A lucid and comprehensive textbook on molecular evolution, it discusses many topics highly relevant to neutral theory, such as nucleotide and amino-acid substitution rates and the molecular clock.


The role of nearly neutral mutations in the origins of genomic complexity is forcefully argued for in this monograph.


A pioneering textbook of molecular evolutionary genetics by a longtime supporter of the neutral theory.


The author proposes a central role of mutation (and drift) rather than selection in evolution in general.


A text on the nearly neutral theory, written by the proposer of the theory. In Japanese.

**Origin of the Theory**

Lewontin 1974 suggests that neutral theory is a continuation of the classical view of population genetics in the classical versus balance controversy on the maintenance of genetic polymorphism. Dietrich 1994 argues that Lewontin’s view is at best partially correct, because the then knowledge of molecular biology provided more evidence for neutral theory. In any case, Kimura 1968 reports that the total rate of nucleotide substitution in a mammalian genome, extrapolated from three proteins, far exceeds the upper limit of adaptive evolution suggested by Haldane 1957 (cited under Initial Response to the Neutral Theory). This observation led Kimura to propose that most genetic changes have been fixed by random drift rather than positive Darwinian selection. Kimura 1968 further states that the large amounts of fruit fly (Lewontin and Hubby 1966) and human (Harris 1966) genetic polymorphism discovered by using protein gel electrophoresis is consistent with the hypothesis that natural polymorphisms are largely neutral. Summarizing evidence from molecular biology, King and Jukes 1969 also argue that most evolutionary changes in DNA sequence are neutral. While the idea of neutral evolution of DNA sequences was previously published, for example, in Sueoka 1962 and Freese 1962, Kimura 1968 and King and Jukes 1969 have certainly caught much wider attention and are commonly regarded as the origin of neutral theory, a response that may be in part owing to the provocative title of King and Jukes 1969.


A science historian’s reconstruction of the origin of the neutral theory; the reconstruction emphasizes the critical role of molecular biology.
Shows the variation of base composition among different species and suggests that many nucleotide changes have no significant selective effect.

One of the first papers revealing high levels of protein-polymorphism in humans.

The nucleotide replacement rate per genome per generation was found to be too high to have been driven by positive selection, prompting the author to hypothesize that most nucleotide replacements must have been neutral. This article is widely regarded as the debut of the neutral theory.

The authors argue for neutral evolution of DNA sequences on the basis of the then knowledge of molecular biology, largely complementary to the evidence used in Kimura 1968.

In this classic book, Lewontin discusses evolutionary explanations of the high genetic polymorphisms revealed by protein gel electrophoresis.

One of the first papers to reveal high intraspecific protein polymorphisms by gel electrophoresis.

The author explains the large variation in base composition among bacteria by mutational pressure without the involvement of selection.

**Initial Response to the Neutral Theory**

The neutral theory received strong opposition from neo-Darwinists immediately after its publication. Maynard Smith 1968 shows that, under truncation selection, the upper limit of the rate of adaptive evolution can be several orders of magnitude higher than that suggested in Haldane 1957 and is not necessarily inconsistent with the observed nucleotide substitution rate. A similar argument is made in King 1967, Milkman 1967, and Sved, et al. 1967 that the observation in Lewontin and Hubby 1966 (cited under Origin of the Theory) of a high heterozygosity of fruit-fly isozymes could be explained by overdominant selection without causing too high a segregational load. Here, segregational load refers to the genetic load (i.e., reduction of population mean fitness compared with the fittest genotype in the population) caused by genes segregating from advantageous heterozygotes to less fit homozygotes. Clarke 1970 and Richmond 1970 assert that some of the phenomena King and Jukes use in supporting neutral evolution are better explained by natural selection. For example, King and Jukes argue that synonymous substitutions, which do not alter protein sequence, are most likely neutral. But, Clarke and Richmond argue that synonymous codon usage is potentially selected to optimize translational efficiency.
were some misunderstandings about the neutral theory in its infancy. Without realizing that the neutral theory allows the predominant role of purifying selection in molecular evolution, some authors, e.g., those of Clarke 1970 and Richmond 1970, have used the inference of purifying selection to argue against neutral theory. It is the limited role of positive selection in molecular evolution that distinguishes neutral theory from neo-Darwinism. Some authors, such as the author of Gatlin 1976, regarded nonrandom genome sequences as evidence against neutral theory, but nonrandom patterns could be due to mutational bias and/or differential purifying selection.

The author argues that the evolution of proteins is driven by natural selection rather than genetic drift.

The author mistakenly argues against the neutral theory because she is confusing mutational bias with selection and positive selection with negative selection.

