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Climbing Mount Probable: Mutation as a Cause of Nonrandomness in Evolution

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Abstract

The classic view of evolution as "shifting gene frequencies" in the Modern Synthesis literally means that evolution is the modulation of existing variation ("standing variation"), as opposed to a "new mutations" view of evolution as a 2-step process of mutational origin followed by acceptance-or-rejection (via selection and drift). The latter view has received renewed attention, yet its implications for evolutionary causation still are not widely understood. We review theoretical results showing that this conception of evolution allows for a role of mutation as a cause of nonrandomness, a role that could be important but has been misconceived and associated misleadingly with neutral evolution. Specifically, biases in the introduction of variation, including mutational biases, may impose predictable biases on evolution, with no necessary dependence on neutrality. As an example of how important such effects may be, we present a new analysis partitioning the variance in mean rates of amino acid replacement during human–chimpanzee divergence to components of codon mutation and amino acid exchangeability. The results indicate that mutational effects are not merely important but account for most of the variance explained. The challenge that such results pose for comparative genomics is to address mutational effects as a necessary part of any analysis of causal factors. To meet this challenge requires developing knowledge of mutation as a biological process, understanding how mutation imposes propensities on evolution, and applying methods of analysis that incorporate mutational effects.

Key words: *evolution, genomics, mutation bias, population genetics*

Climbing Mount Probable

Imagine, as a metaphor for evolution, a climber operating on the jagged and forbidding landscape of Les Drus (Figure 1). A human climber would scout a path to a peak and plan accordingly, but a metaphor for evolution must disallow foresight and planning; therefore, let us imagine a blind robotic climber. The climber will move by a 2-step mechanism. In the "proposal" step, the robotic climber reaches out with one of its limbs to sample a point of leverage, some nearby handhold or foothold. Each time this happens, there is some probability of a second "acceptance" step, in which the climber commits to the point of leverage, shifting its center of mass. Biasing the second step, such that relatively higher points of leverage have relatively higher probabilities of acceptance, causes the climber to ascend, resulting in a mechanism, not just for moving, but for climbing.

What happens if a bias is imposed on the first step, the proposal step? Imagine that the robotic climber (perhaps by virtue of longer or more active limbs on one side) samples more points on the left than on the right during the proposal step. The consequences seem intuitively obvious: The trajectory of the climber will be biased, not just upward, but to the left as well. Because the probability of proposal would be greater on the left, the joint probability of proposal-and-acceptance would be greater (on average). If the landscape is rough, the climber will tend to get stuck on a local peak that is upward and to the left of its starting point. If the landscape is perfectly smooth—a geometrical cone with a single peak—the climber will spiral upward and to the left until the peak is reached.

Either way, one may recognize that the dual causation inherent in the 2-step climbing algorithm allows for 2 components of the climber's trajectory that, in this imaginary case, are separable: an upward bias caused by the bias in the acceptance step and a lateral bias caused by the bias in the proposal step. In order to predict or explain the climber's overall path, one must take into account both causes, for example, it would be incorrect to attribute the entire trajectory to the acceptance step.

This metaphor of Climbing Mount Probable has the fascinating implications that 1) intrinsic biases in variation

Figure 1. Les Drus, a mountain in France that is particularly rugged, with many local peaks (copyright Guillaume Dargaud, used by permission).

are fundamental causes of direction and that 2) by dual causation, adaptive evolution can be—simultaneously—both an adaptive process of fitting to conditions and an expression of intrinsic propensities of change.

Are such effects important in evolution? To answer this question requires more than just understanding a metaphor. First, it must be shown that the metaphor of Climbing Mount Probable faithfully conveys the operation of some proper cause defined, not metaphorically, but in biological terms, that is, something that can be shown theoretically with a population-genetic model, or demonstrated experimentally with a model system. Below we review the conceptual and theoretical basis (Yampolsky and Stoltzfus 2001; Stoltzfus 2006a, 2006b) for recognizing mutational bias in the introduction of variants as a cause of nonrandomness.

Second, one must have evidence that the postulated cause operates in the natural world, and furthermore, accounts for effects that are important rather than trivial. Here, we present 2 narrow but compelling examples, the first one showing that mutation bias is important in determining the course of experimental evolution in a case (Rokyta et al. 2005) that is clearly adaptive, and the second—a new statistical analysis of sequence evolution—showing that mutation is not just a significant factor, but the most important known factor accounting for variance in propensities of amino acid replacement.

From Metaphor to Model

Can mutation impose a bias or be a cause of direction in evolution, and if so, how? In the classic view of the Modern Synthesis, "evolution" is defined as shifting the frequencies of genes in the "gene pool," which maintains an abundance of infinitesimal "random" variation; accordingly, evolutionary causes are conceived as "forces" ("pressures") that cause mass–action shifts, that is, classic evolutionary theory is a theory of forces (Sober 1984). In this view, mutation is seen as a weak force opposed effectively by selection, given that mutation rates are so low (Yampolsky and Stoltzfus 2001; Stoltzfus 2006b). This view suggests that an effect of mutation would depend on special conditions such as unnaturally high rates of mutation, or absence of selection, that is, neutral evolution. Indeed, the research literature associates mutation-biased evolution with neutral evolution (Sueoka 1988; Gillespie 1991; Wolfe 1991; Gu et al. 1998; Lafay et al. 1999; Knight et al. 2001). To this classic conception of mutation as a weak "force," we may add a quite different notion of individual mutation events as a source "chance" or "contingency" that "can cause unpredictability" (Johnson et al. 1995) as in Monod (1972), Gould (1989), Mani and Clarke (1990), Lenski and Travisano (1994), and Wahl and Krakauer (2000).

By contrast, the metaphor of Climbing Mount Probable suggests a predictable effect of a bias in the proposal step (mutation) that does not depend on neutrality or high mutation rates. Thus, to distinguish this way of thinking from pressure and chance (and also the classic idea of mutation as a source of "fuel" or "raw materials"), the key is to look for a predictable effect of a bias in mutation for the case of beneficial (not neutral) changes, where new mutations can occur.

The simplest possible model must allow for 2 types of alleles that are absent initially, so that a bias in their rates of introduction may be imposed. At minimum, then, one may consider a haploid 2-locus 2-allele model in which an initial population of *ab* individuals is subject to mutations that introduce genotypes *Ab* and *aB* at 2 different rates. For fixations to occur, these mutations could be either neutral or beneficial, but as the present goal is to address selective allele fixations, they must be beneficial. Of interest is the nontrivial case in which the alternative genotype of relatively higher fitness is introduced by mutation at the relatively lower rate. Figure 2 (inset) shows a genetic model of this type. Choosing *Ab* arbitrarily as the alternative genotype of higher fitness, the fitnesses of *Ab* and *aB* would be $1 + s_1$ and $1 + s_2$, respectively, with $s_1 > s_2 > 0$, whereas the mutation rates (from *ab*) would be μ_1 and μ_2, respectively, with $\mu_1 < \mu_2$.

