Trends in Ecology & Evolution

CellPress
REVIEWS

Opinion

The Role of Mutation Bias in Adaptive Evolution

Erik I. Svensson [1,*] and David Berger[2]

Mutational input is the ultimate source of genetic variation, but mutations are not thought to affect the direction of adaptive evolution. Recently, critics of standard evolutionary theory have questioned the random and non-directional nature of mutations, claiming that the mutational process can be adaptive in its own right. We discuss here mutation bias in adaptive evolution. We find little support for mutation bias as an independent force in adaptive evolution, although it can interact with selection under conditions of small population size and when standing genetic variation is limited, entirely consistent with standard evolutionary theory. We further emphasize that natural selection can shape the phenotypic effects of mutations, giving the false impression that directed mutations are driving adaptive evolution.

Highlights

Standard evolutionary theory recognizes only one force (natural selection) that can lead to directional evolutionary change towards increased organismal adaptation.

Critics of standard evolutionary theory have argued that mutational bias is an alternative evolutionary process or cause that can increase organismal adaptation independently of natural selection.

We review the theoretical and empirical literature on mutation bias and identify the conditions under which it is likely to be important in adaptive evolution.

Mutation bias is unlikely to play an important role in adaptive evolution, except under conditions of small population sizes and low amounts of standing genetic variation.

Even if novel mutations are random with respect to organismal needs and are mostly deleterious, selection on the phenotypic expression of novel mutations (developmental bias) may cause alignment between mutational variation and the direction of increased fitness and macroevolutionary divergence, creating an illusory impression of adaptive directionality of novel mutations.

Mutation as an Evolutionary Process

In recent years, **standard evolutionary theory** (see Glossary), which has its origin in the **modern synthesis**, has come under criticism from some biologists and philosophers [1–4]. Critics argue that standard evolutionary theory, with its main reliance on four population genetic processes (mutation, genetic drift, recombination and selection) (Figure 1) [5], fails to provide a complete account of adaptation and phenotypic evolution [2]. Critics further argue that **mutation bias** and **developmental bias** can explain the origin of adaptations independently of, or in addition to, natural selection [3,6]. Of the four classical evolutionary processes, selection is currently the only accepted mechanism in standard evolutionary theory that can promote organismal adaptation [5] (Figure 1). Here, we discuss recent suggestions that mutation bias could be a directional force in adaptive evolution, in addition to selection [7,8]. We distinguish between teleological arguments for mutation bias in adaptation through **directed mutation** [9,10], which we firmly refute, and heterogeneous mutation rates, which can lead to **mutation pressure**. We do not deny that mutation pressure can lead to directional change, especially at effectively neutral loci when aided by genetic drift [5,11,12], but we argue that it is unlikely to promote organismal adaptation independently of selection. We also question the view of mutation bias as a novel evolutionary cause or process [7,8,13] above what is already accounted for by standard evolutionary theory, which nowadays includes the neutral theory of molecular evolution [5,11].

The Issue of Non-Random Mutation

Critics of standard evolutionary theory have questioned the assumption that mutations are random [2–4]. We clarify here that 'random' only means that novel beneficial mutations do not arise at a higher relative frequency as a direct response to current organismal needs [9]. Standard evolutionary theory and the modern synthesis do, however, make the non-controversial prediction that mutations will tend to have more beneficial effects on average in maladapted populations that are far from their phenotypic optima [14]. Moreover, mutations are certainly not randomly distributed across the genome, and different regions show different

[1]Department of Biology, Lund University, SE-223 62 Lund, Sweden
[2]Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norrbyvägen 18D, SE-752 36 Uppsala, Sweden

 https://doi.org/10.1016/j.tree.2019.01.015

*Correspondence:
erik.svensson@biol.lu.se (E.I. Svensson).

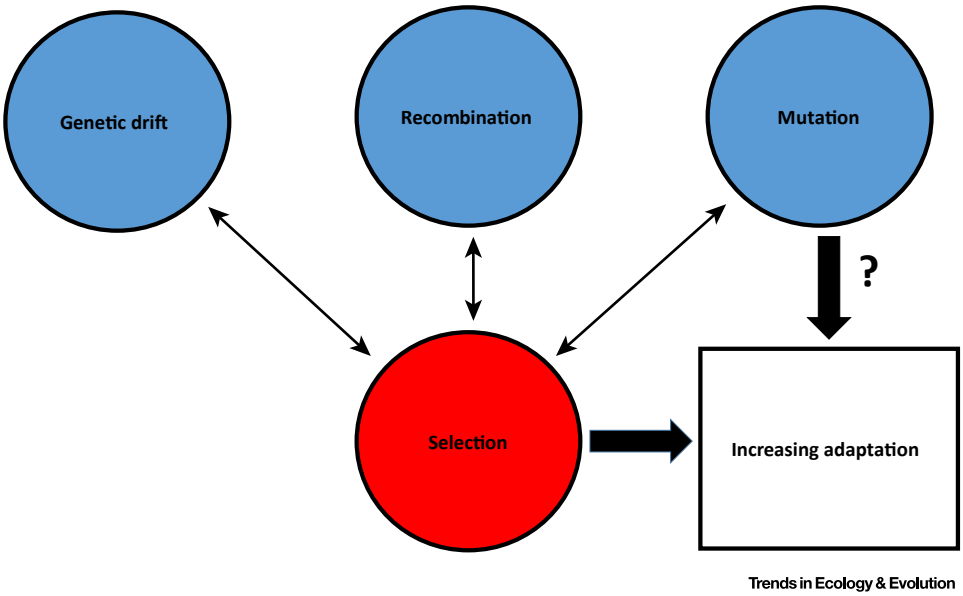

Figure 1. Standard Evolutionary Theory Recognizes Four Main Evolutionary Processes Based on Population Genetic Mechanisms. These are selection (a directional force, red), genetic drift, recombination, and mutation (all non-directional forces, blue). Standard evolutionary theory which grew out of the modern synthesis should thus not be labeled 'neo-Darwinism' because Darwin only discovered the first of these four mechanisms (selection), whereas the other three were discovered by population genetics in the 20th century [5]. Standard evolutionary theory is therefore not pan-selectionist and accepts both deterministic forces such as selection and non-adaptive forces (drift, mutation, and recombination) in adaptive evolution. However, standard evolutionary theory maintains that, in non-neutral evolution, only selection can increase organismal adaptation (unidirectional arrow). Proponents of mutation-driven evolution [1,73] question this view and argue that mutation can also be a directional force in evolution and promote adaptation (shown by a unidirectional arrow with a question mark). Finally, the four evolutionary processes can influence each other, as shown by double-headed arrows.

mutation rates, either due to structural constraints [15] or due to selection for different optimal mutation rates, for example for housekeeping genes versus immune system genes, or in coding versus non-coding regions [16]. Mutation rates can also evolve due to changes in the efficacy of natural selection with changes in effective population size [17], changes in the costs of DNA repair fidelity [18], or via second-order selection during adaptation to novel environments [12]. Likewise, mutation rates are also directly influenced by external environmental factors such as UV radiation and temperature [18,19]. Finally, the phenotypic effects of novel mutations are also shaped by an evolutionary history of past selection on the loci from where they originate as well as by epistatic interactions with other loci across the genome. Novel mutations are therefore not necessarily random in their expression, something which was already recognized by mathematical population geneticists such as Haldane, well before the modern synthesis [20].