Considering the limited reproductive rate and the cost of replacing an amino acid with a fitter one, the author proposes that the rate of adaptive evolution cannot exceed one per 300 generations in mammals.

One of the first papers proposing the possibility of maintaining many balanced polymorphisms without a high segregational load.

Demonstrates that truncation selection substantially reduces the substitution load calculated by Haldane 1957 and thus allows adaptive amino-acid replacements that are as rapid as what Kimura 1968 (cited under Origin of the Theory) observed.

Argues that multiple balanced genetic polymorphisms would not lead to unbearably high segregational load because selection acts on individual organisms rather than individual genes.

The author criticizes the neutral theory by asserting the role of natural selection in molecular evolution.

Argues that a large number of polymorphisms could be maintained by balancing selection without imposing an unbearable segregational load.

Evidence for the Neutral Theory

Over the years, the neutral theory has been supported by a number of lines of evidence. First, Zuckerkandl and Pauling 1965 proposes the concept of “molecular clock” based on the authors’ observation that the amino-acid substitution rate per year for a protein is more or less constant across different evolutionary lineages. Because the rate of neutral substitution equals the rate of neutral mutation, neutral theory can explain the molecular-clock phenomenon if the neutral mutation rate is constant per year. By contrast, under neo-Darwinism, the substitution rate is determined by the product of the effective population size, the selection coefficient, and the rate of adaptive mutation and is thus unlikely to be constant across different evolutionary lineages. Therefore, the molecular-clock phenomenon is considered one of the strongest pieces of evidence for the neutral theory. Second, neutral theory predicts that the evolutionary rate of a gene or a site increases as its functional constraint reduces, because a reduced functional constraint means an increased fraction of neutral mutations. By contrast, neo-Darwinism predicts that the evolutionary rate of a gene or a site increases as its functional importance increases, because high functional importance means high intensity of positive selection. Kimura and Ohta 1974 shows that the functionally important and constrained histone H4 evolves much more slowly than the functionally relatively unimportant and unconstrained fibrinopeptides, consistent with the prediction of the neutral theory. Because synonymous sites are functionally less constrained than nonsynonymous sites, the preponderance of substitutions per synonymous site in the evolution of protein-coding genes also supports the neutral theory, as Kimura 1977 argues. Similarly, Li, et al. 1981 shows exceedingly rapid evolution of functionless genes known as pseudogenes, compared with functional genes, strongly supporting the neutral theory. Third, Nei and Graur 1984 analyzes intraspecific protein polymorphisms measured by gel electrophoresis from seventy-seven species. The authors report that the data are generally consistent with the prediction of neutral theory under population bottlenecks but are incompatible with the model of frequent overdominant selection. More recent evidence is reviewed in Kimura 1991, Takahata 1996, and Nei, et al. 2010.


Argues that faster evolution at functionally less constrained synonymous sites than functionally more constrained nonsynonymous sites supports the neutral theory.


An update on the evidence for the neutral theory since the publication of Kimura 1983 (cited under General Overviews, the Neutral Hypothesis of Phenotypic Evolution, and Outstanding Questions).


This influential paper summarizes five fundamental rules of molecular evolution.


Shows that the extremely rapid evolution of functionless pseudogenes compared with functional genes lends the strongest support to the neutral theory.


Concludes that protein polymorphism levels are consistent with the neutral prediction with frequent population bottlenecks.


An update on the neutral theory in the context of genomic data.

An update on the neutral theory.


An insightful paper reporting an approximately constant rate of protein-sequence evolution known as the molecular clock.

**Neutralist-Selectionist Debate**

The neutralist-selectionist debate started immediately after the debut of the neutral theory in 1968 and has lasted to date. There are four key arguments against neutral theory. First, it was argued that the substitution rate of a protein is far from constant and that this inconstancy was considered to reflect the action of positive selection. Gillespie 1991 summarizes this line of argument. A genomic study in mammals in Kim and Yi 2008, however, finds no evidence for the overdispersed molecular clock for nonsynonymous substitutions.

Second, mutation rate is commonly believed to be constant per generation, rather than per year. Thus, it has been argued that the observed molecular clock per year cannot be explained by the neutral theory. Ohta developed the nearly neutral theory that can explain why the protein evolutionary rate is approximately constant per year, as reviewed in Akashi, et al. 2012. Third, synonymous substitutions were initially believed to be the best example of neutral changes, but Ikemura 1981 finds that codons with high cognate-tRNA concentrations are used more often than other synonymous codons of the same amino acid, suggesting that synonymous substitutions are subject to natural selection in relation to translation. Fourth, according to the neutral theory, the amount of genetic polymorphism in a population increases with the effective population size, but the lack of populations with very high protein polymorphisms, referred to as “invariance of heterozygosity” in Lewontin 1974 (cited under Origin of the Theory), poses a challenge to the neutral theory. This phenomenon is explained by frequent population bottlenecks in Nei and Graur 1984 and stronger selection against slightly deleterious mutations in larger populations in Akashi, et al. 2012. A direct debate between a neutralist and a selectionist can be found in Ohta 1996 and Kreitman 1996. Golding 1994 is an edited volume that presents empirical data supporting non-neutral evolution and statistical methods for testing neutrality.