Of interest for a given set of conditions is the bias in outcomes, that is, the number of times the population evolves to the mutationally favored peak (*aB*), divided by the number of times it evolves to the peak of highest fitness (*Ab*). For convenience, the inequality in fitness of *Ab* and *aB* is defined as a bias in selection coefficients, $K = s_1/s_2$, with the bias in mutation defined oppositely as $B = \mu_2/\mu_1$. Figure 2 shows the bias in outcomes as a function of mutation bias B for various population sizes. Clearly, the bias in outcomes increases as a function of the bias in mutation. The dependence is linear through most of the range examined. Wherever the bias in outcomes exceeds 1, mutation bias may be said to determine the predominant direction of evolution. Yampolsky and Stoltzfus (2001) evaluate this model under a variety of conditions. The general result is that, for a large region of parameter space, the effects of both B (mutation bias) and K (selection coefficient bias) are linear on the bias in outcomes.

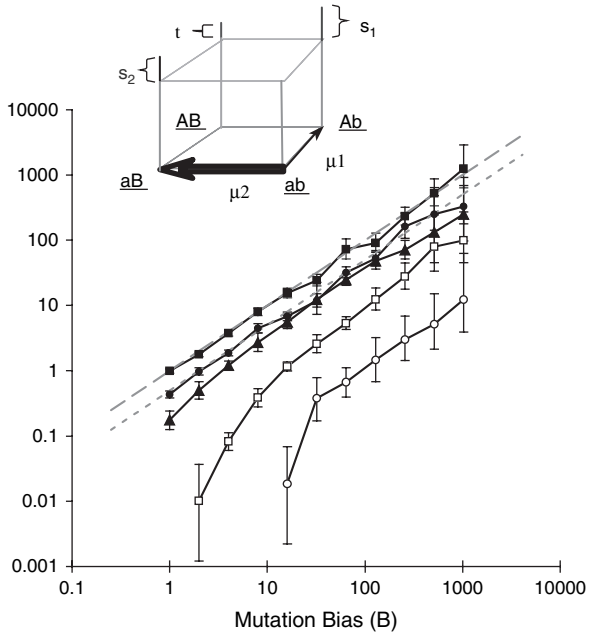

Figure 2. Mutation-biased adaptation in the Yampolsky–Stoltzfus population model. The bias in outcomes (mutationally favored peak vs. alternative peak) is shown as a function of mutation bias for the Yampolsky–Stoltzfus model illustrated in the inset figure. Starting with a pure *ab* population, mutations occurred at a rate of $\mu_2 = 10^{-5}$ and $\mu_1 = \mu_2/B$, with $s_1 = 0.02$ and $s_2 = s_1/K$, where $K = 2$, and the mutation bias B varies along the horizontal axis. The 5 series represent a 10^5-fold range of population sizes: 10^1, black squares; 10^3, filled circles; 10^4, filled triangles; 10^5, open squares; and 10^6, open circles. When $N = 10^1$, the population size is so small that the effect of a new mutation is very nearly neutral ($Ns = 0.1$ or 0.2), and thus, the bias in outcomes corresponds simply to B (dashed gray line). When $N = 10^3$, selection is strong ($Ns = 10$ or 20), and the bias in outcomes is approximately B/K (dotted gray line). Whenever the bias in outcomes exceeds 1, mutation bias has determined the predominant outcome. This occurs even when population sizes are quite large. For results under other conditions, see Yampolsky and Stoltzfus (2001).

The behavior represented in Figure 2 should not be considered mysterious: in the regime where new mutations are rare, that is, when $\mu N \ll 1$, it is an utterly simple matter of "first come, first served." Given that the waiting time to the next new mutation is $1/(\mu N)$, which is inversely proportional to the mutation rate, the first beneficial variant introduced is B times more likely to be the one favored by mutational bias. Even if this first mutant allele gets lost (as is likely, given that the probability of fixation is only $\sim 2s$), the next one (just like the first) is also B times more likely to be the one favored by mutational bias.

This addresses one-step adaptation, but can mutation bias impose a long-term trend? Would such a trend be opposed by some kind of cumulative resistance or backlash? To address this question requires a fitness function that extends beyond immediate alternatives, mapping out some larger region of a "fitness landscape." Accordingly, Stoltzfus (2006a) considers the effect of mutation bias on protein sequence evolution, using an "NK" model (Kauffman 1993), which allows a fitness landscape of tunable ruggedness. Specifically, the model was used to evaluate the influence of a GC:AT mutation bias on amino acid composition, measured by the frequency of amino acids with GC-rich codons (G, A, R, and P) relative to those with AT-rich codons (F, Y, M, I, N, and K). In this case, adaptive walks start from a random position and proceed to a peak, the number of steps typically being on the order of 10^2.

The results (Stoltzfus 2006a) follow what one would expect from the "Climbing Mount Probable" metaphor. A modest mutation bias—in the range of those inferred for actual species by genome analysis—can bias the trajectory of an adaptive walk (composed exclusively of adaptive steps), such that the amino acid composition is biased substantially. Thus, as suggested by the metaphor of Climbing Mount Probable, the trajectory of long-term adaptive change could reflect simultaneously both fitness increases and intrinsic mutation biases.

From Model to Cause

We cannot convey the role of mutation in these models by invoking classic conceptions of mutation as a source of raw materials nor as a mass-action pressure on allele frequencies, nor by invoking mutation as "chance." Why do these classic conceptions fail? Indeed, given the simplicity of the 2-locus 2-allele model, and the fundamental nature of its result, why wasn't this demonstration done 70 years ago? Why, instead, did the architects of the Modern Synthesis reach the opposite conclusion that, because mutation is such a weak force, variation-biased evolution is untenable (Yampolsky and Stoltzfus 2001)?

The answer hinges largely on the fact that Figure 2 reflects a view of evolution as a 2-step process in which distinctive individual mutants, each introduced by an event of mutation, face individual acceptance or loss. Although this conception of evolution may seem familiar to us today, the architects of the "Modern Synthesis" rejected this mutationist or "lucky mutant" conception of evolution, which they perceived to be a non-Darwinian theory giving too much prominence to mutation (Stoltzfus 2006b). Instead, in their view, evolution is a process of shifting the frequencies of alleles already present in a superabundant gene pool of variants with infinitesimal effects. This gene pool view has distinct implications shown in Figure 3. Although the biasing effect of mutation is robust to many different conditions (Yampolsky and Stoltzfus 2001), this effect is utterly destroyed by initial variation (Figure 3, lower curves). When the alleles relevant to the outcome of evolution are present initially, mutation cannot introduce anything new but can influence the outcome only by causing mass-action shifts in frequency, which would require unnaturally high rates of mutation.