All these aspects are recognized in standard evolutionary theory, and no serious evolutionary biologist claims that mutations are completely random with respect to internal or external environmental conditions. The only claim that standard evolutionary theory makes is that mutations are not systematically biased to increase organismal fitness as an immediate response to changed requirements [9], unless environmental changes have been so common and predictable in the evolutionary history of the organism that second-order selection has operated upon variation in mutation rates to increase evolvability [21]. All experimental attempts claiming to demonstrate directed or 'adaptive' mutations [22] have been decisively rejected by

later in-depth studies [9,10,20,23]. The vast majority of mutations within a given environment are neutral or slightly deleterious, as revealed by mutation accumulation (MA) experiments [24] and experimental studies on fitness effects of single mutations [25]. Moreover, species with large population sizes, where selection is more efficient, evolve lower mutation rates [17]. Alternatively, deleterious mutations can promote selection for increased mutational robustness [26]. That the majority of novel mutations are neutral or slightly deleterious is therefore uncontroversial and is supported by massive empirical evidence, and consequently has been incorporated into population genetic theory, studies of life-history trade-offs involving DNA repair [18,27], and sexual selection theory [28,29].

Theoretical Predictions for the Role of Mutation Bias in Neutral and Adaptive Evolution

Heterogeneous mutation rates can arise due to structural constraints across the genome [11] or because of selection [16]. This is recognized in the neutral theory of molecular evolution [11], and is thus part of standard evolutionary theory. Critics of standard evolutionary theory, however, question both the assumption that the origination rate of a beneficial allele does not influence its fixation probability [8,13] and that fixation probability is under exclusive control of selection [7]. Proponents of mutation-driven evolution instead emphasize that the rate by which novel mutations enter the population can affect their fixation rate on a 'first come, first served' basis [7,8,13]. For example, Yampolsky and Stoltzfus [7] showed that mutation bias can affect the fixation probability of two beneficial alleles when their phenotypic effects are functionally redundant, resulting in **reciprocal sign epistasis** for fitness. Similarly, Lynch [5] showed that mutation bias can affect the fixation probability of non-neutral mutations when selection is weak relative to genetic drift (Box 1).

Although these claims may seem distinct from standard evolutionary theory, the above scenario is easily accounted for by classical population genetics (Boxes 1 and 2). The role of mutations in driving adaptive substitutions has been exaggerated by proponents of mutation-driven evolution because they neglect a primary role of selection in limiting the fraction of viable adaptive trajectories. Population genetic theory also gives additional reasons for why mutation bias is unlikely to influence the fixation probability of beneficial alleles. First, pre-existing standing genetic variation [30] makes it unlikely that mutations would bias adaptive evolution. Second, a new beneficial mutation will need many generations to increase in frequency, leaving ample time for alternative alleles with more beneficial fitness effects to arise *de novo* and outcompete the first mutation, counteracting any effect of mutation bias in large populations even in the absence of standing genetic variation (Box 2).

Parallel Genetic Adaptations Is Insufficient Evidence for Mutation Bias

The study of parallel or convergent adaptations can reveal the power of natural selection leading to similar phenotypes and adaptations in similar environments [31]. Parallel phenotypic evolution may (or may not) be caused by parallel genetic changes at the same base positions (i.e., the same mutations), different mutations within the same loci, or mutations at entirely different sets of loci [32,33]. This has stimulated discussions about the relative role of determinism and historical contingency (e.g., stochasticity, mutational history) in evolution [31,34]. Standard evolutionary theory certainly allows for an interplay between the deterministic influence of selection and stochastic factors, including mutations [35–37] (Box 1). For example, speciation in similar environments can proceed by mutation-order divergence, as an alternative to the more deterministic ecological speciation mode where selection is predominant [38]. We stress that parallel genetic change underlying phenotypic convergence is not sufficient evidence for mutation bias being important in causing such convergence. Genetic parallelism in

Glossary

Correlational selection: a form of selection that operates on character combinations rather than on traits in isolation. Correlational selection is of interest because it can promote and maintain adaptive genetic covariance between traits.

Developmental bias: a summary term for the non-random production of phenotypes from underlying genotypes (e.g., novel mutations) and environmental conditions (i.e., phenotypic plasticity).

Directed mutation: a discredited idea that mutations are not random with respect to fitness but arise in relation to the current needs of an organism in a particular environment. Despite many attempts to demonstrate directed mutations, there is no empirical evidence that such directed mutations occur and have any role in adaptive evolution. Sometimes also called 'adaptive mutation'.

***G*-matrix:** a matrix describing genetic variances (along the diagonal) and covariances (the off-diagonal elements) between traits. The *G*-matrix can be used to predict evolutionary responses to selection and can also be shaped by correlational selection. *G* reflects standing genetic variation and covariation in a population.

***M*-matrix:** a matrix of mutational variances and covariances that describe the phenotypic effects of mutations. *M* reflects the input of novel mutations into a population, which is the ultimate source of new genetic variation.

Modern synthesis: the evolutionary synthesis between mathematical and empirical population genetics, systematics, paleontology, and natural history that took place in the 1930s and 1940s under the leadership and initiatives by Dobzhansky, Huxley, Mayr, Simpson, and Stebbins. The modern synthesis relied on the mathematical theories of Fisher, Haldane, and Wright, and recognizes four evolutionary processes based on population genetic mechanisms (Figure 1): selection, mutation, genetic drift, and recombination.

Mutation bias: ousually refers to the potential fixation bias that can arise from mutation pressure and the

Box 1. A Population Genetic Framework for Mutation Pressure

Under a strong selection–weak mutation (SSWM) regime, emphasized in classical population genetic models, adaptation may be mutation-limited due to rapid fixation and low origination rates of beneficial alleles. Under these conditions, Kimura [11] showed that, because the rate of origination of a neutral allele is equal to $N\mu$, where N is the number of replicating gene copies in the population and μ is the mutation rate, and because its probability of ultimately becoming fixed is $1/N$, the rate of fixation R of a neutral mutation arising *de novo* is directly proportional to its mutation rate:

$$R_n = N\mu \ \ 1/N = \mu \quad [I]$$

Thus, heterogeneous mutation rates will bias neutral evolution. However, for a strictly additive beneficial mutation with selection coefficient s, the probability of fixation $P(\text{fix}_b)$ (assuming $N_e \approx N$) is:

$$P(\text{fix}_b) = 2s/1 - e^{-2Ns} \quad [II]$$

For large population sizes and mild mutational effects ($Ns > 1$ but $s << 1$), the probability of fixation approaches $2s$, the classical result attained by Haldane [75] and Fisher [14], and the steady-state rate of fixation simplifies to:

$$R_b = 2N\mu s \quad [III]$$

The relative rate of fixation of two alternative beneficial mutations then becomes [7]:

$$R_{b2}/R_{b1} = 2N\mu_2 s_2/2N\mu_1 s_1 = \mu_2 s_2/\mu_1 s_1 \quad [IV]$$

The influence of heterogeneous mutation rate on substitution bias is illustrated in Figure I. However, this bias is unlikely to influence which of two beneficial allele that ultimately fixes unless these show reciprocal sign epistasis for fitness, and predictions apply only under conditions of SSWM, when mutation supply is limiting (i.e., $s >> \mu << 1/N$) such that the two alternative alleles never co-occur in the population (main text and Box 2). Note also that these results are attained using classic neutral theory and are thus encompassed in standard evolutionary theory.