A thorough review of the nearly neutral theory in the context of protein-sequence evolution.


The author argues that episodic positive selection rather than fixation of neutral mutations by genetic drift explains molecular evolution.


This edited volume contains empirical data that support the action of natural selection as well as statistical methods that can be used to test the null hypothesis of neutrality.

**Ikemura, T. 1981. Correlation between the abundance of Escherichia coli transfer RNAs and the occurrence of the respective codons in its protein genes: A proposal for a synonymous codon choice that is optimal for the *E. coli* translational system. *Journal of Molecular Biology* 151:389–409.**

Finds that codon usage is correlated with the cognate tRNA abundance, suggesting an adaptive nature of biased synonymous codon usage.

A reanalysis, using genomic data, on the overdispersion of molecular clock.


An argument against the neutral theory in a debate with Ohta 1996. Specifically, Kreitman argues that the neutral theory serves as a useful null hypothesis that is nevertheless rejected by empirical data.


Explains the phenomenon of invariance of heterozygosity by frequent population bottlenecks.


An argument for the neutral and nearly neutral theories in a debate with Kreitman 1996. Specifically, Ohta defends the nearly neutral theory, arguing that many data that are seemingly incompatible with the neutral theory are explainable by the nearly neutral theory.

The Nearly Neutral Theory

Kimura defined neutral mutations by \(|2Ns|<<1\), where \(N\) is the effective population size and \(s\) is the selection coefficient. The nearly neutral theory was mainly developed by Ohta and she defines nearly neutral mutations by \(|Ns|~1\). Unlike neutral mutations, whose fate is independent of effective population size, the fate of nearly neutral mutations depends on the effective population size. As reviewed in Akashi, et al. 2012, the nearly neutral theory is able to answer almost each of selectionists’ challenges mentioned in Neutralist-Selectionist Debate. Ohta 1973 is often considered the origin of the theory, which was initially named the slightly deleterious mutation theory. The theory was originally proposed in Ohta 1972a to explain why the protein evolutionary rate is approximately constant per year, while the DNA evolutionary rate shows a generation time effect (i.e., higher rates for species with shorter generations). The nearly neutral theory predicts a negative correlation between the protein evolutionary rate and population size, a correlation verified in Ohta 1972b and Ohta 1995, and was later confirmed in genome-scale analysis, for example, by Rhesus Macaque Genome Sequencing and Analysis Consortium 2007. A comparison between conservative and radical nonsynonymous substitution rates in Zhang 2000 also supports the nearly neutral theory. Comparisons between free-living bacteria and related endosymbiotic bacteria in molecular evolution rates in Moran 1996 strongly support the theory.


A comprehensive summary of the nearly neutral theory.


Contends that accelerated gene evolution of endosymbiotic bacteria, which have small population sizes, supports the nearly neutral theory.

Explains why the protein evolutionary rate is approximately constant per year, while the DNA evolutionary rate is approximately constant per generation.


Suggests that protein evolution is faster in smaller populations.


Argues for the importance of slightly deleterious mutations in evolution.


Analysis of forty-nine mammalian genes shows a higher nonsynonymous-to-synonymous substitution rate ratio in species with small populations than those with large populations, supporting the nearly neutral theory.


Confirms that genomic data show a higher nonsynonymous-to-synonymous substitution rate ratio in primates (with small populations) than rodents (with large populations), consistent with the nearly neutral theory.


Shows that the rate ratio of radical-to-conservative nonsynonymous substitutions is higher in species with smaller effective population sizes, consistent with the nearly neutral theory.

**Neutrality Tests**

While the general validity of the neutral theory remains controversial, almost nobody denies the importance of neutrality as a null hypothesis in the study of evolutionary forces. Many tests of the neutral hypothesis have been developed. They are generally classified into three groups, depending on whether the genetic variation considered is divergence, polymorphism, or both. It should be noted that many neutrality tests applied to individual genes have low statistical power such that failure to reject the neutral hypothesis may not equal a complete lack of selection.