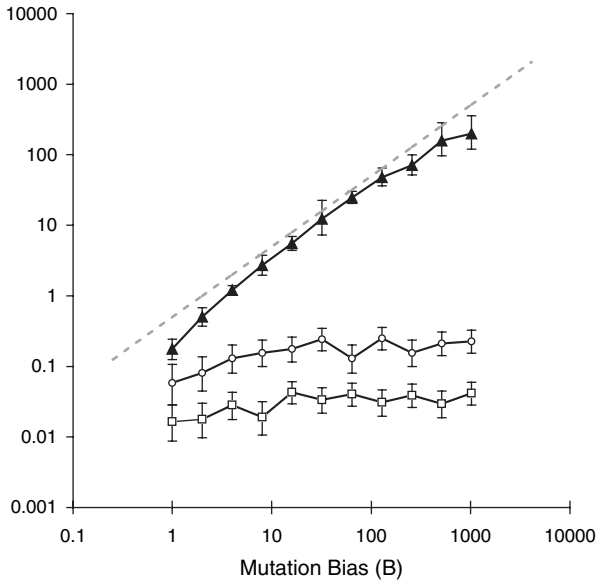

Figure 3. Initial variation forestalls an influence of mutation bias. As in Figure 2, the bias in outcomes is shown as a function of mutation bias for the Yampolsky–Stoltzfus model. The initial population of $N = 10^4$ is either a pure ab population (filled triangles, as in Figure 2), or is seeded with initial frequencies of each of the alternative alleles (aB and Ab) of 0.005 (open circles) or 0.01 (open squares). The presence of initial variation corresponds to the neo-Darwinian conception of evolution in which all the variation that is "needed" is already present in the gene pool, and evolution consists entirely of shifting the frequencies of alleles. The lack of any effect of mutation bias (i.e., the flatness of the lines) where initial variation is present shows that the proper cause of the bias in outcomes, where it occurs, is not mutation bias per se, but a mutational bias in the introduction of new alleles.

Thus, the problem here is not that (e.g., as implied in Orr 2002) the architects of the Modern Synthesis failed to understand the true meaning of population genetics: they simply had a different theory of evolution, which they understood perfectly well. Under their gene pool view, the role of mutation in evolution is precisely as textbooks (e.g., Freeman and Herron 1998) describe it: Mutation is "ultimately necessary" as a source of raw materials in the gene pool, yet it is a "weak pressure" incapable of influencing the outcome of evolution.

Nonetheless, the causal role of mutation is distinctly different when evolution is conceived as an origin-fixation process. We hasten to point out that, for 40 years, theoreticians have been exploring models of evolution as a 2-step mutation-fixation process (Kimura 1983; Bulmer 1991; Gillespie 1991), and such models are fundamental to the field of molecular evolution. Nevertheless, even though the role of mutation in these models does not correspond to the classic conception of mutation as a mass-action pressure, the language of "mutation pressure" and even the "opposing pressures" logic, continue to be used, as explained above (note that the mutation pressure concept remains valid when one is considering persistent deleterious mutation, e.g., in Lynch et al. 2006).

How can we understand the causal role of mutation, and of bias in mutation, in this altered view? In general, scientists accept that for an event or process x to be understood as the cause of another event or process y, 3 conditions must be met: x occurs before (or at the same time as) y; x and y occur in the same place (the "locale" of causation); and x determines y (such that, for instance, y could be computed from x, given background conditions). In the present case, we wish to know what is the cause x of the effect y, where x is something having to do with mutation, and y is a bias on the outcome of evolution relative to the case in which the cause x is absent.

What, precisely, is this new kind of cause? Above it was implied that x is "mutation bias," but Figure 3 shows that this cannot be the proper definition of the cause x, as the criterion of determination fails, that is, y does not always follow x. For all 3 series shown in the figure, mutations continue to occur throughout the simulations, with biased rates determined by B, yet the effect on bias in outcomes only appears in the series where there is no initial variation (Figure 3, upper series). Therefore, the correctly construed cause of the bias in outcomes is not mutation bias per se, but a bias in the introduction of novel alleles. When all genotypes are present initially, there is no introduction process, thus no bias in introduction, thus no effect.

Thus, the notion of an evolutionary bias due to a biased introduction process is not merely a provocative implication of a verbal metaphor, but a fundamental principle of population genetics. Bias in the introduction of novel alleles is a proper evolutionary cause of direction or orientation, but it is not a mass-action force as conceived classically in the evolutionary theory of forces (Sober 1984).

The Case for Mutation-Biased Evolution

Having established that bias in the introduction of variation is a distinctive theoretically possible cause, the next challenge is to determine whether it is an actually important cause. In the field of molecular evolution, many studies suggest that asymmetries in mutation influence the course of evolutionary divergence, contributing to parallelisms, persistent patterns, and directional trends. However, most cases either are 1) not compelling because there is no independent verification of postulated mutational propensities, or 2) of uncertain significance because neutral evolution cannot be ruled out (i.e., so that any mutational effects observed would be superficially consistent with the neutralist interpretation of opposing pressures).

Although neutral evolution cannot be ruled out in most cases, the experiments of Rokyta et al. represent an exception. Rokyta et al. (2005) carried out one-step adaptive walks with a laboratory population of bacteriophages, finding that the likelihood of the observed results given an origin-fixation model of adaptive steps is increased 21-fold

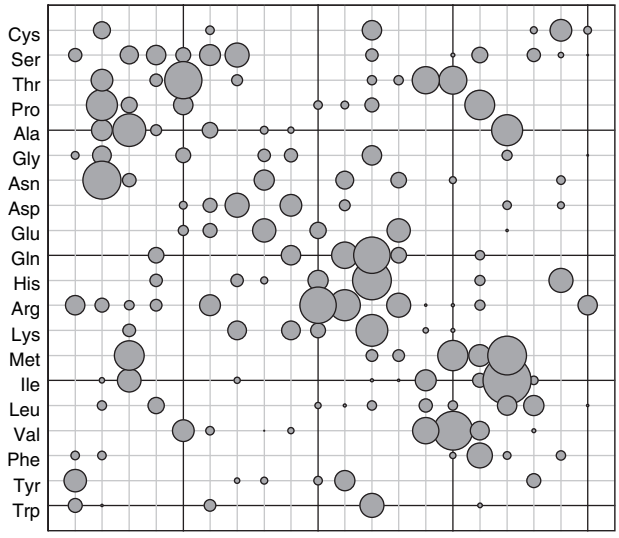

Figure 4. Propensities of amino acid change in human–chimp divergence. The area of the disk at the coordinates for amino acids *x* (horizontal axis) and *y* (vertical axis) is proportional to the number of inferred amino acid replacements from *x* to *y*, normalized by the frequency of amino acid x. The replacements are inferred from 8573 three-way alignments (human, chimpanzee, and mouse) yielding 2 487 858 aligned codon sites, with 17 725 and 14 710 differences assigned to changes in the chimp and human, respectively. As described previously (Yampolsky et al. 2005), sites were removed if 1) any sequence had an indel or uncertainty; 2) the murine amino acid was different from both primate amino acids; and 3) human and chimpanzee differ by more than 1 nucleotide site.

(relative to the model of Orr, which ignores mutational bias) by taking into account mutational effects, which include an approximately 12-fold transition:transversion bias, as well as a maximum 3-fold difference in the multiplicity of mutational paths to alternative amino acid states. That is, the results of an adaptation experiment rule out a model of adaptation precisely because it ignores mutational bias in the introduction process.

As a second case, presented here for the first time, we quantify the responsibility of mutational effects for the tendencies observed in a very large set of changes, namely amino acid changes inferred from human–chimp divergence, shown in Figure 4. Note that this plot (and similar plots below) show only the 150 so-called "singlet" exchanges, that is, the amino acid changes that can take place by a 1-nt mutation. Doublet and triplet changes are ignored here for the sake of simplicity, but these changes actually occur in evolution (and in spontaneous mutation), at a rate just 2 or 3 orders of magnitude less than singlet changes, that is, much more than expected from a null model of independent single-nucleotide changes (Chuzhanova et al. 2003; Smith et al. 2003; Whelan and Goldman 2004).