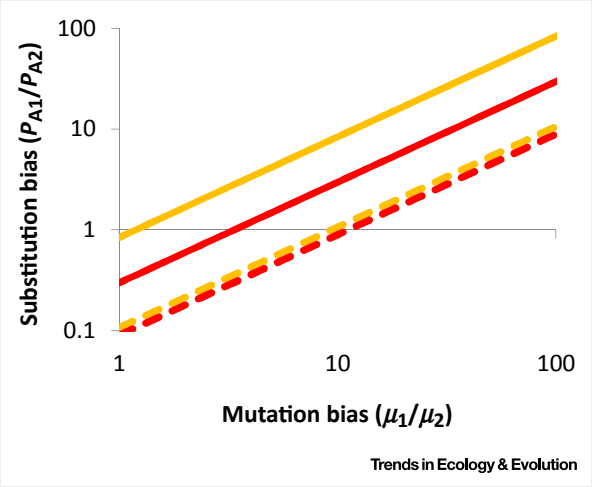

Figure I. The Role of Heterogeneous Mutation Rate in Creating Substitution Bias Between Two Competing Advantageous Alleles. This is shown for small ($N = 10^2$; full lines) and intermediate ($N = 10^4$; hatched lines) population sizes, and for strong ($s = 0.01$; red lines) and modest ($s = 0.001$; yellow lines) selection, with $\mu_1 = 10^{-9}$ and $s_2/s_1 = 10$.

heterogeneity in mutation rateat different genes or parts of the genome, or particular types of base substitutions relative to others, such as transitions versus transversions. Sometimes also refer to directional mutational effects on quantitative traits.

Mutation pressure: changes in the probability of allele-frequency change at a given locus as a result of mutation bias.

Reciprocal sign epistasis: occurs when the phenotypic effect of a mutation is beneficial on one genetic background but deleterious on another, as can happen when two potentially advantageous novel alleles have related phenotypic effects and may start to compete with each other for fixation (Box 2). Reciprocal sign epistasis can result in rugged fitness landscapes and make particular evolutionary trajectories inaccessible.

Standard evolutionary theory: the current general theoretical framework in evolutionary biology with its historical roots in the modern synthesis. Standard evolutionary theory recognizes four evolutionary processes based on population genetic mechanisms: selection, mutation, recombination, and genetic drift (Figure 1). Of these four processes, standard evolutionary theory only considers selection as a directional force that can increase organismal adaptation.

Transition–transversion bias: there are two possible classes of base substitutions. Transitions involve changes from one purine (A/G) or pyrimidine (C/T) to another, and therefore represent less of a structural change than transversions – that involve changes between a purine base and a pyrimidine base. Although transversions are expected to occur about twice as often, transitions are more common in the mutational spectrum.

adaptive radiations could be caused by sorting of, and selection on, standing genetic variation [30,39] with little or no role for mutation bias, in contrast to some claims [2]. In line with this, Conte *et al.* [40] reviewed 25 studies on parallel phenotypic evolution in natural populations and showed that gene reuse was common (underlying 32–55% of all observed adaptive changes). Gene reuse was greater between more recently diverged populations [40], consistent with selection on segregating ancestral polymorphisms [39]. Hence, genetic parallelism in itself and alone does not constitute strong evidence for mutation bias in driving adaptive evolution [37,41]. Even in the absence of standing genetic variation, we expect low convergence at the level of single nucleotides as a result of low mutation rates [21]. The probability of parallel

Box 2. Mutation Supply and Mutation Bias at the Level of Nucleotides, Genes, and Quantitative Traits

Box 1 shows that heterogeneous mutation rates can influence adaptive evolution under SSWM. This premise breaks down if selection is sufficiently weak that there is segregating variation at the loci under selection, or in large populations where there are many gene copies that can mutate into the best allele. This latter point is illustrated by considering the approximate mean number of generations to fixation ($\bar{t}_{fix1}$) for a beneficial mutation that ultimately fixes in the population:

$$\bar{t}_{fix1} = 2\ \log_e(N-1)/s_1 \qquad [I]$$

(assuming purely additive effects and neglecting drift; for a more thorough treatment refer to [92])

For the example in Box 1 where $s = 0.01$ and $N = 10^4$, a new beneficial mutation would take an average of ~1800 generations to reach fixation, but much longer for more weakly selected alleles. The fixation rate of the competing second, more beneficial, mutation during this time interval is dependent on the type of epistasis and the recombination rate between the two loci, once the first mutant (q_1) has increased in frequency. However, we can make a rough approximation of the expected number of times the second mutation will arrive while $q_1 \leq 0.1$, and subsequently fix, by setting $1-q_1 = p \approx 1$ (where q_1 and p is the frequency of the first mutant and the wildtype allele, respectively) such that the origination rate $\approx N\mu_2$ and $s_2(t) = \ln[\omega_2/(\omega_w p_t + \omega_1 q_{1,t})] \approx \ln(\omega_2/\omega_w)$, where ω_1, ω_2, and ω_w are the fitnesses of the first and second mutants and the wild type, respectively. Combining the approximate number of generations of logistic growth until $q_1 = 0.1$, with Equation [III] from Box 1, yields:

$$n\bar{r}_{fix2} \approx 2N\mu_2\ s_2/s_1\ \log_e(0.1N) \qquad [II]$$

The approximation depends on the mutation rate of the second locus only. Moreover, only the relative strength of selection on the two mutations is important because it affects the time to fixation of the first mutation and the probability of fixation of the second. The probability P_{fix2} that the second more beneficial mutation ultimately is fixed ($P_{fix2} = 1-\exp[-n\bar{r}_{fix2}]$, assuming that mutation follows a Poisson process) is illustrated in Figure I. Mutation bias is unlikely for mutation rates expected for quantitative traits ($\mu = 10^{-3}$ [72]), or per gene ($\mu = 10^{-5}$–10^{-6} [93]), provided that $N > 10^5$. For mutation rates expected per base ($\mu = 10^{-9}$ [93]) it is unlikely that a specific mutation will occur before the first mutant has fixed, unless the population size is very large.

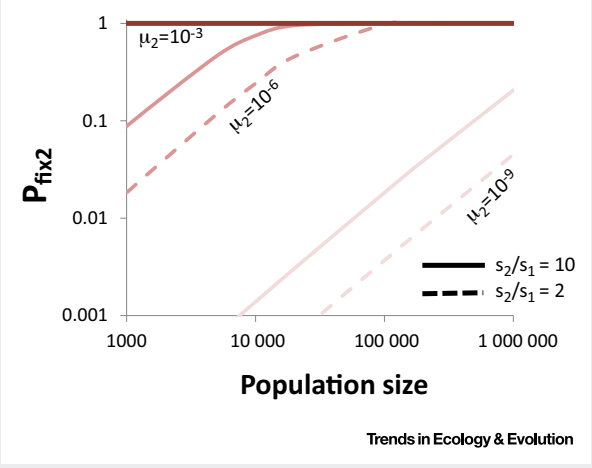

Figure I. Approximated Probability that the More Advantageous, Late-Arriving, Allele Outcompetes the First-Arriving Allele for Different Mutation Rates, Population Sizes, and Ratios of Positive Selection Coefficients.

evolution at the level of single nucleotides as a result of natural selection is actually predicted to be nearly twice as large compared to the neutral expectation [41].

