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REVIEW

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Adaptive evolvability through direct selection instead of indirect, second-order selection

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Abstract

Can evolvability itself be the product of adaptive evolution? To answer this question is challenging, because any DNA mutation that alters only evolvability is subject to indirect, "second order" selection on the future effects of this mutation. Such indirect selection is weaker than "first-order" selection on mutations that alter fitness, in the sense that it can operate only under restrictive conditions. Here I discuss a route to adaptive evolvability that overcomes this challenge. Specifically, a recent evolution experiment showed that some mutations can enhance both fitness and evolvability through a combination of direct and indirect selection. Unrelated evidence from gene duplication and the evolution of gene regulation suggests that mutations with such dual effects may not be rare. Through such mutations, evolvability may increase at least in part because it provides an adaptive advantage. These observations suggest a research program on the adaptive evolution of evolvability, which aims to identify such mutations and to disentangle their direct fitness effects from their indirect effects on evolvability. If evolvability is itself adaptive, Darwinian evolution may have created more than life's diversity. It may also have helped create the very conditions that made the success of Darwinian evolution possible.

KEYWORDS
autoregulation, direct selection, evolvability, gene duplication, robustness

1 | ADAPTIVE EVOLVABILITY AND INDIRECT, SECOND-ORDER SELECTION

Evolvability is the ability of organisms to produce phenotypic variation that is both heritable and adaptive. Some biological systems may be more evolvable than others, because this very property has been subject to natural selection. In other words, evolvability may itself be the subject of adaptive evolution. For brevity, I will refer to such evolvability as *adaptive evolvability*. The existence of adaptive evolvability has been subject to speculation for decades (Arber, 1993, 2005; Bedau & Packard, 2003). Unfortunately, to this day we have little pertinent experimental evidence (Payne & Wagner, 2019).

One can study evolvability from both a genotypic and phenotypic perspective (Houle et al., 2017; Ito et al., 2009; Kaneko, 2007; Sato et al., 2003; Wagner, 2008c). Here I take a genotypic perspective. That is, I focus on the ability of genotypes to produce adaptive heritable variation through DNA mutations. In this context, it is challenging to explain adaptive evolvability for two reasons. First, evolvability, like other traits such as stress-resistance or fertility, is a dispositional trait. In other words, it is a *propensity*. It is the propensity

to produce adaptive variation when mutations occur (Bigelow & Pargetter, 1987; Mills & Beatty, 1979; Mitchell, 1995). Second, these mutations are rare events in the life of an organism. Their incidence is often below one mutation per genome and round of replication (Lynch et al., 2016).

To see why these facts pose a challenge, consider a special kind of DNA mutation, one that alters evolvability itself. Such a mutation need not itself affect fitness, and natural selection need not act directly on the effects of this mutation. Instead such a mutation may only act indirectly, through the effects of other mutations that arise after it, because the evolvability-altering mutation alters the fitness consequences of these mutations. Natural selection that affects such an evolvability-altering mutation has also been called indirect or second-order selection, to distinguish it from first-order selection on a mutation's direct fitness consequences (Raynes et al., 2011; Weber, 1996). Second-order selection is weaker than first-order selection, in the sense that it can operate only under restrictive conditions.

I will illustrate these restrictions with two phenomena closely linked to evolvability. The first is mutation. The rate at which DNA mutations occur in an organism can be increased by "mutator" mutations that impair DNA repair enzymes (Giraud et al., 2001; Raynes et al., 2011; Sturtevant, 1937; Tenaillon et al., 1999; Wielgoss et al., 2013). Such mutators can increase evolvability if they cause a substantial increase in new mutations that are adaptive. In contrast to other kinds of DNA mutations that are themselves either deleterious (maladaptive) or beneficial (adaptive), and thus subject to natural selection, mutators need not have a direct effect on fitness. Because the mutation rate is a propensity—that of producing mutations (Bigelow & Pargetter, 1987; Mills & Beatty, 1979)—it may thus only be subject to indirect selection on the new mutations it helps create. As a result, a mutator can sweep through a population only in restricted circumstances. Specifically, a mutator's prospects are best if an organism finds itself in a new environment to which it is not well adapted, such that some of the mutations caused by the mutator are beneficial. If an individual harboring a mutator allele experiences such a beneficial mutation, the mutation may sweep through the population, and the mutator may hitch-hike to fixation with it, unless recombination separates the mutator from the beneficial mutation. Through this mechanism mutators often rise to high frequency when populations of bacteria like *Escherichia coli* with low recombination rates enter new environments (Chao & Cox, 1983; Giraud et al., 2001; Healey et al., 2016; Raynes et al., 2011; Sniegowski et al., 1997; Tenaillon et al., 1999). However, the advantage of a mutator is usually short-lived. After a population of mutators has become well-adapted to its environment, most or all new mutations become maladaptive and decrease fitness. At that time, the mutator becomes subject to indirect selection for its elimination. Its demise can be even faster when the mutator carries a direct fitness cost, although such costs can be difficult to identify (Raynes et al., 2011). In sum, if a mutator increases evolvability in a new environment, it succeeds through indirect selection via its association with a beneficial mutation.