**Divergence Data of DNA Sequences**

The standard method of testing the neutral hypothesis by using divergence data compares the number of synonymous substitutions per synonymous site \(d_S\) with the corresponding number of nonsynonymous substitutions per nonsynonymous site \(d_N\). Under the null hypothesis of neutrality, \(d_N = d_S\). A significantly greater \(d_N\) than \(d_S\) indicates positive selection, whereas a significantly smaller \(d_N\) than \(d_S\) indicates negative selection. Among the commonly used methods for estimating \(d_S\) and \(d_N\) are those developed in Nei and Gojobori 1986, Li 1993, and Goldman and Yang 1994. Synonymous and nonsynonymous substitution rates can also be compared in one or more branches in a phylogeny instead of between two extant sequences; e.g., see Zhang, et al. 1998 and Yang 1998. Furthermore, synonymous and nonsynonymous substitution rates can be compared for a subset of codons instead of the entire gene; e.g., see Suzuki and Gojobori 1999 and Yang, et al. 2000. Finally, synonymous and nonsynonymous substitution rates can be compared for a subset of...
codons in one or more branches in a tree, as in Zhang, et al. 2005.


A likelihood-based method for estimating synonymous and nonsynonymous substitution rates that has become the standard method in genome-scale analysis.


Develops a method for estimating synonymous and nonsynonymous substitution rates.


Develops one of the simplest and most widely used methods for estimating synonymous and nonsynonymous substitution rates.


Proposes a counting method for testing the neutral hypothesis for a subset of codons along a phylogeny.


Proposes a likelihood-based method for testing the neutral hypothesis in a set of branches in a phylogeny.


Develops a method for detecting positive selection acting on a subset of codons in a gene.


Proposes methods for estimating synonymous and nonsynonymous substitution rates and testing the neutral hypothesis in one or more branches in a phylogeny.


Presents a likelihood method for detecting positive selection acting on a subset of codons in a subset of branches of a phylogeny.
The neutral hypothesis can also be tested by using polymorphism data. The most widely used methods that depend on frequencies of variants at polymorphic nucleotide sites include those developed in Tajima 1989, Fu and Li 1993, and Fay and Wu 2000. Some other methods require additional information on the linkage phase among variant sites and score a haplotype as an allele such as those developed in Ewens 1972, Watterson 1978, and Slatkin 1994. There are also methods that depend on patterns of linkage disequilibrium such as the extended haplotype homozygosity (EHH) test developed in Sabeti, et al. 2002 and its extension proposed in Voight, et al. 2006. Different tests have different properties in terms of the power and accuracy in detecting positive or purifying selection.


This classic paper reports the probabilities associated with counts of how many different alleles are observed a given number of times in a sample under the infinite allele model with no selection.


Adaptation is tested against the null hypothesis of neutrality by comparing two measures of DNA sequence polymorphism.


The null hypothesis of neutrality in a constant-size population is tested by comparing measures of DNA sequence polymorphism that are expected to be equal under the null hypothesis.


Proposes the extended haplotype homozygosity (EHH) test of neutrality.


A neutrality test based on Ewens’ prediction of sampling distribution.


The neutrality hypothesis is tested by comparing two measures of DNA sequence polymorphism that should be equal under neutrality in a constant-size population.


Proposes a modified version of the EHH test named the iHS (integrated haplotype score) test.


Presents a test of neutrality by comparing the observed and expected homozygosity under neutrality.

**Polymorphism and Divergence Data**
The neutral theory can also be tested by comparing polymorphism and divergence data. Hudson, et al. 1987 proposes such a test by comparing the ratio of divergence and polymorphism between two loci. Neutrality is rejected (i.e., at least one locus is under purifying or positive selection) when this ratio is unequal between two loci. McDonald and Kreitman 1991 revises the test in Hudson, et al. 1987 by comparing synonymous sites and nonsynonymous sites of the same gene instead of comparing two loci. Under the assumption that synonymous sites are neutral, a rejection of the null hypothesis by the McDonald-Kreitman test may indicate the action of position selection for or negative selection against nonsynonymous substitutions. Specifically, when the ratio between the number of interspecific nonsynonymous differences (Dn) and the number of intraspecific nonsynonymous polymorphisms (Pn) significantly exceeds the corresponding synonymous ratio (Ds/Ps), positive selection for interspecific nonsynonymous differences is inferred. By contrast, when the nonsynonymous ratio is significantly smaller than the synonymous ratio, negative selection against interspecific nonsynonymous differences is inferred. Because many low-frequency polymorphisms are deleterious, Fay, et al. 2002 proposes to remove low-frequency (e.g., less than 10 percent) derived alleles from the counts of polymorphisms in the McDonald-Kreitman test to improve its power in detecting positive selection.