Replicated experimental evolution studies on microbes adapting from *de novo* mutation have revealed some convergence at the level of phenotypes and genes, but with less convergence at the level of individual nucleotides [42,43], consistent with expectations based on standard

evolutionary theory (Box 2). Interestingly, such studies have also revealed frequent reciprocal sign epistasis for fitness among beneficial mutations [44], fulfilling the basic criteria needed for mutation bias to create substitution bias. Some authors [45–47] have suggested that one could evaluate the importance of mutation bias by comparing per-gene mutation rates based on polymorphism in populations kept under relaxed selection with the observed number of parallel genetic substitutions in the same genes across replicate evolving lineages. The few studies available [45–47], all on microorganisms, collectively suggest only a minor role for mutation bias relative to selection at the gene level.

Transition–Transversion Bias and Other Forms of Mutation Bias in Adaptation

A common argument for mutation bias as a directional evolutionary force is the **transition–transversion bias** [48]. Stoltzfus and McCandlish [6] compiled a large dataset from several taxa to quantify the role of transition–transversion bias among adaptive substitutions. Because the rate of transition mutations is higher than the rate of transversions, they argued that the former should be over-represented among adaptive substitutions. They found some evidence for such a pattern, and therefore concluded that these putatively adaptive amino acid replacements were partly influenced by mutation bias [6]. However, that transitions are over-represented among adaptive substitutions is not in itself strong evidence for mutation bias because transitions could be favored over transversions owing to selection on genomic base composition [49]. Selectively beneficial transitions and selectively beneficial transversions could also have different distributions of fitness effects, in which case there would be no reason to expect that they will contribute to adaptive evolution at equal rates, even if there were no mutation bias, and regardless of whether or not purifying selection is stronger against transversions [48].

Two recent studies, one on altitudinal adaptation in the hemoglobin gene in Andean house wrens [50], and the other on the *pel* regulatory gene encoding pelvic fins in stickleback [21] highlight the putative role of mutational hotspots underlying genetic parallelism. Specifically, the stickleback study highlights how mutation rates several orders of magnitude higher than typical can greatly increase the chance of parallel adaptation at specific genomic sites, leading to recurrent loss of pelvic fins upon independent freshwater colonization events and suggesting a role for both 'arrival of the fittest' via mutation and 'survival of the fittest' via selection [21]. Because loss-of-function mutations are expected to be relatively common, and can sometimes be conditionally beneficial in novel environments with relaxed selection [51], such mutations are particularly likely to contribute to parallel adaptation. Despite the importance of mutations in these two studies, we emphasize that selection ultimately drove these adaptive allele frequency changes, rather than evolution being 'mutation-driven' as some might claim [1,7,8,13]. These studies therefore only exemplify how historical contingency and mutational history interact with selection during adaptation to novel environments [31,38,52], entirely in line with standard evolutionary theory and the uncontroversial insight that different genomic regions contribute differentially to adaptation driven by selection, with mutations merely providing the genetic input [53].

Alignment between Mutation and Adaptation as a Result of Past Selection

We have argued that mutation bias is unlikely to drive adaptive evolution alone, and particularly not when standing genetic variation is abundant (Box 2). We have also emphasized that genetic parallelism is unlikely to result from mutation bias alone in the absence of any major role for selection in narrowing down the available evolutionary trajectories. A further finding suggestive of prominent roles for mutation bias or developmental bias in adaptive evolution is the observed

alignment between phenotypic divergence and mutational effects [54]. However, we argue that such adaptive alignment instead reflects past **correlational selection** on standing genetic variation or selection for mutational robustness (Box 3). Thus, we suggest that the causality is reversed and that alignment is more likely to reflect past and current selection on quantitative genetic architecture, rather than mutation bias being the cause of the alignment [55–60] (Box 3).

Selection for robustness in the face of frequent environmental and genetic perturbation can bias the phenotypic effects of novel mutations [61]. The key point here is that developmental systems may become robust to novel mutations [62] as a result of selection for genetic or

Box 3. Alignment between *M* and *G* by Correlational Selection and Selection for Mutational Robustness

Consider a 2D adaptive landscape in the form of a fitness surface between two traits with a ridge of high fitness (Figure IA). In such a scenario, correlational selection [56,58,71] favors the buildup and maintenance of a genetic correlation that becomes aligned with the ridge in the fitness surface (Figure IB). Such a genetic correlation can come about, for instance, through the spread of novel pleiotropic mutations with favorable influences on both traits or through the formation of physical linkage [58,60]. *G* can thus evolve [81], and correlational selection can shape the variance–covariance matrix of standing genetic variation. Such correlational selection can lead to the formation of a more ellipse-shaped structure (Figure IB) from an ancestral condition where there is no genetic correlation between the two traits (Figure IA). Arrows in Figure IA indicate maladaptive genetic combinations which are opposed by correlational selection. In this scenario, the mutational variance–covariance matrix (*M*) can evolve to become aligned with the fitness ridge, both as an indirect effect of correlational selection on *G* and as a result of direct selection for mutational robustness [15,26,63].

Note also that the assumption of isotropic phenotypic effects of novel mutations in Figure IA does not necessarily logically follow from the fact that mutations are random with respect to organismal fitness, in contrast to what was argued in a recent review on developmental bias [62]. These authors argued that the default assumption in standard evolutionary theory is that phenotypic variation from novel mutations is unbiased (isotropic) and they cited Lande [66] as an example of how quantitative genetic theory assumes such uniform mutational effects on the phenotype [62]. However, closer reading of both Lande's early quantitative genetic model [66] and more recent models [64,65] gives little support for this interpretation. On the contrary, Lande [66] explicitly considered mutational pleiotropy, and thus he did not assume isotropic phenotypic effects of novel mutations. Hence, developmental bias does not in any way contradict the assumption in standard evolutionary theory that novel mutations are neutral or slightly deleterious, and are not systematically biased towards high fitness [9]. Quantitative genetics therefore readily accommodates developmental bias and such non-random mutational effects [57,64,65,78].

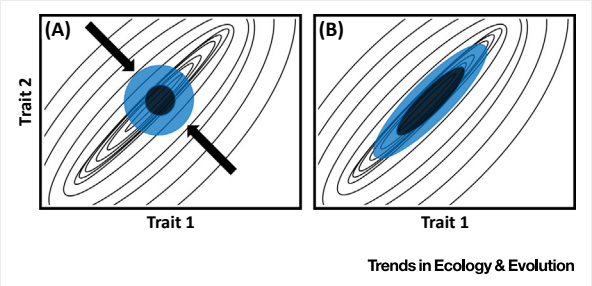

Figure I. How Correlational Selection and Selection for Mutational Robustness Can Shape both the Genetic Variance–Covariance Matrix (*G*, Blue) and the Mutational Variance–Covariance Matrix (*M*, Black). For simplicity, only two traits are shown (trait 1 and trait 2), and they experience correlational selection in the form of a fitness ridge that favors the buildup of a positive genetic correlation between these two traits. (A) At outset there is no genetic correlation between trait 1 and trait 2, as indicated by cylindrical spheres of both *G* and *M*, but correlational selection shapes *G* and may also results in mutational robustness (*M*) by selecting against particular trait combinations (arrows). (B) As a result, both *G* and *M* evolve to become aligned with the fitness surface, resulting in an ellipsoid structure and positive genetic and mutational covariance between the traits.

