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Half a Century of Controversy: The Neutralist/Selectionist Debate in Molecular Evolution

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Abstract

The neutral and nearly neutral theories, introduced more than 50 yr ago, have raised and still raise passionate discussion regarding the forces governing molecular evolution and their relative importance. The debate, initially focused on the amount of within-species polymorphism and constancy of the substitution rate, has spread, matured, and now underlies a wide range of topics and questions. The neutralist/selectionist controversy has structured the field and influences the way molecular evolutionary scientists conceive their research.

Key words: neutral theory, nearly neutral theory, within-species polymorphism, substitution rate, genome size, GC-biased gene conversion.

Significance

Whether genome evolution is mainly driven by natural selection or largely reflects random, nonadaptive processes is arguably the central issue of molecular evolution as a field. Over the last 40 yr, the question has been hotly debated in the 2 Society for Molecular Biology and Evolution journals, *Molecular Biology and Evolution* and *Genome Biology and Evolution*. Initially focusing on the amount of within-species polymorphism and the rate of amino acid substitution, the controversy now touches every aspect of molecular biology, including genome size, content, and function.

Introduction

This perspective is part of a series of articles celebrating 40 years since our sister journal, *Molecular Biology and Evolution*, was founded (Russo et al. 2024). The perspective is accompanied by virtual issues, a selection of papers on the neutralist/selectionist debate published by *Genome Biology and Evolution* and *Molecular Biology and Evolution*.

The neutral and nearly neutral theories have polarized the field of molecular evolution since they were first proposed by Kimura (1968) and Ohta (1973). The controversy was intense at the end of the 20th century and remains palpable since the beginning of the 21st century. The 2 Society for Molecular Biology and Evolution (SMBE) journals

were key vehicles for the expression of these contrasting viewpoints. The neutral theory was based mainly on 2 observations: (i) that within-species genetic polymorphism is substantial and (ii) that proteins evolve at a roughly constant rate. This led Kimura and others (e.g. King and Jukes 1969) to postulate that most of the observable variations at a molecular level were neutral and governed primarily by drift. This view was revolutionary and challenged the dominant idea stating that, because genes determine phenotypes, genes must evolve like phenotypes, i.e. be mainly directed by natural selection. Interestingly, the 2 cornerstones of the neutral theory are still very much alive and important aspects of current molecular evolutionary thinking.

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Within-Species Polymorphism and Lewontin's Paradox

The among-species variation in genetic polymorphism has been the subject of in-depth analyses, mostly revolving around the meaning and pertinence of the concept of effective population size (N_e). The neutral model predicts that within-species polymorphism should equal the product of mutation rate and N_e (Kimura 1971). However, it was quickly realized that measured amounts of polymorphism do not scale proportionally with species census population size, N_c —the so-called Lewontin's paradox (Lewontin 1974; Leffler et al. 2012; Romiguier et al. 2014; Corbett-Detig et al. 2015; Buffalo 2021). Potential explanations for this conundrum include the suggestions that N_c might strongly differ from N_e (Filatov 2019), that N_e might be inversely related to the mutation rate (Lynch 2007), and that recurrent population bottlenecks might result in frequent drops of diversity in otherwise large N_c populations, which never reach equilibrium (Charlesworth and Jensen 2022). These hypotheses account for the narrow range of the level of genetic polymorphism observed across species, while being compatible with the neutral theory.

Another family of explanations for Lewontin's paradox invokes selective effects and, more specifically, linked selection. It is common knowledge that selection applied to a specific locus will also affect genetic variation at neighboring, linked loci. This idea was already present in the early 70s, when the neutral theory was intensely debated. At that time, the literature focused on the suggestion that elevated amounts of polymorphism could be maintained by associative overdominance, i.e. the effect on linked loci of weakly deleterious alleles maintained by mutation pressure (Ohta 1971) or by heterozygote advantage (Kimura 1971). Since then, the focus has moved to the effect on linked loci of strong directional selection, either positive (selective sweeps, Maynard Smith and Haigh 1974; Wiehe and Stephan 1993; Enard et al. 2014) or negative (background selection, Charlesworth et al. 1993). There is convincing evidence that linked directional selection tends to decrease the neutral genetic diversity, particularly in large N_e species (Corbett-Detig et al. 2015; Castellano et al. 2018; Chen et al. 2020). One such piece of evidence is the report in several species of an across-loci correlation between recombination rate and heterozygosity (Begun and Aquadro 1992; Nachman 2001; Elyashiv et al. 2016)—although the most recent literature also points to alternative explanations (Smith et al. 2018; Barroso and Dutheil 2023). When recombination is low or absent, large chunks of DNA cosegregate with selected alleles, and the resulting local reduction in polymorphism extends widely across chromosomes. A pervasive effect of linked selection might explain why heterozygosity never reaches extremely high values, even when N_e is very large and drift negligible.