A second phenomenon closely linked to evolvability—and subject to indirect selection—is mutational robustness, a genetic system's propensity to preserve its phenotype when perturbed by mutation. Both theory and empirical data demonstrate that increased robustness can enhance evolvability (Bloom et al., 2006; Ferrada & Wagner, 2008; Masel & Siegal, 2009; Najafabadi et al., 2017; Payne & Wagner, 2014; Starr et al., 2017; Wagner, 2008c). One kind of empirical evidence comes from laboratory experiments on enzymes. For example, an engineered variant of a cytochrome P450 enzyme that stabilizes the enzyme's tertiary structure and enhances its robustness to mutations can help the enzyme tolerate further mutations that create new enzymatic activities, while simultaneously destabilizing the enzyme (Bloom et al., 2006). In other words, the variant is a more evolvable enzyme.

Another kind of evidence comes from the evolutionary history of proteins (Ferrada & Wagner, 2008; Starr et al., 2017). Consider steroid hormone receptors, proteins that bind DNA and regulate gene expression in response to steroid hormones. The common ancestor of these receptors existed more than 450 million years ago. Reconstruction of this ancestral receptor shows that it regulated its target genes via binding to DNA motifs called estrogen responsive elements (ERE), which are similar to the DNA binding sites of today's estrogen receptors. During its evolutionary history, the gene encoding this receptor duplicated. The duplicates diversified and evolved into a new class of receptors that respond to androgens, progesterone, and corticosteroids. These new receptors bind to different motifs called steroid responsive element (SREs). Robustness played an important role in the switch from ERE to SRE binding specificity, because of 11 mutations that occurred during the evolution of specific SRE binding. These mutations did not affect the specific DNA sequence that a receptor binds, because they occurred outside the part of the receptor that recognizes DNA. Instead, they increased the mutational robustness of the ancestral receptor's ability to bind DNA. In doing so, they increased the proportion of mutant receptors that are capable of binding the SRE by more than 20-fold, shortened the evolutionary paths to SRE specificity, and facilitated its evolution (Starr et al., 2017).

Robustness-increasing mutations that enhance evolvability are subject to indirect selection, because of their dispositional nature—they alter the effects of other mutations. In the language of population genetics, the descendants of any one such mutation experience a higher incidence μ_n of neutral mutations and a lower incidence of deleterious mutations. Evolutionary theory shows that such a mutation can sweep through a population only under restrictive conditions. Specifically, the population mutation rate, the product of population size N and neutral mutation rate μ_n per genome and generation, $N\mu_n$, must exceed one (van Nimwegen et al., 1999; Wagner, 2005), because only in this case will a population be polymorphic for multiple neutral mutations (Kimura, 1983). The descendants of robust individuals will harbor more neutral and fewer deleterious mutations than those of less robust individuals, and less robust individuals will therefore slowly be eliminated by selection against these deleterious mutations. In contrast, when the mutation supply is low ($N\mu_n \ll 1$), a population will harbor very little genetic variation at any one time, and the occasional mutation that occurs will not be sufficient to help a robustness-enhancing mutation sweep to fixation by reducing the detrimental effects of later mutations. The mutation pressure $N\mu_n$ required to evolve mutational robustness may be high enough for some viruses and microbes with both large populations and high mutation rates, but it is too low for many eukaryotes (Lynch et al., 2016). In sum, the indirect nature of selection for mutational robustness means that robustness-mediated

evolvability can evolve only under high mutation rates or in large populations.