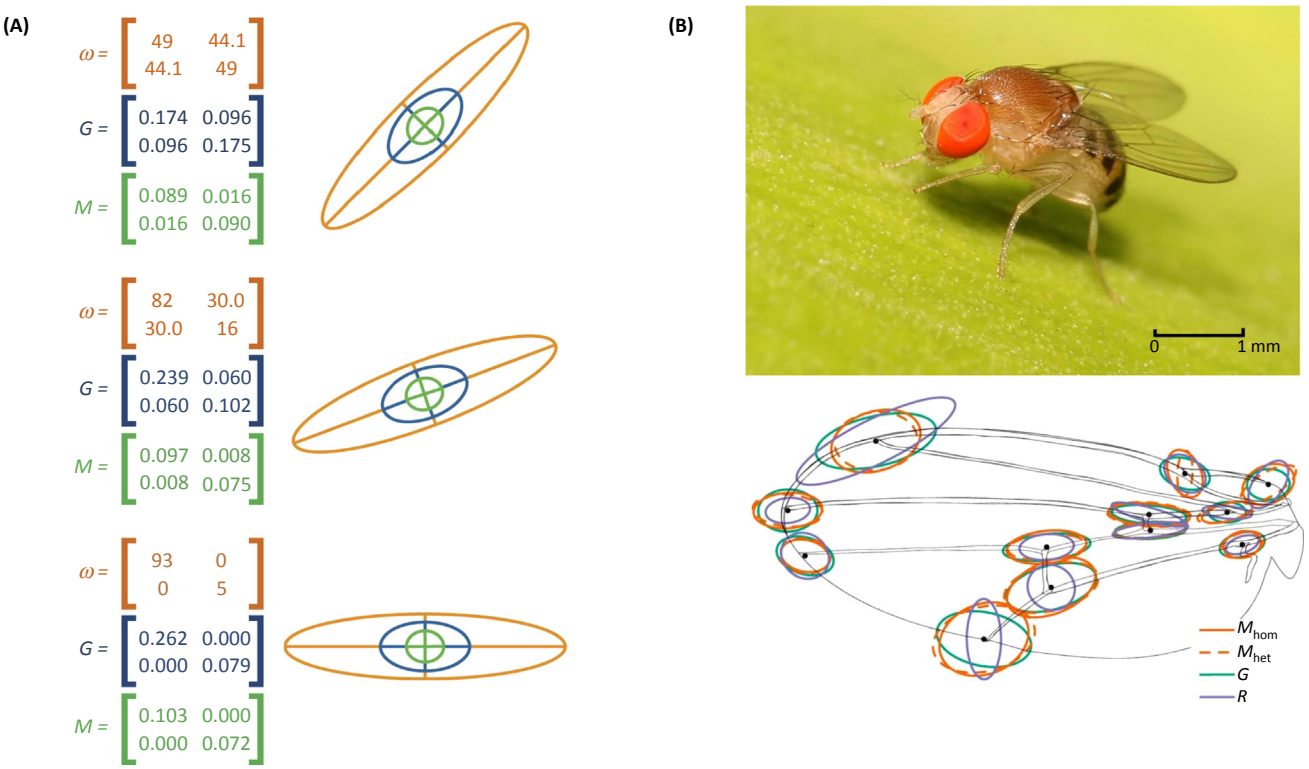

Figure 2. Novel Mutations Can Evolve Adaptive Pleiotropy as a Result of Correlational Selection [66]. (A) A two-locus simulation model, where the strength of correlational selection [56,58] and the shape of the multivariate fitness surface (ω) predicts alignment between the mutational variance–covariance matrix (*M*) and the matrix of standing genetic variation (*G*) [65]. Shown are the evolved matrices of G and M, following simulation of ω (left column), with the off-diagonal elements representing allelic variances for *G* and *M*. Shown also is a visualization of the evolved ellipses of *M* and *G* (right column). (B) Empirical results from a quantitative-genetic study of the genetic variance–covariance structure of *Drosophila* wing venation patterns [54] that is consistent with the model predictions by Jones *et al.* [65]. The authors found that *M*, *G*, and the divergence matrix between species (*R*) showed remarkable similarity and strong alignment, which is consistent with correlational selection that is operating on both short and long timescales. Shown are two estimates of *M*: the homozygous (M_{hom} total) and heterozygous (M_{het} non-segregational) mutational variance matrices. Images reproduced, with permission, from [65] and [54] and Wikimedia Commons (*Drosophila melanogaster*; photograph by Muhammad Mahdi Karim).

environmental canalization [61,63]. Indeed, recent quantitative genetic models predict alignment between the phenotypic effects of novel mutations, as captured by the mutational variance–covariance matrix (***M*-matrix**), standing genetic variation, as captured by the genetic variance–covariance matrix (***G*-matrix**), and multivariate selection [64,65] (Figure 2A). Thus, epistasis and correlational selection [58] can shape standing genetic variation [60] and thereby also the phenotypic effects of novel mutations, even though these have not yet been exposed to selection [65] (Box 3).

These models have recently obtained partial empirical support in a large-scale study on the quantitative genetic architecture of wing morphologies in fruit flies (*Drosophila*) by Houle *et al.* [54]. The authors demonstrated strong alignment between the direction and orientation of *M*, *G*, and between-species divergence (Figure 2B). This suggests that long-term macroevolutionary change over 40 million years can at least partly be predicted from *M* and *G* obtained from one species in this clade [54]. Importantly, however, the mutational variance was

much greater than would be expected if mutation supply had limited phenotypic evolution [46], but was consistent with long-term stabilizing selection around a shared and stable fitness peak. Therefore, this is relatively weak support for mutation bias having influenced adaptive evolution in this clade, despite *M* being aligned with between-species divergence (Figure 2B).

Mechanistically, alignment between *M*, *G*, and the fitness surface can arise because novel mutations at loci that have been subject to past correlational selection will also inherit the pleiotropic properties of the ancestral alleles from which these novel mutations originated [66] (Box 3). Hence, the phenotypic expression of such novel mutations is biased in the direction away from fitness valleys as a result of their inherited adaptive pleiotropy, even though these novel mutations themselves have never been subject to selection [51]. Superficially, the inherited pleiotropic effects of the novel mutations at such loci might then create a false impression that mutation bias drove adaptive evolution. However, the bias of the phenotypic expression of novel mutations in this scenario does not result from non-random or directed ('adaptive') mutations, but instead results from downstream phenotypic effects on their expression, often alluded to as developmental bias [62]. Therefore, neither developmental nor mutation bias disproves the random nature of mutations, as emphasized by standard evolutionary theory, although there is some disagreement on this issue [2,4,9,20]. We re-emphasize that there is no strong empirical evidence that directed mutations play any role in the evolutionary process [9,10]. Moreover, we question a recent interpretation of standard evolutionary theory that random mutation implies isotropic phenotypic effects of novel mutations [62]. We emphasize that quantitative genetic theory incorporated mutational pleiotropy a long time ago [66], and therefore does not necessarily assume isotropic mutational effects [64,65] (Box 3). Indeed, in a quantitative genetic framework, developmental bias is not a very useful concept, and it is often not meaningful to distinguish between the initial random mutation and its non-random downstream effects because both are captured by the mutational variance–covariance matrix (*M*).