Distinguishing between the demographic and linked selection hypotheses is not an easy task since the 2 categories of models often make similar predictions (Wright and Gaut 2005; Schrider et al. 2016; Wang et al. 2020; Johri et al. 2022). Understanding the variation in genetic diversity, both within and among genomes, remains an important, central goal of the field of molecular evolution, and the neutralist/selectionist debate underlies this vivid body of literature. The issue, however, is no longer phrased in terms of corroborating versus challenging the neutral theory, but rather in terms of quantifying the relative contribution of neutral and selective processes to the observed patterns.

Substitution Rates and Protein Adaptation

The second, major argument at the basis of the (nearly) neutral theory relies on the approximate clock-like evolution of protein sequences. This would appear unexpected if adaptation was governing protein evolution—phenotypes do not evolve at a constant rate. A constant rate, however, appears consistent with a prediction of the neutral model, which is that the substitution rate is independent of N_e and equal to the mutation rate—which can plausibly be assumed to be approximately constant. This result, associated with the observations that synonymous substitutions are more common than nonsynonymous ones and that the amino acid composition of proteins reflects the nucleotide composition of genomes, led to the suggestion that most amino acid substitutions might be neutral or weakly selected and driven by genetic drift (Kimura 1969; King and Jukes 1969; Ohta 1973).

The rate of protein evolution and its meaning in terms of neutral versus selected evolution have been heavily debated over the last 50 yr. Gillespie (1989) repeatedly argued that the protein evolutionary rate is overdispersed—i.e. not perfectly clock-like—and that just a modest departure from the molecular clock hypothesis is expected under a number of models in which selection is the main driver. His 1989 article in *Molecular Biology and Evolution* was one of the last to explicitly focus on the validity of the rationale that initially underpinned the neutral theory. The debate took another dimension after the suggestion was made to approach the rate of adaptive amino acid substitution by comparing polymorphism and divergence patterns (McDonald and Kreitmann 1991). Elaborate statistical methods exploiting this idea were developed (Eyre-Walker and Keightley 2009; Messer and Petrov 2013) and applied to various species of microbes, plants, and animals. This led to estimates of the fraction of adaptive amino acid substitution that varied from nearly zero (Gossmann et al. 2010) up to roughly 90% (Galtier 2016)—an impressively wide range, likely in part explained by differences in the number and nature of analyzed genes, as well as in methodological choices.

Despite these uncertainties, it is a fact that, in many taxa, the ratio of nonsynonymous to synonymous changes is higher between species than within species, despite the probable existence of slightly deleterious mutations, whose fixation probability is lower than that for neutral mutations. This strongly suggests that a substantial fraction of amino acid substitutions are nonneutral, contradicting early postulates by the founders of the neutral theory. This, in turn, opens a new issue, which is the interpretation of the excess of nonsynonymous substitutions, compared with the nearly neutral expectation. A fraction of these substitutions probably reflects the response of the proteome to environmental changes, as is usually considered. Some may instead correspond to compensatory substitutions, i.e. changes restoring the function of a protein previously impaired by a population bottleneck, hitchhiking, or selfish processes such as GC-biased gene conversion (gBGC; Bolivar et al. 2018; Latrille et al. 2023). Quantifying the contribution of these many processes to the overall (or apparent) adaptive rate is an exciting challenge for the forthcoming years. Of note, the theory predicts that, if a mutation does limit adaptation, beneficial mutations should accumulate at a faster rate in large than in small N_e species—a prediction that received some empirical support (Gossmann et al. 2012; Rousselle et al. 2020).

Another interesting aspect is the determination of the among-lineage variation in substitution rate. The nearly neutral theory predicts that the rate of amino acid substitution should be negatively correlated to N_e —in small populations, drift is expected to reduce the efficacy of selection, thus allowing more mildly deleterious mutations to reach fixation (Ohta 1973; Lanfear et al. 2014). N_e in natural populations is hard to measure but is likely correlated to traits such as body size, longevity, or reproductive systems. Empirical analyses in plants and animals have provided extensive evidence that the ratio of nonsynonymous to synonymous changes is indeed higher in presumably small N_e than in presumably large N_e species, which is in agreement with the nearly neutral theory (Bromham and Leys 2005; Nabholz et al. 2013; Figuet et al. 2016; Chen et al. 2017). Therefore, we have reached this peculiar situation where the analysis of amino acid substitution rates has both led to a clear rejection of a major claim of the neutral theory—that most observable changes are neutral—and at the same time indisputably demonstrated the importance of drift in protein evolution.