Proposes a modification of the McDonald-Kreitman test (McDonald and Kreitman 1991) to improve the power of detecting positive selection in the presence of deleterious mutations.

Neutrality is tested by comparing the divergence to polymorphism ratio between two loci.

One of the most widely used tests of neutrality, it compares nonsynonymous and synonymous variations between and within species at a gene.

Adaptive Molecular Evolution

Numerous tests of the hypothesis of neutral evolution have been conducted for DNA sequences. In a number of cases, neutrality is rejected in favor of adaptation driven by positive Darwinian selection. The best studied type of molecular adaptation, investigated in many individual genes as well as genomes, involves beneficial nonsynonymous nucleotide changes. Genome-wide fraction of amino-acid substitutions that are adaptive has been estimated for some species. In addition, other types of molecular adaptations, such as those involving gene duplication or gene loss, have also been discovered. It should be noted that neutral theory allows the prominent role of positive selection in the evolution of a minority of genes. Thus, rejecting the neutral hypothesis for a small fraction of genes does not refute the neutral theory.

Adaptive Protein Evolution Detected by Neutrality Tests from Studies of Individual Genes

A number authors reported the rejection of the neutral hypothesis by using the methods described in Neutrality Tests. Hughes and Nei 1988 shows that dN is significantly greater than dS in the antigen recognition regions of the human major-histocompatibility-complex (MHC) genes, suggesting the action of positive selection, which is most likely related to the immune function of MHC. Numerous genes have since been shown to be subject to recurrent or episodic positive selection on nonsynonymous changes. Best-known examples include the hemagglutinin gene in human influenza viruses reported in Bush, et al. 1999; abalone sperm lysoin reported in Lee, et al. 1995; Drosophila “speciation genes” Odysseus reported in Ting, et al. 1998 and Nup96 reported in Presgraves, et al. 2003; colobine-monkey pancreatic ribonuclease gene reported in Zhang, et al. 2002b; and human speech gene FOXP2 studied in Enard, et al. 2002 and Zhang, et al. 2002a.

Amino-acid sites in influenza hemagglutinin that are subject to recurrent positive selection are identified.


A gene critical to human speech is found to have evolved under positive selection during human origins.


Discovers one of the first examples of molecular adaptation.


Reports very high \(d_N/d_S\) ratios between sperm lysins of different abalones.


Reports that adaptive divergence of a nuclear pore protein underlies the hybrid inviability between two *Drosophila* species.


Reports that rapid evolution of a homeobox gene is responsible in part for hybrid sterility in *Drosophila*.


Reports substantial acceleration and action of positive selection in the evolution of a human speech gene.


Reports that adaptive evolution of a duplicate pancreatic ribonuclease gene followed the evolution of leaf eating in colobine monkeys.

**Adaptive Protein Evolution Detected by Neutrality Tests from Genomic Studies**

Identifying genes subject to positive selection using the methods described in Neutrality Tests has been a routine practice in comparative genomic studies. One of the first genome-wide scans of positive selection, Clark, et al. 2003 reports positively selected genes in the human lineage since its divergence from the chimpanzee lineage about six million years ago. Sabeti, et al. 2007 reports genes subject to comparatively recent position selection within humans. Bakewell, et al. 2007 reports that more genes underwent positive selection in chimpanzees than in humans since their separation, probably because of a larger effective population size and hence more effective
selection in the former than the latter. Qiu, et al. 2012 reports positively selected genes related to hypoxia and hence high-altitude adaptation in the yak genome. The authors of Zhang, et al. 2014 have detected numerous bird genes under positive selection. Mackay, et al. 2012 identifies positively selected genes in the fruit fly *Drosophila melanogaster*. Huang, et al. 2012 identifies fifty-five selective sweeps during rice domestication. Raffaele, et al. 2010 reports positively selected genes in the fungal pathogen *Phytophthora infestans* that causes potato blight, a serious potato disease. These experiments are just a few examples of a very large number of genome scans of positive selection.


Reports more positively selected genes in chimpanzee evolution than in human evolution.


Infers positive selection for hundreds of genes in the human branch after the human-chimpanzee separation.


Identifies fifty-five selective sweeps during rice domestication from the genome sequences of 446 geographically diverse accessions of wild rice and 1,083 cultivated varieties.


Identifies positively selected genes in the genome sequences of 168 lines from one outbred fruit-fly population.


A comparison between yak and cow genomes revealing that positively selected genes in the yak lineage are enriched in functional categories and pathways related to hypoxia and nutrition metabolism.


Positively selected genes are identified from the Irish-potato-famine pathogen.


More than 300 positively selected regions of the human genome are identified on the basis of polymorphism data.