2 | A DIRECT PATH TO HIGH EVOLVABILITY IN PROTEIN EVOLUTION

Mutators and robustness illustrate, for different reasons, that indirect selection cannot be a universal source for adaptive evolvability. A more direct route to adaptive evolvability is suggested by a recent directed evolution experiment (Zheng et al., 2020). In this experiment, we studied the evolvability of a yellow fluorescent protein, which emits yellow light when exposed to light of a specific wavelength. We were interested in how selection might affect the evolvability of a new (green) color phenotype in this protein. To this end, we subjected populations of this protein expressed in *E. coli* to multiple rounds ("generations") of directed evolution, in which we alternated mutagenesis of the protein population with selection on its phenotype. More specifically, we subdivided the experiment into two phases (Figure 1a). In Phase I, we evolved each of four replicate populations under strong selection, in which we allowed only the top 20% of yellow-fluorescing cells to survive. In parallel, we evolved each of four replicate populations under weak selection on the ancestral yellow phenotype, by allowing all cells that fluoresced in

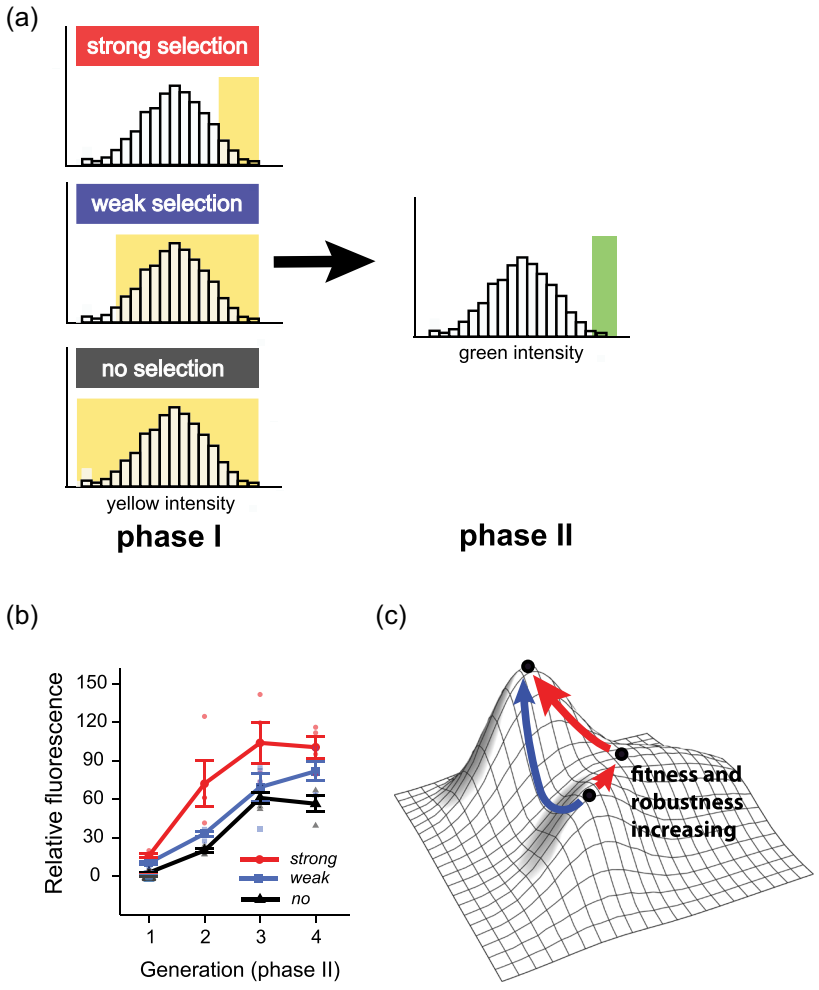

FIGURE 1 Strong selection enhances evolvability of a new color phenotype in a fluorescent protein by favoring mutations that enhance both fitness and robustness-mediated evolvability. (a) Schematic description of the experiment (Zheng et al., 2020). See text for details. (b) Fold-change of green fluorescence intensity relative to ancestral yellow fluorescence protein (vertical axis) in each generation of Phase II (horizontal axis), for populations that had been under strong, weak, or no selection in Phase I. Error bars represent one standard error of the mean, from four replicate populations (single small symbols). (c) A hypothetical fitness landscape illustrating how different modes of selection may affect the evolution of a new phenotype that is located at the highest peak of the landscape. Weak selection (blue arrows) may allow deleterious "stepping stone" mutations to survive and thus help a population traverse an adaptive valley on the path to the adaptive peak. Strong selection (red arrows) favors mutations that enhance fitness but also enhance robustness. In doing so, it can help an evolving population reach a region of the landscape that is not only elevated but also flatter, indicating that on such a plateau mutations affect fitness to a lesser extent, that is, robustness is higher. From such a region, the fitness peak can be reached more easily than for weak selection. Figure panels modified from Zheng et al. (2020)

yellow to survive, regardless of their fluorescence intensity. Finally, we also evolved four populations under no selection. After Phase I, we started Phase II, in which we evolved all 12 populations under equally strong selection for the new phenotype of green fluorescence, allowing only the top 0.01% of green fluorescing cells to survive each generation. Each phase lasted for four generations.