Of note, this research was sustained by a remarkable effort to develop models of sequence evolution and statistical methods of substitution rate estimation (e.g. Thorne et al. 1998; Yang and Nielsen 2008; Lartillot and Poujol 2011), which, in turn, opened exciting opportunities such as, among other things, the characterization of the distribution of fitness effects of mutations (Castellano et al. 2019), the reconstruction of ancestral N_e and ancestral traits (Brevet

and Lartillot 2021), and the connection between the biochemistry and the evolution of amino acids and proteins (Glaser et al. 2003; Goldstein 2013).

Genome Size and the Drift Barrier Hypothesis

The neutralist/selectionist debate has not been restricted to the topics that gave rise to the neutral theory. The exploration of the growing amount of genomic data led to a number of discoveries that fueled the controversy. As noncoding DNA sequences became available, it appeared that the effects of N_e on genome evolution are not limited to proteins. Regulatory sequences, such as promoters, are evolutionarily conserved among distant species in relatively large N_e species such as rodents but decay rapidly in small N_e species like primates (Keightley et al. 2005). Besides sequences, the way the genome functions also seems to respond to variation in N_e . The drift barrier hypothesis generally states that the efficiency of the machinery regulating genome biology—e.g. DNA replication, repair, transcription, and splicing—is determined by N_e , i.e. optimal in large N_e species and suboptimal in small N_e ones (Lynch 2011; Sung et al. 2012). Analyses of patterns of gene expression (Brawand et al. 2011; Meer et al. 2020) and alternative splicing (Bénitière et al. 2023) tend to be consistent with this advanced version of the nearly neutral theory. Codon usage bias is another good illustration: early analyses in large N_e bacteria and yeasts uncovered the existence of selection for synonymous codon choice in highly expressed genes, implying a finely tuned translation machinery, whereas codon usage in large vertebrates was found to be mainly determined by the genomic context and independent of gene function (Sharp et al. 1995).

Lynch (2006, 2007, 2012) argued that the complex architecture of eukaryotic genomes and proteomes—a large size, the presence of introns, repeated elements, and numerous multimeric protein complexes—results from their reduced N_e , compared with prokaryotes. This claim, however, was criticized based on the observation that N_e is not a good predictor of genome size in prokaryotes (Batut et al. 2014) and that phylogenetic nonindependence is not being properly accounted for (Whitney et al. 2011). In animals, genome size has been found to respond to N_e in some (Lefébure et al. 2017; Fuselli et al. 2023) but not all (Mohlhenrich and Mueller 2016; Roddy et al. 2021) of the examined contrasts, and analyses in plants yielded similarly equivocal results (Whitney et al. 2010; Bromham et al. 2015). It is puzzling to note that, >50 yr after the so-called C-paradox was coined, we still have no good understanding of the forces governing the evolution of genome size (Elliott and Gregory 2015). One difficulty could be that the types of mutations affecting genome size are diverse, apply at various time scales, and

might be pleiotropic. The appearance of a novel, active transposable element, a whole-genome duplication, or a mutation modifying the efficiency of structural change repair, for instance, should influence genome size in the long run but will likely be selected on other grounds. Such mutations might be under strong selection, and their evolution, therefore, is largely independent of N_e . Small insertions or deletions of noncoding DNA, on the other hand, presumably have extremely small fitness consequences and might behave effectively neutrally across a wide range of the existing N_e s. This might explain why the drift barrier hypothesis fails to generally predict the among-species variation in genome size. Experimental assessments of the fitness consequences of a change in genome size (e.g. Stelzer et al. 2023) might help progress here.

Base Composition and gBGC

GC content varies considerably within and among genomes, an observation that has intrigued molecular evolutionary scientists for decades. Interest in this topic has probably been heightened by the fact that the human genome shows a particularly high variance in GC content, which varies from ~30% to ~60% at a 100-kb scale. The causes of this pattern, at that time called the isochore structure, was the subject of a vigorous debate in the 1980s and the 1990s. The discovery that, among vertebrates, the warm-blooded mammals and birds, but not the cold-blooded fish and amphibians, harbored isochores led to the suggestion that this trait was an adaptation to homeothermy (Bernardi and Bernardi 1986). This hypothesis lasted for a remarkably long time, despite receiving no empirical support (Hughes et al. 1999; Belle et al. 2002) and being theoretically implausible (Piganeau et al. 2001). The climax was reached when the first DNA sequence polymorphism data in mammals revealed a segregation bias: GC alleles appeared to be more frequent, on average, than AT alleles (Eyre-Walker 1999), and especially so in the GC-richest regions of the human genome (Duret et al. 2002), suggesting that isochores were selected—a confusing situation.