The pattern of evolutionary tendencies in amino acid replacement shown in Figure 4 is not trivial. Scientists who study protein-sequence evolution are exposed to its consequences on a daily basis. Tendencies of amino acid replacement have been considered an interesting subject for analysis and interpretation for over 40 years. For example, Zuckerkandl and Pauling remarked in 1965 that

> The inadequacy of a priori views on conservatism and nonconservatism [of amino acid replacements] is patent. Apparently chemists and protein molecules do not share the same opinions regarding the definition of the most prominent properties of a residue (Zuckerkandl and Pauling 1965, p. 355)

Such ideas of "conservatism and nonconservatism" continue to be relevant today, in practical problems of biomedical inference, such as how to interpret naturally occurring protein variants in the human population (Ng and Henikoff 2003). Therefore, it is neither trivial nor unimportant to ask the question, to what extent are tendencies of amino acid replacement due to propensities of variation, and to what extent are they due to fitness effects?

This question can be addressed in this case because it is possible to formulate a largely successful model of mean relative rates of amino acid changes based entirely on prior knowledge of mutational and selective effects in the context of a simple population-genetic model. The population-genetic model is the steady-state rate of an origin-fixation process (Kimura 1983), which is the product of the rate of introduction, $N\mu$, and the probability of fixation, π. In the present context, N is constant and can be ignored (given that we are interested only in relative rates). The basis of predictable effects on π is that some types of changes are less likely than others to be disruptive, or more likely to be beneficial, for example, a change from Serine to Threonine is a change between chemically similar residues, whereas a change from Serine to Proline is not. The basis of effects of mutation is 2-fold, reflecting both biases inherent in mutation and biases imposed by the genetic code. For instance, to change a Serine codon to a Proline codon requires a T-to-C nucleotide mutation, which (due to transition:transversion bias, explained below) typically occurs at a higher rate than the mutation required to change a Serine codon to a Threonine codon (which is either T-to-A or G-to-C, depending on the Serine codon).

The predicted rate (unscaled) for changing from amino acid i to amino acid j is then

$$r_{ij} = \Sigma_n \Sigma_m \mu_{mn} \pi_{ij}, \quad (1)$$

where m and n are the indexes over the sets of codons for amino acids i and j, respectively. Thus μ_{mn} is a relative rate of codon mutation from codon m to codon n, and π_{ij} is the probability of fixation for a mutation that changes amino acid i to j.

Mutation parameters relevant to primates or mammals have been estimated from studies of natural variation and of divergence in noncoding sequences (Nachman and Crowell 2000; Ebersberger et al. 2002; Kondrashov 2003; Zhang and Gerstein 2003). Here, we consider only mutation biases known to have a 1.5-fold or greater effect, a category that

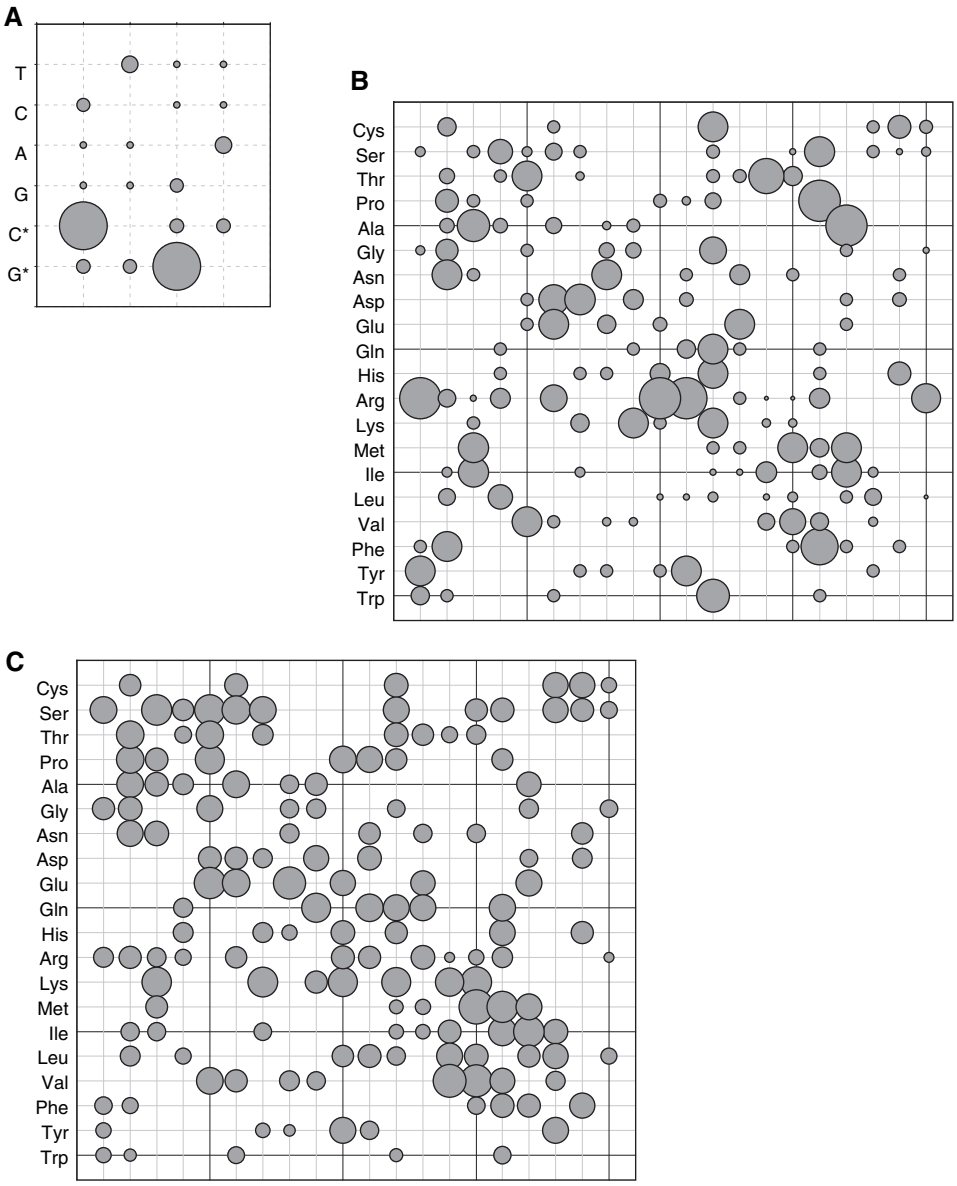

Figure 5. Components of a predictive model. (**A)** The nucleotide mutation model incorporates only effects of 1.5-fold or more, as described in the text, including transition:transversion bias, the effect of a CpG dinucleotide context (represented by C* and G*) on transitions and (separately) on transversions, and the effect of a bias in forward versus reverse rates of mutations from strong (G or C) to weak (A or T) base pairs. (**B)** Combining nucleotide mutation and the genetic code, weighting each synonymous codon equally, yields relative mean rates of mutation from a codon for amino acid x (horizontal axis) to a codon for amino acid y (vertical axis). (**C)** Values of EX, an unbiased measure of amino acid exchangeability in proteins (Yampolsky and Stoltzfus 2005), are shown here for singlet exchanges only (EX_{ij} is the exchangeability from the amino acid in column *i* to row *j*). In the absence of any better predictor, the probability of fixation for a mutation from amino acid *i* to *j* is assumed to be a simple function of EX_{ij}.