The experiment showed that strong selection on the old, yellow phenotype increased evolvability of the new phenotype most strongly (Figure 1b): Populations under strong selection evolved the green color phenotype faster and to a higher level. The reasons became clear through a combination of high-throughput DNA sequencing, protein engineering of selected mutants, and biochemical assays. In populations under strong selection, five specific mutations rose to a higher frequency. Several of these mutations had an important dual role. First, they increased fluorescence and did so at least partly by increasing protein foldability— the likelihood that a translated protein folds into its native tertiary structure and thus displays its color phenotype. Second, these mutations also increased the robustness of both the yellow and green color phenotype to DNA mutations. In doing so, they increased evolvability by reducing the effects of deleterious mutations on fluorescence, and thus allowed protein variants with more intense green fluorescence to sweep more rapidly through the population. In the graphical metaphor of fitness landscapes (Figure 1c), these mutations give evolving populations access to a region of a higher elevation (greater fluorescence) in the landscape than the ancestor. Importantly, this region is also flatter (greater robustness), which facilitates the ascent to the green fluorescence fitness peak. In sum, these mutations increased both fitness and evolvability.

These observations show that natural selection can increase evolvability through a combination of direct, first-order selection on fitness and indirect, second-order selection on evolvability. This is important, because a synergism between direct and indirect selection makes it easier to explain evolvability as a product of adaptive evolution. However, this explanation has a price: When a mutation increases both fitness and evolvability, an increase in evolvability cannot be the sole product of selection on evolvability itself. It will result at least partly from direct selection on a phenotype. In the experiment in question, mutational robustness accounted for the majority (>75%) of each mutation's benefit for adaptive evolution (Zheng et al., 2020). In other words, the direct fitness benefit was smaller than the indirect evolvability benefit, such that one cannot explain increased evolvability solely as a by-product of increased fitness.

3 | A RESEARCH PROGRAM ON THE ADAPTIVE EVOLUTION OF EVOLVABILITY

These observations suggest a largely unexplored research program on the adaptive evolution of evolvability. Instead of focusing on indirect selection as a source of adaptive evolvability, this program would study genetic changes with the dual role of enhancing both fitness and evolvability. The program could be highly productive, because the mutations we observed are far from the only candidates for such changes. Other candidates include mutations that increase the thermodynamic stability of proteins, that is, the free energy needed to unfold a protein. I already mentioned a study that compared more and less stable variants of a cytochrome P450 enzyme. The more stable variant was more evolvable, because when it experienced random mutations, it was more likely to catalyze reactions with several new substrate molecules than the less stable variant (Bloom et al., 2006).

More generally, mutations that increase protein stability can directly increase fitness, because they cause a larger fraction of newly synthesized protein molecules to adopt and retain their native tertiary structure. Consistent with this notion, stabilizing mutations often help improve proteins with a given activity through directed evolution or targeted mutagenesis (Bloom & Arnold, 2009; Brown et al., 2010; Fasan et al., 2007, 2008; Heinzelman et al., 2009; Salazar et al., 2003; Wang et al., 2002). Most existing studies, however, focus on the effects of such mutations on an already existing protein activity. We know little about the joint fitness and evolvability-changing effects of such mutations.