Adaptive Directionality and Similarities between Evolution and Learning

Based on the many past unsuccessful attempts to demonstrate any positive directionality of novel mutations on organismal fitness, one would perhaps expect the issue to now have become finally settled. However, recently there has been a new set of theoretical studies based on neural networks and machine learning [67] that claim to demonstrate that evolution might 'learn' from past environments and thereby generalize to novel environments [68]. According to these models, novel mutations could under some circumstances be beneficial in novel environments if there is some structural similarity between these novel and past environments [67]. Apart from the criticism that novel environments are then not entirely novel, we have several reservations against the interpretations of these models by the authors [67,68]. First, we question the analogy between evolution and learning because analogy does not necessarily reflect the same underlying process. Second, introducing the learning analogy into evolutionary theory opens the way for teleological arguments and gives the erroneous impression that evolution has foresight [69]. It has been a long uphill battle in evolutionary biology to eliminate teleological arguments – and develop a mechanistic theory that is not based on purpose [70] but instead is based on selection and other well-characterized evolutionary processes (Figure 1) [5]. The machine-learning approach by Watson [67], although novel and interesting, would benefit from closer integration with theoretical and empirical methods in evolutionary quantitative and population genetics [58–60,71] which are based on a well-understood mathematical framework and well-characterized processes of genetic drift, mutation, and correlational selection [5,17,55,72] (Figure 1 and Box 3).

Challenging Historical Narratives about Mutation Bias in the Modern Synthesis and Standard Evolutionary Theory

We question here some historical narratives around the modern synthesis that are sometimes voiced by critics of standard evolutionary theory [2,3], including those arguing for mutation bias as a novel mechanism that can direct adaptive evolution [8,13,73]. According to some of these narratives, the modern synthesis exclusively focused on selection as an evolutionary process [2,3] and only considered standing genetic variation ('shifting gene frequencies') [8], while ignoring the role of novel mutations [13]. We take issue with these narratives, which we consider as caricatures of both the modern synthesis and standard evolutionary theory. It is simply not true that the architects of the modern synthesis were unaware of the potential role of novel mutations in the evolutionary process because mutational bias was already discussed in Haldane's and Dobzhansky's early papers [19,74,75]. For instance, Dobzhansky discussed the possible role of mutation bias (expressed as 'similarity in germ plasms') as a potential explanation for parallel geographic variation in various species of lady-bird beetles (Col.: Coccinellidae) [74]. Moreover, theoretical models of the genetics of adaptation have traditionally focused on novel mutations rather than on standing genetic variation [35,76,77], building on the famous 'microscopy analogy' of Fisher [14]. It is also only relatively recently that evolutionary biologists have turned their attention more towards adaptation from standing genetic variation [30], again in contrast to claims that the modern synthesis only focused on shifting allele frequencies in a closed gene pool while ignoring mutations [8,13]. Today, standard evolutionary theory incorporates both adaptive and non-adaptive processes [5,78]. Associating standard evolutionary theory with an assumption of isotropic effects of random mutations [62] goes back to Gould [79], but, as pointed out by Charlesworth *et al.* [20] and Futuyma [80], this is a false claim. On the contrary, evolutionary quantitative genetics theory has long since recognized non-isotropic effects of novel mutations [66] and that genetic correlations can evolve through correlational selection [55–58,60,81] (Box 3) or through genetic drift, which in turn can bias subsequent evolutionary trajectories [57].

Environment → Selection ↔ Mutation

Although we argue that mutation bias is unlikely to be a major and independent force in driving convergent evolutionary responses, we suggest that environmental influences on population genetic parameters have been somewhat neglected. Specifically, the interplay between mutation and selection in driving evolutionary divergence should be investigated in more depth. For example, the role of mutation bias versus fixation bias is somewhat paradoxically turned in favor of selection as baseline mutation rates increase due to unlimited mutation supply (Box 2). Moreover, because fixation bias is crucially dependent on the strength of selection versus that of chance and genetic drift (Box 1), environments that increase the mean strength of selection should exhibit more adaptive convergence. For instance, organisms living in hotter climates could show stronger convergent evolution if higher temperatures increase the strength of genome-wide selection [82]. These ideas can partly be traced to as far back as Haldane [19]. Haldane speculated that elevated mutation rates at high temperatures could explain differences between the tropics and the temperate regions in rates of molecular divergence [19]. Such consequences of temperature on mutation rates and molecular evolution for biogeography, macroecology, and macroevolution are still subject to much interest [83–85]. Finally, recent studies have demonstrated that mutation rates may be higher in individuals of low fitness that cannot afford costly DNA repair [18,27], suggesting that harsh conditions may simultaneously increase mutation supply. As a result, the deterministic force of selection may not decrease relative to genetic drift during adaptation to novel environments, despite presumably lower population sizes.

Concluding Remarks

We do not deny a role for mutation bias in adaptation in mutation-limited populations [5,6], but this role is not independent of selection and such evolution is certainly not mutation-driven. We therefore question if mutation bias should be elevated to become a novel cause or evolutionary process, as argued by some authors [7,73] (see Outstanding Questions). Stochasticity is already well incorporated into standard evolutionary theory, which has moved well beyond panselectionism [5], as exemplified by exciting research on historical contingencies, mutational history, and mutation-order speciation [31,38,50,52,53]. In the few cases where mutational bias plays a role independent of selection and genetic drift, it would be more natural to view it as a lower-level internal drive mechanism [5] by which some alleles increase in frequency against the force of organismal (higher-level) selection, similar to segregation distorters and meiotic drive [5,86]. Obviously, with respect to fitness, mutations will be negatively biased given that organisms show some level of adaptation to their environments. Such negative mutation bias is an expected outcome of random mutations (with respect to their phenotypic effects) in standard evolutionary theory [14,41], although the effects can differ between the sexes, being more negative in males than in females [87,88]. Indeed, negative mutation bias for fitness is so widely observed and acknowledged [24] that it serves as a basis for theories about sexual selection for good genes [29], the maintenance of sex [89,90] and recombination [91], and extinction via mutational meltdown [12]. In standard evolutionary theory, selection is therefore the only force that can increase organismal fitness, with mutations generally opposing selection rather than promoting adaptation.

Outstanding Questions

How common and pronounced is mutation bias? While some figures are available, such as for transitions versus transversions, it remains unclear how often mutation bias is of the magnitude necessary to generate visible genomic signatures during adaptive evolution.

How common is reciprocal sign epistasis among the beneficial mutations available for evolution? Mutation bias may affect the rate of evolution at different loci, but is unlikely to affect the ultimate fixation of alternative alleles unless there is reciprocal sign epistasis.

Despite evidence for an environmental influence on population genetic parameters such as population size, mutation rate, recombination rate, and the mean strength of selection, the role of the environment in driving parallelism through such changes is largely unexplored.

Does selection affect mutation rate across the genome, and to what extent? Population genetic theory would suggest that mutation rates will generally be too low at individual sites for selection to act, even in organisms with large population sizes such as bacteria. However, if mechanisms exist that allow selection to act on groups of mutations, a role for selection in shaping heterogeneous mutation rates becomes more likely.