includes effects of transition:transversion bias, the added mutability of CpG sites, and an asymmetry of mutations between G:C versus A:T base pairs. These parameters, as illustrated in Figure 5A, are defined such that an A-to-T transversion mutation occurs at the base rate μ. Given a transition:transversion bias of α, a transition mutation occurs at the rate $\mu\alpha\delta^{0.5}$ if it increases GC content (i.e., T-to-C and A-to-G), and $\mu\alpha\delta^{-0.5}$ if it decreases GC content, thus δ is the GC:AT bias parameter (the ratio of forward to reverse rates). These terms are multiplied by β for a transition in a CpG context or by γ for a transversion in a CpG context. The estimated values of these parameters are $\alpha = 3.82 \pm 0.034$ (95% confidence interval), $\beta = 9.09 \pm 0.16$, $\gamma = 3.52 \pm 0.13$, and $\delta = 1.68 \pm 0.018$ (from data for noncoding regions kindly provided by Dick Hwang based on the data of Hwang and Green 2004).

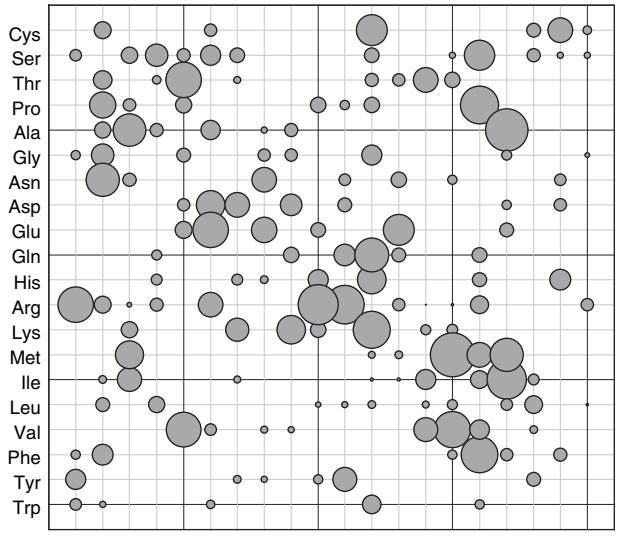

Figure 6. Predicted relative mean rates of amino acid replacement. The predicted relative rate of amino acid replacement can be computed from the simple origin-fixation model explained in the text (see Equation 1 and accompanying explanation), given the models for mutation and exchangeability shown in Figure 5. The resulting predicted values, shown here, correlate strongly ($R^2 = 0.63$) with the mean rates based on observed human–chimp differences (see Figure 4).

The mutation model alone implies a considerable nonrandomness in the mean codon mutation rate from one amino acid to another, as shown in Figure 5B.

In principle, the probability of fixation for a nucleotide mutation in a protein-coding region may reflect a variety of effects on mRNA stability, splicing, translation efficiency, protein operation, metabolic cost of amino acids, and so on. In the absence of a complete quantitative model of these effects, here the dominant effect is assumed to be the role of amino acids in protein structure and operation. This is implicit in Equation 1 in that π_{ij} is defined in terms of the starting and ending amino acids (not codons). Thus, π_{ij} may be defined as some increasing function of EX_{ij}, a measure of amino acid exchangeability based on a meta-statistical analysis of nearly 10 000 amino exchanges engineered and assayed under controlled conditions (Yampolsky and Stoltzfus 2005). Unlike amino acid similarity measures computed from patterns of evolution, EX is derivationally free of effects of mutation.

The predicted relative rates are shown in Figure 6, assuming that π_{ij} is a linear function of EX_{ij}. The correlation between the predicted pattern and the pattern inferred from observed human–chimp differences (Figure 4) is remarkably strong, $R^2 = 0.63$. That is, the prediction model accounts for 63% of the variance in propensities of amino acid replacement.

How much of the predictability of amino acid replacement preferences is due to prior knowledge of mutation? One way to ask this question is to consider the mutational component of the model by itself, shown in Figure 5B. The predicted pattern is quite similar to the pattern inferred from human–chimp alignments (Figure 4), indeed the correlation is $R^2 = 0.45$. This similarity is due largely to transition:transversion bias. By comparison, EX alone accounts for 13% of the variance in propensities of amino acid change. Prior results (Yampolsky and Stoltzfus 2005) suggest that an exponential transformation of EX might be more appropriate and indeed, exp(EX) accounts for a larger fraction of the variance, 24%.

Perhaps an even stronger effect of exchangeability would emerge with a more accurate version of EX, which has considerable uncertainty (Yampolsky and Stoltzfus 2005). Also, by focusing solely on amino acid exchangeability, the fitness model might be ignoring some key factors, such as metabolic cost of amino acids or codon fitness. Nevertheless, although a better model might reveal that fitness effects account for more variance than the 13% or 24% attributable to EX, it will not substantially reduce the 45% attributable to mutation, unless the missing factors happen to correlate strongly with mutation (EX does not). Given this one qualification, it seems rather safe to conclude that in this case, not only are propensities of mutation important, but their importance is also comparable with that of selection.

A Firm Grasp of the Obvious

It is worthwhile to state a few conclusions that follow directly from the foregoing results and that previously were not known, not accepted, or simply not stated explicitly.

First, evolution has tendencies or propensities, an idea that has faced resistance from those committed to the idea that evolution is unpredictable (e.g., see Beatty 2008 for an analysis of the resistance to the idea of trends or directions), and that is not obvious in other fields of evolution. For instance, paleontologists debate this issue, because patterns of change in features inferred from the fossil record typically are consistent with a random walk model (Bookstein 1987). By contrast, in the field of comparative genomics, this point is demonstrated literally thousands of times each day when sequence alignments are computed: such alignments depend on alignment scoring matrices (e.g., the classic Dayhoff matrix)—which merely distill the patterns of change inferred from a previous set of alignments in order to apply them to a new set—so that whenever alignment succeeds on this basis, the recurrence or consistency of evolutionary tendencies has been demonstrated.

Second, these tendencies are predictable, contrary to the oft-stated notion that evolution is inherently unpredictable. The tendencies are predictable, not merely in the weak sense (above) that they are recurrent, but in the stronger sense that we can construct a largely successful statistical predictor by incorporating logically prior estimates of parameters within the framework of a causal model. Of course, this still falls short of temporally prior predictions—the gold standard of prediction in science—a challenge that is difficult for the evolutionary biologist not necessarily because evolution is unpredictable, but because it takes too long to do the experiments.

Third, causal responsibility (for evolutionary tendencies) can be apportioned to multiple causes, in the same manner that, in the context of the nature-nurture debate, one may

apportion causal responsibility for tendencies of behavior to genes and to the environment (for discussion, see Sober 1994). It is absurd to ask whether an evolutionary change, such as a change from Arginine to Lysine at some site in some protein, is due to mutation or to fixation, as it is caused by both. Yet when many changes are observed, tendencies emerge, such that a given tendency (e.g., a tendency for Arginine to be replaced by Lysine more often than by Alanine) might be attributable entirely to mutation, or entirely to selection, or to some mixture of the 2.