4 | EVOLVABILITY THROUGH GENE DUPLICATION

Amino acid changes in proteins are not the only kind of genetic change that can affect evolvability. Another is the duplication of protein-coding genes. Such gene duplications occur at high rates as by-products of DNA repair and recombination processes (Lynch & Conery, 2003; Lynch, 2007). As a result, genomes are replete with the remnants of past duplication events. For example, some 50% of human genomic DNA is duplicated (Consortium, 2001), and 65% of plant genes are duplicated (Panchy et al., 2016). After a duplication has created identical copies of a gene, these copies are redundant. Because of their redundancy, most new duplicates are eventually deactivated by point mutations or eliminated by DNA deletions. A small fraction of duplicates, however, experience neofunctionalizing mutations—mutations that help create proteins with new functions. This process is facilitated by the very redundancy of identical gene duplicates, which causes them to be more robust to DNA mutations, and can help an organism to tolerate neofunctionalizing mutations in the duplicates (Wagner, 2008a). Not surprisingly then, gene duplications have been implicated in the evolutionary diversification of many organisms and their organs (Ohno, 1970). Examples include the evolution of plant morphological diversity via the duplication of genes encoding MADS box transcriptional regulators (Irish & Litt, 2005; Theissen et al., 1996), the evolution of vertebrate diversity through the duplication of Hox genes (Carroll et al., 2001), as well as the evolution of complex vertebrate organs like the four-chambered mammalian heart (Olson, 2006).

Many models exist that predict how duplicates diversify in function (Conant & Wolfe, 2008). Among them, the experimentally

validated innovation-amplification-diversification (IAD) model best illustrates the potential synergism between the fitness- and evolvability enhancing effects of gene duplications (Andersson et al., 2015; Bergthorsson et al., 2007; Copley, 2020; Nasvall et al., 2012). The IAD model relies on the observation that many proteins and especially enzymes are functionally promiscuous. That is, in addition to catalyzing one main reaction at a high rate, such enzymes also catalyze multiple side reactions at a much lower rate (Andorfer et al., 2017; Copley, 2020; Huang et al., 2015; Khersonsky & Tawfik, 2010; Martínez-Martínez et al., 2018; O'Brien & Herschlag, 1999). The model envisions a gene encoding a promiscuous protein with a main and a side reaction. It considers the side reaction as an innovation that may provide a benefit to an organism in the right environment. However, if the side reaction is catalyzed at a low rate, its benefit may be too small to be visible to natural selection. This is where gene amplification—the creation of not just two but multiple gene copies through duplication—comes in. When multiple genes express the promiscuous protein, its concentration increases, which can render the catalytic rate of the innovative reaction sufficiently high to become visible to selection. If so, gene amplification may be favored and maintained by natural selection. Subsequently, adaptive mutations can begin to accumulate in individual gene copies and help them catalyze the novel reaction more rapidly. It is sensible to argue that an organism with the gene amplification is more evolvable than one without it. First, multiple gene copies cause robustness of the main reaction, which is catalyzed by multiple redundant enzymes. Second, by increasing the catalytic rate of the innovative side reaction, they render its benefits visible to selection. Third, multiple gene copies present a much larger target for mutations that alter the side reaction than the ancestral, single copy gene. In consequence, mutations have many more opportunities to increase the encoded enzyme's ability to catalyze the novel reaction (Andersson et al., 2015).

Direct experimental evidence for the IAD model exists in the bacterium *Salmonella enterica* for enzymes in the biosynthesis of the amino acids histidine and tryptophan (Nasvall et al., 2012). A pertinent experiment started from a mutant of the gene HisA, which encodes an enzyme that catalyzes a step in the biosynthesis of histidine, and does so at a rate similar to the wild-type enzyme. In addition, this HisA mutant can also catalyze, at a low rate, a reaction in tryptophane biosynthesis that is normally catalyzed by the product of the TrpF gene. When this mutant HisA gene is expressed in a strain of *Salmonella enterica* that lacks both the wild-type HisA and TrpF genes, and whose environment contains neither histidine not tryptophane, both of its catalytic activities are necessary for survival. Within 3000 generations of experimental evolution, efficient catalysis of the TrpF reaction evolves. This process begins with amplification of the mutant HisA gene. Subsequently, some duplicates of the gene accumulate mutations that increase the catalytic rate of the TrpF reaction. Other duplicates help preserve the ability to catalyze the equally essential HisA reaction. The IAD process has also been implicated in the evolution of antifreeze proteins in Antarctic fish, of enzymes that metabolize isomaltose in yeast, as well as of multiple other enzymes (Andersson et al., 2015; Copley, 2020; Deng et al., 2010; Voordeckers et al., 2012).

In the IAD process, gene amplification increases fitness directly by increasing the expression of a beneficial protein. In addition, it increases the evolvability of fast catalysis by increasing both robustness and the mutational target size of the amplified genes. The IAD process relies on promiscuity as a source of the primary fitness benefit, but the same argument applies to any one of multiple diversification processes in which gene duplication can cause increased fitness (Conant & Wolfe, 2008). In sum, gene duplication exemplifies another synergism between fitness-increasing and evolvability-increasing mutations.