Are there general differences in how important mutation bias is between unicellular haploid organisms such as bacteria compared to multicellular diploids? Theory would suggest this because these two groups are likely to differ in many important aspects such as population size, recombination rate, mutation rate, and standing genetic variation. However, no study that we know of has systematically compared mutation bias across the two groups.

How common is alignment between standing genetic variation (G) and novel mutations (M)? What are the mechanisms behind such alignment, and when is it found? What is the relative importance of correlational

Acknowledgments

The ideas put forward in this paper and the topics discussed emerged from discussions with Troy Day, Magne Friberg, Thomas F. Hansen, Stephen De Lisle, Tobias Uller, and many others who have directly or indirectly influenced our thinking about mutation bias. We thank Martin Lascoux and Claus Rüffler for input to Boxes 1 and 2. Funding for our research has been provided by the Swedish Research Council (VR) to E.I.S. (grant 2016-03356) and D.B. (grant 2015-05223).

References

1. Nei, M. (2013) *Mutation-Driven Evolution,* Oxford University Press
2. Laland, K. *et al.* (2014) Does evolutionary theory need a rethink? *Nature* 514, 161–164
3. Laland, K.N. *et al.* (2015) The extended evolutionary synthesis: its structure, assumptions and predictions. *Proc. Biol. Sci.* 282, 20151019
4. Noble, D. (2017) Evolution viewed from physics, physiology and medicine. *Interface Focus* 7, 20160159
5. Lynch, M. (2007) The frailty of adaptive hypotheses for the origins of organismal complexity. *Proc. Natl. Acad. Sci. U.S.A.* 104, 8597–8604
6. Stoltzfus, A. and McCandlish, D.M. (2017) Mutational biases influence parallel adaptation. *Mol. Biol. Evol.* 34, 2163–2172
7. Yampolsky, L.Y. and Stoltzfus, A. (2001) Bias in the introduction of variation as an orienting factor in evolution. *Evol. Dev.* 3, 73–83
8. Stoltzfus, A. (2006) Mutationism and the dual causation of evolutionary change. *Evol. Dev.* 8, 304–317
9. Futuyma, D.J. (2017) Evolutionary biology today and the call for an extended synthesis. *Interface Focus* 7, 20160145
10. Sniegowski, P.D. and Lenski, R.E. (1995) Mutation and adaptation: the directed mutation controversy in evolutionary perspective. *Annu. Rev. Ecol. Syst.* 26, 553–578
11. Kimura, M. (1983) *The Neutral Theory of Molecular Evolution,* Cambridge University Press
12. Lynch, M. *et al.* (1995) Mutation accumulation and the extinction of small populations. *Am. Nat.* 146, 489–518
13. Stoltzfus, A. and Cable, K. (2014) Mendelian-mutationism: the forgotten evolutionary synthesis. *J. Hist. Biol.* 47, 501–546
14. Fisher, R.A. (1930) *The Genetical Theory of Natural Selection,* Clarendon Press
15. Wagner, A. (2005) Distributed robustness versus redundancy as causes of mutational robustness. *BioEssays* 27, 176–188
16. Martincorena, I. *et al.* (2012) Evidence of non-random mutation rates suggests an evolutionary risk management strategy. *Nature* 485, 95–98
17. Lynch, M. (2010) Evolution of the mutation rate. *Trends Genet.* 26, 345–352
18. Berger, D. *et al.* (2017) Temperature effects on life-history trade-offs, germline maintenance and mutation rate under simulated climate warming. *Proc. R. Soc. B.* 284, 20171721
19. Haldane, J.B.S. (1933) The part played by recurrent mutation in evolution. *Am. Nat.* 67, 5–19
20. Charlesworth, D. *et al.* (2017) The sources of adaptive variation. *Proc. Biol. Sci.* 284
21. Metzgar, D. and Wills, C. (2000) Evidence for the adaptive evolution of mutation rates. *Cell* 101, 581–584
22. Cairns, J. *et al.* (1988) The origin of mutants. *Nature* 335, 142–145
23. Maisnier-Patin, S. and Roth, J.R. (2015) The origin of mutants under selection: how natural selection mimics mutagenesis (adaptive mutation). *Cold Spring Harb. Perspect. Biol.* 7, a018176
24. Halligan, D.L. and Keightley, P.D. (2008) Spontaneous mutation accumulation studies in evolutionary genetics. *Annu. Rev. Ecol. Evol. Syst.* 40, 151–172
25. Bataillon, T. and Bailey, S.F. (2014) Effects of new mutations on fitness: insights from models and data. *Ann. N.Y. Acad. Sci.* 1320, 76–92