A comparison of forty-eight bird genomes that detects many genes under positive selection.
Fraction of Adaptive Amino-Acid Substitutions

When the author of Kimura 1968 (cited under Origin of the Theory) inferred the rate of amino-acid substitution per generation per mammalian genome by using only three proteins, he was unaware that only 1.5 percent of the mammalian genome is protein coding and mistakenly assumed that this number is 100 percent. On the basis of the estimate of evolutionary rates from thousands of proteins, Zhang 2010 calculates that Haldane’s upper limit of the rate of adaptive evolution (Haldane 1957, cited under Initial Response to the Neutral Theory) is not violated even when 33 percent of amino-acid substitutions in mammals are adaptive. Smith and Eyre-Walker 2002 proposes that one can estimate the fraction of amino-acid substitutions that are adaptive by \( \alpha = 1 - (D_{Sn})/(D_{nP}) \) (see Neutrality Tests: Polymorphism and Divergence Data for notation). Eyre-Walker 2006 reviews the estimates of \( \alpha \) in a number of species, finding that it varies from nearly zero in humans and Arabidopsis to over 50 percent in Drosophila and some microbes and viruses. By modifying the McDonald-Kreitman test (McDonald and Kreitman 1991, cited under Neutrality Tests: Polymorphism and Divergence Data), the authors of Bustamante, et al. 2002 develop a hierarchical Bayesian method to estimate the mean scaled selection coefficient and its variance for nonsynonymous substitutions of each gene. The authors of Sawyer, et al. 2003 further develop this method to allow variation in selection coefficient among nonsynonymous substitutions within a gene. Using this method, Sawyer, et al. 2007 shows that approximately 95 percent of amino-acid substitutions in Drosophila is adaptive. The finding in Drosophila and several microbes that most amino-acid substitutions in a genome have been driven by positive selection seriously challenges the neutral theory.

Develops a method for estimating the distribution of selection coefficient from polymorphism and divergence data.

A review of the fraction of adaptive amino-acid substitutions in multiple species.

Develops a Bayesian random-effect model to estimate the distribution of selection coefficient from polymorphism and divergence data.

Estimates that 95 percent of amino-acid substitutions in Drosophila are adaptive.

First estimation, using relatively large data, of the fraction of adaptive amino-acid substitutions.

A review of the progress of evolutionary genetics from the 1950s to the 2000s.

Other Types of Molecular Adaptation Detected by Neutrality Tests

In addition to the adaptive evolution of protein sequences, adaptive evolution of promoters that affect gene expression has also been...
reported. The best known examples include the human prodynorphin gene promoter reported in Rockman, et al. 2005 and human lactase gene promoter reported in Tishkoff, et al. 2007. The authors of Haygood, et al. 2007 have conducted a genome-wide scan of human promoters to detect signals of positive selection. Gene loss may also be subject to positive selection, as reported in Wang, et al. 2006 for human CASPASE12 and reported in MacArthur, et al. 2007 for human ACTN3. In addition to point mutations, insertions/deletions may be subject to positive selection, as reported in Podlaha and Zhang 2003 for mammalian CATSPER1, via the comparison between insertion/deletion substitution rates in coding and noncoding regions.


Reports genomic scan of positive selection in human promoters.


Reports that loss-of-function mutants of ACTN3 are positively selected in humans.


Reports greater insertion/deletion substitution rates in the coding sequence of a cation channel regulating sperm motility than in presumably neutral regions.


Presents evidence for the action of positive selection on cis-regulation of human prodynorphin.


Reports adaptive evolution of the lactase promoter in Africans.


Presents population genetic evidence for the action of positive selection on a null allele of human CASPASE12.

Molecular Adaptations Revealed by Other Methods

In addition to the methods mentioned in Neutrality Tests, several other methods have been designed to detect positive selection in molecular evolution. First, parallel and convergent amino-acid substitutions have been reported in some proteins. The authors of Zhang and Kumar 1997 have developed a statistical method to test whether the observed numbers of parallel and convergent substitutions exceed the neutral expectations. An example of parallel protein-sequence evolution can be found in Li, et al. 2010 on the hearing gene Prestin of echolocating bats and whales. Christin, et al. 2010 reviews empirical evidence for parallel and convergent molecular evolution. Second, gene expression noise, defined by the variation in the mRNA or protein expression level of a gene among isogenic individuals in the same environment, has been shown to be ubiquitous. Zhang, et al. 2009 proposes a theory on how a high level of gene expression noise may be advantageous for some genes under certain conditions and provides evidence that yeast plasma-membrane transporters are subject to positive selection for elevated expression noise. In principle, nothing rejects neutrality more
convincingly than the demonstration that a substitution improves organismal fitness, but because it is usually difficult to know the exact genetic background and environmental context in which the substitution occurred, fitness data might or might not be relevant. Nevertheless, when combined with other information such as gene expression or functional changes and/or population genetic evidence, fitness data can be quite powerful. For example, Qian and Zhang 2014 shows by using fitness data and a series of gene expression and protein function data that gene duplication frequently leads to adaptation. This finding complements an earlier case study in Hittinger and Carroll 2007, showing, on the basis of expressional, functional, and fitness data that the duplication of a key player in the yeast galactose use pathway was adaptive. Beyond the single gene level, it has been argued that the expansions/contractions of certain gene families were subject to positive selection; e.g., see Demuth, et al. 2006 for a genomic study of mammalian gene families and Shi and Zhang 2007 for a case study of vertebrate vomeronasal receptor gene families.