Fourth, when causal responsibility is apportioned, the result is that mutational effects are quantitatively important. This is a key result. If the mutational component had accounted for only $R^2 \sim 1/100$ of the pattern, one could conclude that mutation is unimportant, consistent with the confident claim of Ford (1971) from the hey-day of neo-Darwinism that "if ever it could have been thought that mutation is important in the control of evolution, it is impossible to think so now." If the result had been $R^2 \sim 1/10$, it would have been possible to conclude that mutational causes have a significant yet distinctly minor impact. Instead, the actual finding of $R^2 = 0.45$ indicates that (in this case) mutation is a primary cause of nonrandomness, comparable with natural selection in the magnitude of its influence on why some types of changes occur more often than others.

Fifth, mutational effects are graduated, not absolute; thus, their influence cannot be described by invoking "constraints." For instance, the effect of transition:transversion bias does not arise because of a constraint that prevents transversion mutations from happening: Instead, they simply occur at a lower rate.

Beyond Mutation Bias

The concept of a bias in the introduction of variants is more general than the concept of a mutation bias, which one might define narrowly as an inequality in rates of mutational transformation between unique genetic states. Biases in the introduction of variation could take many different forms, as illustrated in Figure 7. The first 2 examples invoke conventional nucleotide mutation biases. However, the second 2 examples are more interesting, showing cases in which the starting type and its alternatives are defined, not in terms of unique genotypes, but in terms of unique phenotypes. In Figure 7C, the starting type is the amino acid Phenylalanine. Whether this is encoded by TTT or TTC, there are twice as many mutational paths to Leucine as there are to Tyrosine. Assuming (for the sake of example) that all individual mutation rates are the same, this means that, in a population of Phe-encoding genotypes, there will be a 2-fold bias in the introduction of alternative Leucine phenotypes, relative to Tyrosine phenotypes.

Extending the concept of bias in this way makes it more valuable for understanding the evolution of genomes and other molecular features. The causes of a bias in the introduction process may include, not just mutation bias conceived narrowly, but also effects of asymmetries in the representation of alternative states in local (mutationally accessible) genotype space. This kind of asymmetry may play a large role in molecular evolution (Stoltzfus 1999).

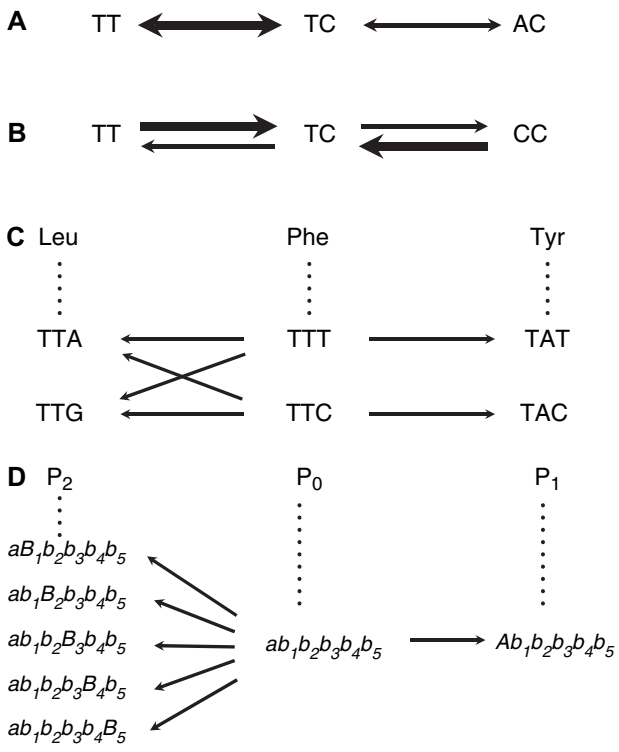

Figure 7. Different forms of bias in mutation effects. The model in Figures 2 and 3 can be given various interpretations in which mutually exclusive alternatives are accessible by mutation at different rates. In (**A**) and (**B**), there are 2 nucleotide loci with the starting genotype "TC." In (**A**), the alternative genotype on the left is reached by a C-to-T transition mutation at the second site, whereas the alternative (right) is reached by a T-to-A transversion at the first site. Thus, the leftward change would be favored by a transition:transversion bias. In (**B**), the alternative genotype on the left is reached by a C-to-T mutation at the second site, whereas the other alternative is reached by a T-to-C mutation at the first site. Thus, the leftward change would be favored by a bias favoring AT over GC base pairs. In (**C**), the starting type is the amino acid Phe. Whether this is encoded by TTT or TTC, there are twice as many mutational paths to Leu (left) as there are to Tyr (right). This asymmetry is imposed by the genetic code, a nonuniform developmental mapping of amino acid phenotypes to codon genotypes. In (**D**), this concept of a nonuniform mapping is interpreted in terms of a starting phenotype P_0 that has 5 times as many ways to mutate to P_2 as it does to P_1, with the result that there is a 5-fold bias toward P_2. For clarity, reverse mutations are not shown in (**C**) and (**D**).

Formally, this kind of asymmetry is not different from a conceivable form of developmental bias. An observation that is stressed repeatedly in the evo–devo literature is that some phenotypes are more likely to arise by mutation than others (Emlen 2000). Figure 7D gives this concept a precise interpretation, in which, given the starting phenotype P_0, there are 5 times as many ways to mutate to P_2 as to P_1. In the

language of genetics, the mutants that generate P_2 are phenocopies of each other. In some scenario where P_2 and P_1 are alternatives favored under a set of conditions, the evolutionary emergence of phenotype P_2 is favored by a developmentally mediated bias in the introduction of variation.

This point is relevant to the way in which, at various times, evo–devo enthusiasts have attempted to suggest that "integrating development into evolutionary theory" might require something more than just adding facts to a set of preestablished principles. In some cases, these claims clearly implicate a causal principle based on biases in variation, for example,

> The whole thrust of the developmentalist approach to evolution is to explore the possibility that asymmetries in the introduction of variation at the focal level of individual phenotypes, arising from the inherent properties of developing systems, constitutes a powerful source of causation in evolutionary change (Thomson 1985).

Nevertheless, this kind of claim has met with stiff resistance, on 2 main grounds. For Mayr (Mayr 1994), such evo–devo claims are "hopelessly mixed up" because they confound what he believes to be proximate biological causes such as development with ultimate or "evolutionary" causes (Mayr 1961). Why development cannot be construed as an evolutionary cause is often explained as an issue of causal locale, the demand (invoked above as a criterion of causation) that cause and effect occur in the same place:

> If we are to understand evolution, we must remember that it is a process that occurs in populations, not in individuals. Individual animals may dig, swim, climb, or gallop, and they also develop, but they do not evolve. To attempt an explanation of evolution in terms of the development of individuals is to commit precisely that error of misplaced reductionism of which geneticists are sometimes accused. (Maynard Smith 1983)

A second criticism of evo–devo is based on interpreting evo–devo as a claim about the importance of developmental biases in variation, and then appealing to the logic of opposing pressures to argue that such biases would be ineffectual, as when (Reeve and Sherman 1993) ask, in their rebuttal of evo–devo, "why couldn't selection suppress an 'easily generated physicochemical process' if the latter were disfavored?"