5 | THE EVOLVABILITY OF GENE REGULATION

We know much less about evolvability in regulatory evolution, but the following, more speculative examples suggest that synergisms between direct and indirect selection can be found there as well. I mentioned that adaptive evolution in an ancestral steroid hormone receptor helped change its DNA binding specificity from an ERE to a SRE with the help of 11 mutations. These mutations increased evolvability of the new binding affinity by increasing robustness, but they did more than that. They also increased the receptor's DNA binding to both the ERE and SRE, and may thus have had a direct fitness benefit mediated by stronger gene activation. If so, they increased both fitness and evolvability of SRE binding (Starr et al., 2017).

Another regulatory example involves a transcriptional regulation circuit that is as simple as it is abundant in cells (Rosenfeld et al., 2002; Thieffry et al., 1998). It is a negative autoregulation circuit, in which a transcriptional regulator binds a regulatory region near its own gene and represses its own expression. In *E. coli*, some 40% of transcriptional regulators display negative autoregulation (Rosenfeld et al., 2002; Thieffry et al., 1998), which helps reduce gene expression noise that results from a variety of sources, among them stochastic protein-DNA binding (Becskei & Serrano, 2000).

The *E. coli lexA* gene exemplifies such a negative autoregulator. LexA represses not only itself but also multiple genes that are important for the bacterium's SOS response to DNA damage (Little & Mount, 1982). Mutations that eliminate *lexA* autoregulation are harmful for at least two reasons. First, they slow down the SOS response. Second, such mutations lead to energetically costly *lexA* overexpression even in the absence of DNA damage (Kozuch et al., 2020). In other words, the negative autoregulation of *lexA* carries a direct fitness benefit. In addition, *lexA* autoregulation also increases mutational robustness. Specifically, when *lexA* is autoregulated, deleterious mutations that impair *lexA* function have less impact on *lexA* expression and on a cell's resistance to DNA damage (Marciano et al., 2014). This suggests that autoregulation helps *lexA* explore a greater proportion of DNA sequence space than it could otherwise. In doing so, it may have helped *lexA* evolve the ability to regulate expression from a wide diversity of DNA binding sites across different species (Erill et al., 2007; Mazon et al., 2004). Consistent with this suggestion,

evolution has diversified the amino acid sequence of *lexA* and of other transcriptional regulators with negative autoregulation (but not with positive autoregulation) more rapidly than that of their target genes (Marciano et al., 2014). In sum, if negative autoregulation indeed helps regulatory diversity evolve, it would be another case of a trait with both a direct fitness and an indirect evolvability benefit.

6 | TRADE-OFFS BETWEEN DIRECT AND INDIRECT SELECTION

I have thus far focused on genetic change that enhances both fitness and evolvability, because it is the most promising route toward adaptive evolvability. However, it is not the only route, and probably not even the predominant route in which mutations can affect both properties. Figure 2 schematically identifies three other possibilities, together with their propensity to become established in a population.

The upper left quadrant represents changes that enhance both fitness and evolvability and that I just discussed. The lower right quadrant represents changes that impair both fitness and evolvability. I will not discuss them further, because they are most likely eliminated by natural selection.

The lower left quadrant refers to changes that impair fitness but increase evolvability. Such changes are not rare. Consider those stability-enhancing amino acid mutations that increase protein evolvability by increasing robustness. I discussed some examples where such changes also enhance fitness, but this is not universally true. For example, a single amino acid change that increases the stability of a *Bacillus subtilis* adenylate kinase at 20°C reduces its activity at the same temperature (Counago et al., 2008). Likewise, three different amino acid changes in a bacterial ribonuclease increase stability and decrease activity (Meiering et al., 1992), as do multiple mutations in the bacterial AmpC β-lactamase (Beadle & Shoichet, 2002). Examples like these rely on a modest number of mutations, whereas high throughput mutagenesis and phenotyping can assess the relationship between stability and activity more comprehensively. One pertinent study generated thousands of mutations in two enzymes, a beta-lactamase and a levoglucosan kinase. It showed that more than 40% of mutations that enhance solubility—a compound quantifier of stability, foldability, and aggregation propensity—reduce fitness (Klesmith et al., 2017).