A review of various types of molecular convergence in evolution.

A genomic analysis demonstrating the potential role of positive selection in some gene family expansions.

A detailed analysis of expression, function, and fitness that demonstrates the adaptive evolution of a key regulator of the yeast galactose use pathway.

A case of adaptive convergent evolution of a hearing protein in echolocating mammals.

Genomic analysis of the fitness of yeast strains with one or two genes deleted demonstrates that gene duplication facilitates adaptation.

An analysis of vertebrate vomeronasal receptor genes suggests the involvement of positive selection in gene family size expansion.

Develops a statistical test of adaptive convergent evolution of protein sequences.

Evidence that positive selection drives the elevation of expression noise of a small group of yeast genes.
Neutral Evolution of Genomic Architecture

One of the most important extensions of the (nearly) neutral theory of molecular evolution is its application to the study of the origin of genomic architecture, including gene number, intron content, transposable element content, gene structure, etc. As the primary advocate, Lynch 2007 summarizes evidence for the role of nearly neutral mutations in the origin of genomic complexity. The chief tenet of this thesis, more formally referred to as the mutational-hazard hypothesis, is that many complex elements of the genome, such as introns, are slightly deleterious. As the organism size increases in long-term evolution, the effective population size decreases, making it possible for slightly deleterious mutations to fix by chance. These elements may be subsequently tinkered by evolution to provide apparent utility, such as the existence of enhancers in introns. One good example is the evolution by gene duplication. It was commonly believed that gene duplication leads to the origin of new gene functions. Force, et al. 1999 proposes that duplicate genes are initially retained because of subfunctionalization (i.e., subdivision of ancestral functions to daughter genes) via degenerate mutations rather than neofunctionalization (i.e., acquisition of new functions) by advantageous mutations. In other words, gene number in a genome can increase via a pure neutral process. Based on an analysis of protein-protein interactions and among-tissue gene expressions, He and Zhang 2005 provides empirical evidence that subfunctionalization plays the major role in the initial fixation and retention of duplicates, whereas neofunctionalization likely occurs more slowly. Qian, et al. 2010 finds that a duplicate gene tends to have a lower expression than its progenitor gene, supporting subfunctionalization with regard to the expression level. Lynch and Conery 2003 provides evidence that genome size increases as the effective population size decreases, and that intron number and size, half-life of duplicate genes, and number of transposable elements all increase with genome size. Lynch’s theory is not without controversy. For instance, Daubin and Moran 2004 argues that bacterial genome size and effective population size are actually positively correlated rather than negatively correlated, because genome size tends to decrease under relaxed selection as a result of mutational deletion bias in bacteria. Whitney and Garland 2010 asserts that Lynch and Conery failed to correct for phylogenetic non-independence in their statistical analysis; all significant correlations reported in Lynch and Conery 2003 are gone after the correction. Lynch 2011 rebuts the need for correcting phylogenetic non-independence in this case and argues that the correction was not done properly.


The authors contend that, in bacteria, genomic complexity decreases rather than increases with the reduction of effective population size.


Subfunctionalization is proposed as an alternative to neofunctionalization in explaining the retention of duplicate genes.


Analysis of yeast protein-protein interaction data and human tissue gene-expression data supports the role of subfunctionalization in duplicate-gene retention, but also identifies substantial neofunctionalization, which occurs more slowly than subfunctionalization.


Extending the (nearly) neutral theory to the evolution of genomic architecture, this is one of the most influential books on evolutionary biology since Kimura 1983 (cited under General Overviews, the Neutral Hypothesis of Phenotypic Evolution, and Outstanding Questions).


Lynch’s rebuttal of the view in Whitney and Garland 2010.

Proposes that a reduction in effective population size facilitated the fixation of slightly deleterious mutations and led to genomic complexity.