It is now possible to explain why these criticisms are misplaced. The flaw in the opposing pressures schema has been explained already: To the extent that the role of development in evolution is to mediate biases in the introduction of variation, this role is not part of a zero-sum contest with an opposing force of selection, because introduction and fixation are 2 different steps in the evolutionary process (Yampolsky and Stoltzfus 2001). The notion that a developmental cause cannot satisfy a demand from causal locale also is mistaken: even if we accept the doctrine that evolutionary causes must happen "in a population," the introduction of a novel phenotype by mutation and altered development is an event that happens in a population, and thus, a bias in the introduction process can be an evolutionary cause by this criterion. It follows that the notion that causation in biology includes a single ultimate cause, selection (Mayr 1961; Ayala 1970), and various other proximate or nonevolutionary causes, is false.

Discussion

Bias in the introduction of variation is a theoretically possible cause of evolutionary orientation. Because this cause acts via the introduction (not fixation) step in a 2-step process, it is not an analog or competitor of selection (i.e., not a mass-action pressure, not an opposing pressure) and its impact does not require neutral evolution. Instead, by virtue of dual causation, evolutionary change may be both an adaptive adjustment to external conditions and, simultaneously, an expression of inherent propensities of variation. The inherent propensities of variation that are relevant to this kind of effect are not limited to mutational biases per se but may include biases in the emergence of alternative phenotypes mediated by development.

On several occasions, we have been told that the preceding claims, to the extent that they are consistent with theoretical population genetics, are consistent with the views of Fisher, Haldane, et al., and pose no threat to neo-Darwinism; that the results presented above, though perhaps interesting in some ways, are unsurprising and do not represent anything new in terms of principles or concepts. As evidence of the priority of Haldane, one participant at the AGA conference referred to the following passage from Haldane's "The Causes of Evolution":

> A selector of sufficient knowledge and power might perhaps obtain from the genes at present available in the human species a race combining an average intellect equal to that of Shakespeare with the stature of Carnera. But he could not produce a race of angels. For the moral character or for the wings, he would have to await or produce suitable mutations.

Here, Haldane contrasts 2 modes of evolution: 1) selection of available variation, which may be expected to produce natural wonders such as Shakespeare or Carnera (i.e., the Modern Synthesis view) and 2) waiting for new mutations, which is associated with the production of imaginary creatures such as angels (mutationism). We see nothing in this passage that represents an understanding of the principles enumerated in the first paragraph of this section. Indeed, Haldane (along with Fisher) was a progenitor of the opposing pressures argument against a role of mutation as a directional cause (Yampolsky and Stoltzfus 2001).

Such attempts to assign recent innovations to long-dead authorities perhaps reflects a hindsight bias, as well as an insufficient awareness of what Orr (2002) calls the "curious disconnect between the verbal theory that sits at the heart of neo-Darwinism and the mathematical content of most evolutionary genetics". In the case of evolutionary biology, the verbal theory (e.g., Darwin's theory) predates any mathematical content and, for instance, plays an exclusive role in Gould's magnum opus on "the structure of evolutionary theory" (Gould 2002), which contains (in its 1400 pages) not a single equation of population genetics.

One aspect of the "disconnect" between verbal and mathematical is a generic process of science by which different representations (verbal, graphical, mathematical, and analog) of nominally the "same" thing are discovered to differ, and in particular, a process by which relatively more formal representations ("mathematical content," simulations) are interpreted to have implications that contradict less formal—but often more intuitively appealing—representations. For instance, to consider an example from molecular biology, textbooks typically invoke a "pacemaker" ("rate-limiting step") concept by which the flux through a metabolic pathway is imagined to be controlled by one step. Yet, over 30 years ago, Kacser and Burns (1973), working solely from first principles of enzyme kinetics, showed that the pacemaker concept is invalid and that flux control is distributed (unequally; dependent on conditions) among all steps in a pathway (for explanation, see Chapter 12 of Cornish-Bowden 2004).

Apparently, scientists like the idea of a rate-limiting step much more than that of distributed, condition-dependent control; they make use of this flawed concept and may do so without contradiction in contexts where its flaws are not exposed. We suggest similarly that, for instance, evolutionists are familiar with the conceptual schema of mutation and selection as opposing pressures, and that the flaws in this way of thinking are rarely exposed (due to the low level of interest in mutational explanations).

Another part of this disconnect (between verbal and mathematical) is peculiar to evolutionary biology, which has made a number of explorations into areas of mathematical theory that clash with classic views of evolution, while continuing to use the old language in barely modified form, as though nothing had changed. This is particularly jarring in research where an implicitly mutationist conception of evolution is relevant. A recent article asks the question of "whether mutation or selection is responsible for evolution at silent sites" (Yang and Nielsen 2008). This is a question about causal responsibility that makes sense if mutation and selection are classical forces (which act as the cause of an evolutionary change by driving a gene to fixation), but which does not make sense within the origin-fixation view implicit in the model represented mathematically in the very same article. Another recent article (Hermisson and Pennings 2005) defines adaptation in classic Darwinian fashion as a process initiated by a change in conditions ("environmental change or the colonization of a new niche"), then presents, as alternative models for this process, both the classic model based on standing variation and the "new mutations" model, even though the latter model is not a model of adaptation so defined, because the new mutation (not the change in conditions) initiates the process. Although one might redefine adaptation as an increase in "aptness," so that it might include both models, Darwin clearly saw adaptation as a response to "altered conditions of life" and likewise rejected the notion of a process initiated by a variational "sport," yet recent authors have taken to describing their mutation-fixation models as "Darwinian" (Orr 2005; Weinreich et al. 2006). Orr (2005) blames the popularity of the Neutral Theory for a decades-long delay in the emergence of mutation-fixation models of "Darwinian adaptation," not realizing—precisely due to using the same words for different concepts—that, during this time, the prevailing view (the Modern Synthesis) had ruled out his mutationist conception of evolution, and that the fixation of newly arising beneficial mutations is not in the same category as the classic understanding of adaptation as a response to an external stimulus.

For such reasons, the development of an accurate unifying verbal theory is a major outstanding problem in evolutionary biology. The arguments presented here are intended to advance a theory that has solid links to mathematical and empirical results. Although this theory is not the same as the Modern Synthesis, clearly it relates to prior work. Previously (Stoltzfus 2006b), we discussed some of these sources (King 1971; Vrba and Eldredge 1984; Mani and Clarke 1990; Stoltzfus 1999). The most compelling of these, King's (1971) article "The Role of Mutation in Evolution," gives a verbal depiction of mutation-biased adaptation and explains why mutation-biased evolution would not require neutral evolution.

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References

Ayala FJ. 1970. Teleological explanations in evolutionary biology. Phil Sci. 37:1–15.

Beatty J. 2008. Chance variation and evolutionary contingency: darwin, Simpson, the Simpsons, and Gould. In: Ruse M, editor. Oxford handbook of the philosophy of biology. Oxford: Oxford University Press.

Bookstein FL. 1987. Random walk and the existence of evolutionary rates. Paleobiology. 13:446–464.

Bulmer M. 1991. The selection–mutation–drift theory of synonymous codon usage. Genetics. 129:897–907

Chuzhanova NA, Anassis EJ, Ball EV, Krawczak M, Cooper DN. 2003. Meta-analysis of indels causing human genetic disease: mechanisms of mutagenesis and the role of local DNA sequence complexity. Hum Mutat. 21:28–44.