What holds for amino acid changes also holds for gene duplications. Some of them may be immediately beneficial, but many others are detrimental, because of various costs they incur. These costs include detrimental imbalances in protein concentrations, additional time needed to replicate a duplicated gene, extra energy to synthesize its DNA nucleotides, and most importantly, additional energy expended in expressing an RNA transcript and translating this transcript into protein (Adler et al., 2014; Lynch & Marinov, 2015; Schrider et al., 2013; Veitia, 2005; Wagner, 2007). Based on such costs, it has been estimated that every additional kilo base pair of duplicated *E. coli* genomic DNA carries a fitness cost of 0.15% (Adler et al., 2014). Whenever such costs are not offset by benefits like those of enhancing a promiscuous protein function, they will help create an antagonistic relationship between fitness and evolvability. Such antagonism may be widespread, at least in some species. For example, it has been estimated that almost 99% of DNA duplications in *Drosophila melanogaster* are deleterious (Schrider et al., 2013).

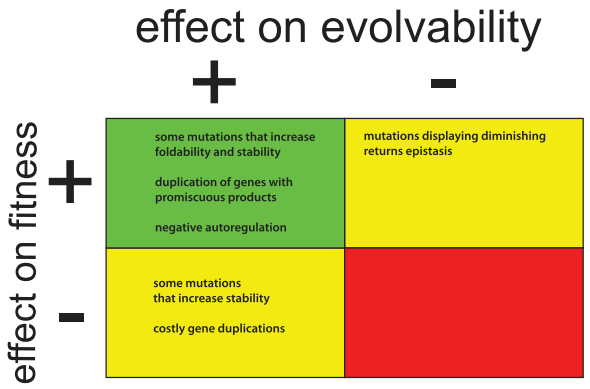

FIGURE 2 A simple schematic illustrating how natural selection may affect genetic changes that increase ("+") or decrease ("−") fitness or evolvability. The green color in the upper left rectangle indicates that mutations which increase both fitness and evolvability are most likely to be favored by natural selection. The upper right yellow rectangle refers to mutations that reduce evolvability but increase fitness, whereas the lower left rectangle refers to mutations that decrease fitness but increase evolvability. Whether either mutations can invade an evolving population depends on the relative strengths of their effects on fitness and evolvability. The red color in the lower right rectangle indicates that mutations which reduce both fitness and evolvability are likely to be eliminated by natural selection

An antagonistic relationship between fitness and evolvability reduces the chances that a mutation affecting both will succeed. However, it does not eliminate these chances. Experimental proof is provided by experimental evolution in *E. coli* (Díaz Arenas & Cooper, 2013; Phillips et al., 2016; Woods et al., 2011). In one such experiment, two mutations arose that were initially detrimental, but that eventually succeeded in sweeping through the population (Woods et al., 2011). These mutations facilitated the origin of further mutations that helped elevate their own fitness through nonadditive (epistatic) interactions. Perhaps such examples are rare, but they show that evolvability can sometimes increase as an adaptation in its own right and without help from direct selection.

Finally, the upper right quadrant harbors mutations that increase fitness but decrease evolvability. Such mutations are frequently observed in laboratory evolution experiments, where fitness increases rapidly at first, while an evolving population's mean fitness is still low. Later during evolution, when the population's mean fitness has increased, fitness increases more slowly. Individual mutations in such populations display a phenomenon called diminishing returns epistasis (Chou et al., 2011; Jerison et al., 2017; Khan et al., 2011; Kryazhimskiy et al., 2014; Wünsche et al., 2017). Epistasis refers to the nonadditive effects of two or more mutations on fitness. Diminishing returns epistasis means that one mutation that increases

fitness will cause subsequent mutations to increase fitness by a lesser extent than they would have without the first mutation. This form of epistasis is easily explained by the adaptive landscape metaphor (Svensson & Calsbeek, 2012): As a population approaches an adaptive peak, fewer and fewer mutations will increase fitness, and those that do will increase fitness by a smaller amount. In other words, mutations that increase fitness reduce an organism's ability to bring forth adaptive mutations in the future. They reduce evolvability.

In sum, Figure 2 and the examples above illustrate a total of four categories of mutations that affect fitness and evolvability. Only in two of them does evolvability increase, and only in one of them do both evolvability and fitness increase. Mutations that increase both fitness and evolvability are most likely to succeed in Darwinian evolution. However, it remains an open empirical question how many of the remaining two kinds of mutations succeed, especially since their numbers may be far greater than those of mutations that enhance both fitness and evolvability.