Reports that reduction of expression level after gene duplication, a special type of subfunctionalization, is prevalent in yeast and mammals and explains the long retention of duplicate genes.


Questions Lynch and Conery’s statistical analysis (Lynch and Conery 2003) that was reported to support the relationship between effective population size and genomic complexity.

The Neutral Hypothesis of Phenotypic Evolution

The author of Kimura 1983 clearly limits his neutral theory to molecular evolution. However, at least as a null hypothesis, neutrality applies to phenotypic evolution such as the evolution of morphological and physiological traits. Several statistical methods have been developed to test the neutral hypothesis for phenotypic traits, including, for example, Lande 1976, Chakraborty and Nei 1982, Lynch and Hill 1986, Turelli, et al. 1988, and Spitze 1993. A major problem in testing the neutral hypothesis of phenotypic evolution is that the published phenotypic data are highly biased as a result of a general interest in adaptive traits. Nei 2007 proposes that the neutral theory also applies to phenotypic traits in general.


It is proposed that the ratio of interpopulational genetic variance to intrapopulational genetic variance of a quantitative trait be used for testing the neutral evolution hypothesis of the trait.


The most comprehensive and authoritative book on the neutral theory of molecular evolution.


The author derives the expected amount of phenotypic evolution in a given time when the trait is not under selection.


Proposes a neutrality test for phenotypic traits by comparing within- and between-population genetic variances of the traits.

Proposes that mutation and drift play more important roles than positive selection in phenotypic evolution.


Proposes a neutrality test of phenotypic traits by comparing $Q_{ST}$ of phenotypes with $F_{ST}$ of neutral loci.


Proposes a test of neutral evolution of quantitative traits, on the basis of the neutral expectation in the rate of phenotypic divergence.

Outstanding Questions

An important question regarding the neutral theory is the definition of neutral mutations. Kimura 1983 defines neutral mutations as those with selection coefficients $< 1/(2N)$, where $N$ is the effective population size. In many unicellular organisms, $N$ exceeds $10^7$. Thus, mutations with selection coefficients not much smaller than $5 \times 10^{-8}$ would be non-neutral by Kimura's definition. Nei, et al. 2010 suggests that the definition be loosened to selection coefficients $<1/\sqrt{2N}$, which equals $2.2 \times 10^{-4}$ when $N=10^7$. The authors further suggest that, for practical purpose, any selection coefficient $< 0.001$ be considered more or less neutral, because of expected environmental fluctuation over generations and the stochasticity in progeny number of a given genotype. The change of the definition, if accepted, will certainly alter the prevalence of neutral evolution. As mentioned, the fraction of adaptive amino-acid substitutions has been estimated for a number of species. But the estimators are dependent on certain simplifying assumptions such as the constancy in population size for the evolutionary history concerned for most estimators. How often these assumptions are violated and how much the violations affect the estimates will be an important subject of future study. Apart from protein coding regions, for which the neutral theory was initially proposed, it is important to ask what fraction of nucleotide substitutions in noncoding regions have been driven by positive selection. Andolfatto 2005 suggests that a large fraction of nucleotide substitutions in noncoding regions of the *Drosophila* genome are adaptive. Whether this result applies to other species, especially mammals, which have a much larger noncoding fraction of the genome, is unclear. Thanks to rapid technological development, many previously hidden variations at the molecular level have now been revealed. Most of these variations are currently explained as adaptations, but the possibility of neutrality has started to be considered. One example is the finding in Xu and Zhang 2014 that, contrary to the initial belief by many, human coding RNA editing is generally nonadaptive. The neutralist-selectionist debate started in the late 1960s, but strongly opposing views can still be found in the modern literature. For example, Lynch 2007 criticizes the default use of adaptation to explain almost any aspect of biodiversity and argues that adaptation is not only unnecessary but also insufficient for explaining many observations, whereas Hahn 2008 argues that the neutral hypothesis has been rejected by such overwhelming molecular evidence that its continued use as a null hypothesis is not only disingenuous but also harmful to the field of molecular evolution. The debate is unlikely to be resolved soon.


Reports that *Drosophila* population genomic data suggest a role of positive selection in many noncoding changes.


Calls for a selection theory of molecular evolution because the author sees overwhelming evidence against the neutral hypothesis.


A classic book on the neutral theory authored by the chief proponent of the theory.

Criticizes the default use of adaptive explanation of almost any aspect of biodiversity and suggests that adaptation is neither necessary nor sufficient to explain many phenomena.


A review of the neutral theory in the context of genomic data, it also redefines neutrality.


An analysis of genome-wide coding RNA editing data from humans suggests that most of the editing is nonadaptive and reflects mistakes made by editing enzymes.