Cornish-Bowden A. 2004. Fundamentals of enzyme kinetics. London: Portland Press.

Ebersberger I, Metzler D, Schwarz C, Paabo S. 2002. Genomewide comparison of DNA sequences between humans and chimpanzees. Am J Hum Genet. 70:1490–1497.

Emlen DJ. 2000. Integrating development with evolution: a case study with Beetle Horns. BioScience. 50:403.

Ford EB. 1971. Ecological genetics. London: Chapman & Hall.

Freeman S, Herron JC. 1998. Evolutionary analysis. Upper Saddle River (NJ): Prentice-Hall.

Gillespie JH. 1991. The causes of molecular evolution. New York: Oxford University Press.

Gould SJ. 1989. Wonderful life: the Burgess shale and the nature of history. New York: Norton.

Gould SJ. 2002. The structure of evolutionary theory. Cambridge (MA): Harvard University Press.

Gu X, Hewett-Emmett D, Li WH. 1998. Directional mutational pressure affects the amino acid composition and hydrophobicity of proteins in bacteria. Genetica. 103:383–391.

Hermisson J, Pennings PS. 2005. Soft sweeps: molecular population genetics of adaptation from standing genetic variation. Genetics. 169:2335–2352.

Hwang DG, Green P. 2004. Bayesian Markov chain Monte Carlo sequence analysis reveals varying neutral substitution patterns in mammalian evolution. Proc Natl Acad Sci USA. 101:13994–14001.

Johnson PA, Lenski RE, Hoppensteadt FC. 1995. Theoretical analysis of divergence in mean fitness between initially identical populations. Proc R Soc Lond B Biol Sci. 259:125–130.

Kacser H, Burns JA. 1973. The control of flux. Symp Soc Exp Biol. 27:65–104.

Kauffman SA. 1993. The origins of order: self-organization and evolution. New York: Oxford University Press.

Kimura M. 1983. The neutral theory of molecular evolution. Cambridge: Cambridge University Press.

King JL. 1971. The role of mutation in evolution. In: Le Cam LM, Newman J, Scott EL, editors. Sixth Berkeley Symposium on Mathematical Statistics and Probability. Berkeley (CA): University of California Press. p. 69–88.

Knight RD, Freeland SJ, Landweber LF. 2001. A simple model based on mutation and selection explains trends in codon and amino-acid usage and GC composition within and across genomes. Genome Biol. 2:research0010.0011–research0010.0013.

Kondrashov AS. 2003. Direct estimates of human per nucleotide mutation rates at 20 loci causing Mendel Ian diseases. Hum Mutat. 21:12–27.

Lafay B, Lloyd AT, McLean MJ, Devine KM, Sharp PM, Wolfe KH. 1999. Protease composition and codon usage in spirochetes: species-specific and DNA strand-specific mutational biases. Nucleic Acids Res. 27:1642–1649.

Lenski RE, Travisano M. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. Proc Natl Acad Sci USA. 91:6808–6814.

Lynch M, Koskella B, Schaack S. 2006. Mutation pressure and the evolution of organelle genomic architecture. Science. 311:1727–1730.

Mani GS, Clarke BC. 1990. Mutational order: a major stochastic process in evolution. Proc R Soc Lond B Biol Sci. 240:29–37.

Maynard Smith J. 1983. Evolution and development. In: Goodwin BC, Holder N, Wylie CC, editors. Development and evolution. New York: Cambridge University Press. p. 33–46.

Mayr E. Response to John Beatty. Biol Phil. 9:357–358.

Mayr EM. 1961. Cause and effect in biology. Science. 134:1501–1506.

Monod J. 1972. Chance and necessity: an on the natural philosophy of modern biology. New York: Vintage Books.

Nachman MW, Crowell SL. 2000. Estimate of the mutation rate per nucleotide in humans. Genetics. 156:297–304.

Ng PC, Henikoff S. 2003. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 31:3812–3814.

Orr HA. 2002. The population genetics of adaptation: the adaptation of DNA sequences. Evil Into J Org Evil. 56:1317–1330.

Orr HA. 2005. Theories of adaptation: what they do and don't say. Genetica. 123:3–13.

Reeve HK, Sherman PW. 1993. Adaptation and the goals of evolutionary research. Quart Rev Biol. 68:1–32.

Rokyta DR, Joyce P, Caudle SB, Wichman HA. 2005. An empirical test of the mutational landscape model of adaptation using a single-stranded DNA virus. Nat Genet. 37:441–444.

Smith NG, Webster MT, Ellegren H. 2003. A low rate of simultaneous double-nucleotide mutations in primates. Mol Biol Evil. 20:47–53.

Sober E. 1984. The nature of selection: evolutionary theory in philosophical focus. Cambridge (MA): MIT Press.

Sober E. 1994. From a biological point of view. New York: Cambridge University Press.

Stoltzfus A. 1999. On the possibility of constructive neutral evolution. J Mol Evol. 49:169–181.

Stoltzfus A. 2006a. Mutation-biased adaptation in a protein NK model. Mol Biol Evol. 23:1852–1862.

Stoltzfus A. 2006b. Mutationism and the dual causation of evolutionary change. Evol Dev. 8:304–317.

Sueoka N. 1988. Directional mutation pressure and neutral molecular evolution. Proc Natl Acad Sci USA. 85:2653–2657.

Thomson KS. 1985. Essay review: the relationship between development and evolution. Oxford Surv Evol Biol. 2:220–233.

Vrba ES, Eldredge N. 1984. Individuals, hierarchies and processes: towards a more complete evolutionary theory. Paleobiology. 10:146–171.

Wahl LM, Krakauer DC. 2000. Models of experimental evolution: the role of genetic chance and selective necessity. Genetics. 156:1437–1448.

Weinreich DM, Delaney NF, Depristo MA, Hartl DL. 2006. Darwinian evolution can follow only very few mutational paths to fitter proteins. Science. 312:111–114.

Whelan S, Goldman N. 2004. Estimating the frequency of events that cause multiple-nucleotide changes. Genetics. 167:2027–2043.

Wolfe KH. 1991. Mammalian DNA replication: mutation biases and the mutation rate. J Theor Biol. 149:441–451.

Yampolsky LY, Kondrashov FA, Kondrashov AS. 2005. Distribution of the strength of selection against amino acid replacements in human proteins. Hum Mol Genet. 14:3191–3201.

Yampolsky LY, Stoltzfus A. 2001. Bias in the introduction of variation as an orienting factor in evolution. Evol Dev. 3:73–83.

Yampolsky LY, Stoltzfus A. 2005. The exchangeability of amino acids in proteins. Genetics. 170:1459–1472.

Yang Z, Nielsen R. 2008. Mutation-selection models of codon substitution and their use to estimate selective strengths on codon usage. Mol Biol Evol. 25:568–579.

Zhang Z, Gerstein M. 2003. Patterns of nucleotide substitution, insertion and deletion in the human genome inferred from pseudogenes. Nucleic Acids Res. 31:5338–5348.

Zuckerkandl E, Pauling L. 1965. Evolutionary divergence and convergence in proteins. In: Bryson V, Vogel HJ, editors. Evolving genes and proteins. New York: Academic Press.

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