The solution came from a hitherto little-documented molecular process: gBGC. gBGC is a recombination-associated transmission distorter, by which AT versus GC heterozygotes produce more GC-bearing than AT-bearing gametes, conferring a population advantage to GC alleles (Eyre-Walker 1993; Galtier et al. 2001). The last 2 decades have demonstrated that gBGC is pervasive and the major determinant of GC content variation in all domains of life (e.g. Pessia et al. 2012; Lassalle et al. 2015; Clément et al. 2017; Galtier et al. 2018; Boman et al. 2021). In vertebrates, the across-species variation in mean and variance of genomic GC content (“isochores”) was found to be mainly determined by the distribution and dynamics of the recombination rate, which, in turn, is influenced by

karyotype evolution, small chromosomes experiencing a higher per base pair recombination rate (Mugal et al. 2015).

gBGC is neutral in the sense that it is unrelated to the fitness of organisms. gBGC, however, leaves a population genetic signature that resembles that of selection, and for this reason, it is a major confounding factor to be accounted for (Ratnakumar et al. 2010). The expected strength of gBGC depends on the product of the transmission bias by N_e —just like selection, gBGC is less effective if drift is strong. A phylogenetic analysis of GC content variation can, therefore, inform ancient recombination maps and ancient N_e (Lartillot 2013). The origin and evolution of gBGC is an open issue. Why did this meiotic bias evolve in the first place? How does its strength remain moderate across taxa differing in N_e by orders of magnitude? These are promising avenues of research requiring an explicit incorporation of gBGC in evolutionary genomic models (Capra et al. 2013; Borges et al. 2019). gBGC is an important evolutionary force that had gone unnoticed for decades and was revealed thanks to the failure of the preexisting hypotheses, both neutral and nonneutral—perhaps one of the greatest successes of the neutralist/selectionist controversy.

Why a Controversy?

In the late 1960s, despite the tiny amount of data at their disposal, a few brilliant geneticists correctly realized that the classical view of evolution, in which natural selection is the dominant force, does not apply as is at a molecular level and that the role of neutral mutations and drift had been underestimated. This marked a critical turning point in molecular evolutionary thinking. Enthused by their discovery, they slightly overstated the prevalence of neutrality. With the gradual accumulation of DNA sequence data, subsequent research converged toward a more balanced view of molecular evolution, the relative contribution of neutral and nonneutral forces being assessed with increasing accuracy (Pouyet and Gilbert 2021) for an increasingly wide range of biological problems (Ohta 2011; Austerlitz and Heyer 2018; Cannataro and Townsend 2018; Yoder et al. 2018).

The above looks like a classical process of collective knowledge advancement, of which the early stages could have been largely forgotten. Instead, the neutral and nearly neutral theories have polarized the field for half a century (Ohta and Gillespie 1996) and still arouse strong feelings (Graur et al. 2013; Kern and Hahn 2018; Jensen et al. 2019). Why is this so? The limited data sets available for decades probably played a role—it is easy to take extreme or dogmatic positions when no data can contradict them. It could also be that scientists working on relatively small N_e species, such as large vertebrates, tend to adhere to the neutral theory, whereas, say, drosophilists or

microbiologists might be more attracted to hypotheses involving selection—simply because drift has a limited impact in these taxa. Below, I consider yet another hypothesis and speculate that the duration and heat of the controversy might in part be explained by cultural differences among scientists.

There are several pathways for a researcher to get to study evolution at the molecular level. Many are passionate about nature and biodiversity and use DNA as a tool to better understand how organisms function and adapt to their environment. For them, natural selection is what we want to study, and building a neutralist theory is just pointless. These researchers may be frustrated by the feedback from picky reviewers who repeatedly ask for more controls and show no apparent interest in their results. Others, instead, are fascinated by genomes as biological objects and aim at understanding why they are what they are. For them, ignoring neutral and nearly neutral processes is a logical flaw, or even scientific malpractice. These scientists can be irritated by colleagues easily jumping to selectionist explanations, which often attract more attention than their subsequent refutation.

This author cannot help considering that scientists from the latter category get closer to the truth, while also recognizing that the imagination and diligence of more phenotype-oriented researchers brings exciting hypotheses to the test. Controversy is not bad, after all, and the plurality of viewpoints makes molecular evolutionary biology a lively field to work in, as illustrated year after year by the content of SMBE journals and meetings.

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