26. Rajon, E. and Masel, J. (2011) Evolution of molecular error rates and the consequences for evolvability. *Proc. Natl. Acad. Sci.* 108, 1082–1087
27. Sharp, N.P. and Agrawal, A.F. (2012) Evidence for elevated mutation rates in low-quality genotypes. *Proc. Natl. Acad. Sci.* 109, 6142–6146
28. Pomiankowski, A. *et al.* (1991) The evolution of costly mate preferences: I. Fisher and biased mutation. *Evolution* 45, 1422–1430
29. Rowe, L. and Houle, D. (1996) The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 263, 1415–1421
30. Barrett, R.D. and Schluter, D. (2008) Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23, 38–44
31. Huey, R.B. *et al.* (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287, 308–309
32. Hoekstra, H.E. and Nachman, M.W. (2003) Different genes underlie adaptive melanism in different populations of rock pocket mice. *Mol. Ecol.* 12, 1185–1194
33. Mundy, N.I. (2005) A window on the genetics of evolution: MC1R and plumage colouration in birds. *Proc. Biol. Sci.* 272, 1633–1640
34. Rosenblum, E.B. *et al.* (2014) The molecular basis of phenotypic convergence. *Annu. Rev. Ecol. Evol. Syst.* 45, 203–226
35. Chevin, L.-M. *et al.* (2010) Fisher's model and the genomics of adaptation: restricted pleiotropy, heterogenous mutation, and parallel evolution. *Evolution* 64, 3213–3231
36. Storz, J.F. (2016) Causes of molecular convergence and parallelism in protein evolution. *Nat. Rev. Genet.* 17, 239–250
37. Lässig, M. *et al.* (2017) Predicting evolution. *Nat. Ecol. Evol.* 1, 0077
38. Schluter, D. (2009) Evidence for ecological speciation and its alternative. *Science* 323, 737–741
39. Meier, J.I. *et al.* (2017) Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nat. Commun.* 8, 14363
40. Conte, G.L. *et al.* (2012) The probability of genetic parallelism and convergence in natural populations. *Proc. R. Soc. B* 279, 5039–5047
41. Orr, H.A. (2005) The probability of parallel evolution. *Evolution* 59, 216–220
42. Tenaillon, O. *et al.* (2016) Tempo and mode of genome evolution in a 50,000-generation experiment. *Nature* 536, 165–170
43. Weinreich, D.M. *et al.* (2006) Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* 312, 111–114
44. Berger, D. and Postma, E. (2014) Biased estimates of diminishing-returns epistasis? Empirical evidence revisited. *Genetics* 198, 1417–1420
45. Streisfeld, M.A. and Rausher, M.D. (2011) Population genetics, pleiotropy, and the preferential fixation of mutations during adaptive evolution. *Evolution* 65, 629–642
46. Bailey, S.F. *et al.* (2017) What drives parallel evolution? *BioEssays* 39, e201600176
47. Marek, A. and Tomala, K. (2018) The contribution of purifying selection, linkage, and mutation bias to the negative correlation between gene expression and polymorphism density in yeast populations. *Genome Biol. Evol.* 10, 2986–2996
48. Stoltzfus, A. and Norris, R.W. (2016) On the causes of evolutionary transition:transversion bias. *Mol. Biol. Evol.* 33, 595–602
49. Hildebrand, F. *et al.* (2010) Evidence of selection upon genomic GC-content in bacteria. *PLOS Genet.* 6, e1001107
50. Galen, S.C. *et al.* (2015) Contribution of a mutational hot spot to hemoglobin adaptation in high-altitude Andean house wrens. *Proc. Natl. Acad. Sci. U. S. A.* 112, 13958–13963
51. Lahti, D.C. *et al.* (2009) Relaxed selection in the wild. *Trends Ecol. Evol.* 24, 487–496
52. Good, B.H. *et al.* (2017) The dynamics of molecular evolution over 60,000 generations. *Nature* 551, 45–50
53. Xie, K.T. *et al.* (2019) DNA fragility in the parallel evolution of pelvic reduction in stickleback fish. *Science* 363, 81–84
54. Houle, D. *et al.* (2017) Mutation predicts 40 million years of fly wing evolution. *Nature* 548, 447–450
55. Cheverud, J.M. (1984) Quantitative genetics and developmental constraints on evolution by selection. *J. Theor. Biol.* 110, 155–171
56. Phillips, P.C. and Arnold, S.J. (1989) Visualizing multivariate selection. *Evolution* 43, 1209–1266
57. Schluter, D. (1996) Adaptive radiation along genetic lines of least resistance. *Evolution* 50, 1766–1774
58. Sinervo, B. and Svensson, E. (2002) Correlational selection and the evolution of genomic architecture. *Heredity* 16, 948–955
59. Blows, M.W. *et al.* (2004) Orientation of the genetic variance–covariance matrix and the fitness surface for multiple male sexually selected traits. *Am. Nat.* 163, E329–E340
60. Roff, D.A. and Fairbairn, D.J. (2012) A test of the hypothesis that correlational selection generates genetic correlations. *Evolution* 66, 2953–2960
61. Siegal, M.L. and Leu, J.-Y. (2014) On the nature and evolutionary impact of phenotypic robustness mechanisms. *Annu. Rev. Ecol. Evol. Syst.* 45, 495–517
62. Uller, T. *et al.* (2018) Developmental bias and evolution: a regulatory network perspective. *Genetics* 209, 949–966
63. de Visser, J.A.G.M. *et al.* (2003) Perspective: evolution and detection of genetic robustness. *Evolution* 57, 1959–1972
64. Jones, A.G. *et al.* (2007) The mutation matrix and the evolution of evolvability. *Evolution* 61, 727–745
65. Jones, A.G. *et al.* (2014) Epistasis and natural selection shape the mutational architecture of complex traits. *Nat. Commun.* 5, 3709
66. Lande, R. (1980) The genetic covariance between characters maintained by pleiotropic mutations. *Genetics* 94, 203–215
67. Watson, R.A. *et al.* (2014) The evolution of phenotypic correlations and 'developmental memory'. *Evolution* 68, 1124–1138
68. Watson, R.A. and Szathmary, E. (2016) How can evolution learn? *Trends Ecol. Evol.* 31, 147–157
69. Žliobaitė, I. and Stenseth, N.C. (2016) Improving adaptation through evolution and learning: a response to Watson and Szathmáry. *Trends Ecol. Evol.* 31, 892–893
70. Gardner, A. (2017) The purpose of adaptation. *Interface Focus* 7, 20170005
71. Svensson, E.I. *et al.* (2009) Intralocus sexual conflict over immune defense, gender load, and sex-specific signaling in a natural lizard population. *Evolution* 63, 3124–3135
72. Lynch, M. and Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits,* Sinauer Associates
73. Stoltzfus, A. and Yampolsky, L.Y. (2009) Climbing mount probable: mutation as a cause of nonrandomness in evolution. *J. Hered.* 100, 637–647
74. Dobzhansky, T. (1933) Geographical variation in lady-beetles. *Am. Nat.* 67, 97–126
75. Haldane, J.B.S. (1927) A mathematical theory of natural and artificial selection: Part V: selection and mutation. *Math. Proc. Camb. Philos. Soc.* 23, 838–844
76. Orr, H.A. and Coyne, J.A. (1992) The genetics of adaptation: a reassessment. *Am. Nat.* 140, 725–742
77. Orr, H.A. (1998) The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution* 52, 935–948
78. Svensson, E.I. (2018) On reciprocal causation in the evolutionary process. *Evol. Biol.* 45, 1–14
79. Gould, S.J. (2002) *The Structure of Evolutionary Theory,* Harvard University Press
80. Futuyma, D.J. (2011) Expand or revise? The evolutionary synthesis today. *Q. Rev. Biol.* 86, 203–208
81. Eroukhmanoff, F. and Svensson, E.I. (2011) Evolution and stability of the G-matrix during colonization of a novel environment. *J. Evol. Biol.* 24, 1363–1373

selection on standing genetic variation and possible 'carry-over effects' on mutations versus selection for mutational robustness?

What is the role of mutation bias in adaptation via compensatory mutations arising as a result of deleterious pleiotropy of the first adaptive changes?

Mutations are generally slightly deleterious rather than beneficial. Mutation bias should thus result in differences in the contribution to mutation load from different genomic regions or types of base substitutions. To what extent does such mutation bias generate systematic fitness costs across the genomes of different organisms? It follows that any gene more likely to contribute to adaptive evolution owing to a higher mutation rate should also contribute more to the mutation load, but we know of no study testing this hypothesis.

82. Berger, D. *et al.* (2018) A universal temperature-dependence of mutational fitness effects. *bioRxiv*
83. Brown, J.H. (2014) Why are there so many species in the tropics? *J. Biogeogr.* 41, 8–22
84. Rohde, K. (1992) Latitudinal gradients in species diversity – the search for a primary cause. *Oikos* 65, 514–527
85. Allen, A.P. *et al.* (2006) Kinetic effects of temperature on rates of genetic divergence and speciation. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9130–9135
86. Okasha, S. (2006) *Evolution and the Levels of Selection,* Oxford University Press
87. Sharp, N.P. and Agrawal, A.F. (2013) Male-biased fitness effects of spontaneous mutations in *Drosophila melanogaster*. *Evolution* 67, 1189–1195
88. Grieshop, K. *et al.* (2016) Strong sexual selection in males against a mutation load that reduces offspring production in seed beetles. *J. Evol. Biol.* 29, 1201–1210
89. Agrawal, A.F. (2001) Sexual selection and the maintenance of sexual reproduction. *Nature* 411, 692–695
90. Siller, S. (2001) Sexual selection and the maintenance of sex. *Nature* 411, 689–692
91. Kondrashov, A.L. (1988) Deleterious mutations and the evolution of sexual reproduction. *Nature* 336, 435–440
92. Crow, J.F. and Kimura, M. (1970) *An Introduction to Population Genetic,* Theory Harper & Row
93. Drake, J.W. *et al.* (1998) Rates of spontaneous mutation. *Genetics* 148, 1667–1686