For simplicity, Figure 2 does not include mutations that do not affect fitness, even though such neutral mutations are relevant for evolvability in at least two ways (Kimura, 1983; Ohta, 1992, 2011; Payne & Wagner, 2019; Starr et al., 2017; Wagner, 2008b; Zheng et al., 2019). First, some fitness-neutral mutations may alter evolvability. On the vertical axis of Figure 2, these neutral mutations would fall between beneficial (+) and deleterious (−) mutations, and the figure could thus be easily extended to accommodate them. They can spread either through genetic drift or through their indirect benefits for evolvability. Second, a mutation that increases evolvability by increasing mutational robustness (whether the mutation itself alters fitness or not) may increase the incidence of neutral mutations. In doing so, the mutation increases the size of a "neutral region" around the genotypes of a population that explores a genotype space. By being able to explore this region without adverse consequences, the chances that the population encounters adaptive mutations increase (Wagner, 2008b).

The main reason why I do not discuss such mutations more explicitly is a practical one: Their detection in any kind of evolving population poses serious technical challenges for currently available technologies. In evolving populations with large effective population size N, fitness differences among individuals that are of the order of $1/N$ are non-neutral and thus visible to selection (Lynch, 2007). These differences can be much smaller than the detection limit of any available and foreseeable experimental technology. Thus, proving that a specific DNA mutation is strictly fitness-neutral is difficult with current tools.

7 | CHALLENGES AND OUTLOOK

Any research program on adaptive evolvability must be able to disentangle the direct fitness benefit of a mutation from its more indirect benefit on evolvability. To be sure, quantifying the fitness benefit of mutations is routine, at least in model organisms where a mutation can be engineered into a genome, and where its effect can be easily measured. The same cannot be said for distinguishing a mutation's effect on fitness from that on evolvability. To do so requires that two challenges are met.

First, it is necessary to experimentally control variables that affect evolvability. For example, when evolvability is mediated by robustness to mutations, one will have to study the effect of a mutation in the presence and the absence of other mutations. This is straightforward to do in the directed evolution of individual proteins, where molecular cloning tools like the polymerase chain reaction can be used to turn mutation pressure on and off at will (Dalby, 2011; Zheng et al., 2020). It is much harder in the experimental evolution of whole organisms, where mutation rates can be controlled only to a limited extent via mutator strains.

Second, partitioning a mutation's effect into direct fitness effects and indirect evolvability effects, requires an appropriate scale to quantify these effects and compare their relative magnitudes. For example, one may choose to ask by how much the frequency p of an allele changes in a single generation if the allele (i) increases only fitness, or (ii) increases both fitness (by the same amount as in the first scenario) but additionally also evolvability. If Δp and Δp_e denote this allele frequency change in scenario (i) and (ii), respectively, then the difference $\Delta p_e - \Delta p$ is one candidate quantifier of the allele's evolvability benefit. However, it is sensible only if the evolvability benefit manifests itself within a single generation, which can be the case if the introduction of new variation by DNA mutations is frequent. If that is not the case, other quantities need to be used. Candidates include the number of generations that an evolvability-altering allele requires to exceed a given frequency threshold, the time the allele needs to go to fixation, or the likelihood that the allele can invade a population when rare. The relative fitness and evolvability benefits may differ among these quantifiers. It is also relevant here that an evolvability-altering mutation creates two subpopulations that differ in their potential for future adaptive evolution. To disentangle fitness benefits from evolvability benefits, it may be useful to study the traits of not just individuals but of entire subpopulations. Fortunately, sociobiology has developed the tools to do just that. Because sociobiology is concerned with the behavior of groups of organisms, it needs to distinguish between traits of groups and those of individuals, and how these traits influence a population's evolutionary dynamics. Among its relevant tools are the Price equation (Frank, 1998) and contextual analysis (Heisler & Damuth, 1987).

It is too early to say which experimental systems and theoretical approaches will prove most useful to a research program in adaptive evolvability. However, even if such a program discovers only a few fitness-enhancing mutations that also enhance evolvability, Darwin's core idea would have proven itself in a new and unexpected way. It would mean that natural selection has not just been central to create the diversity of life. It may also have helped make life evolvable, and thus create the very conditions that allowed Darwinian evolution to succeed in the first place.

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CONFLICT OF INTERESTS

The author declares that there are no conflict of interests.

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Data sharing is not applicable to this article as no new data were created or analyzed.

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