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Are mutations usually deleterious? A perspective on the fitness effects of mutation accumulation

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Abstract

All adaptive alleles in existence today began as mutations, but a common view in ecology, evolution, and genetics is that non-neutral mutations are much more likely to be deleterious than beneficial and will be removed by purifying selection. By dramatically limiting the effectiveness of selection in experimental mutation accumulation lines, multiple studies have shown that new mutations cause a detectable reduction in mean fitness. However, a number of exceptions to this pattern have now been observed in multiple species, including in highly replicated, intensive analyses. We briefly review these cases and discuss possible explanations for the inconsistent fitness outcomes of mutation accumulation experiments. We propose that variation in the outcomes of these studies is of interest and understanding the underlying causes of these diverse results will help shed light on fundamental questions about the evolutionary role of mutations.

Keywords

Distribution of fitness effects; life history traits; beneficial mutations; fitness landscape

Introduction

Mutations can be good or bad. While many genetic changes will disrupt organism function in ways that are detrimental, mutation is ultimately the source of all adaptive variation as well. To understand the impact of the mutation process on individuals and populations, and to predict how the mutation rate itself will evolve, we need to understand how often beneficial versus deleterious mutations occur. A simplified prediction is that beneficial mutations should eventually be depleted in populations adapting to a stable environment, as they become fixed by positive selection. In a perfectly adapted genotype, we would expect mutations to be either deleterious or neutral. In practice, interactions between genes, environment, and the ever-changing landscape of adaptation makes predicting

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the distribution of fitness effects (DFE) of mutations in natural populations far less straightforward. Nevertheless, there has been intense interest in understanding the DFE, due to its relevance for both predicting trajectories of adaptation and managing the conservation of threatened species (Lande 1994; Eyre-Walker and Keightley 2007; Halligan and Keightley 2009).

One way to study the effects of spontaneous mutations is with a mutation accumulation (MA) approach. In a typical MA experiment, a given genotype is replicated into many "lines", which are each repeatedly bottlenecked for many generations. Bottlenecking ensures that the effective population size (N_e) in each MA line is very low. As a result, most new mutations that arise have a probability of fixation approximately equal to that of a neutral mutation (Keightley and Caballero 1997; Lynch and Walsh 1998). In other words, the fate of mutant alleles in populations with small N_e is due primarily to genetic drift, rather than natural selection, unless their effects on fitness are large.

Some simple equations are useful for understanding the results of MA (reviewed in Halligan and Keightley 2009). Following MA, the expected number of mutations in a line is Ut, where U is the mutation rate per genome and t is the number of generations of MA. If we measure some trait (typically a life history trait), the expected trait value for MA lines is $z_t = z_0 + UtE[s]$, where z_0 is the pre-MA (control) trait value, and $E[s]$ is the average effect of a mutation on the trait (or $E[hs]$ in heterozygous diploids, where h is the dominance coefficient). The expected genetic variance among MA lines is $UtE[s^2]$. We can describe the rate of change in the mean per generation as $\Delta M = (z_t - z_0)/t = UE[s]$. Similarly, the rate of change in genetic variance is $\Delta V = UE[s^2]$, as there is presumed to be no genetic variance prior to MA. Modelling approaches have been applied that use MA data to estimate specific values for the mutation rate and complex distributions of mutational effects (e.g., Keightley 1994; García-Dorado 1997; Shaw et al. 2002; Böndel et al. 2019; Böndel et al. 2021), but because U cannot be negative, the sign of ΔM must always reflect the sign of $E[s]$. While approaches to modelling the DFE vary, we consider the sign of ΔM a "first order" metric of mutational effects that can always be compared among MA studies.

In many MA experiments, mean fitness declines (negative ΔM using the formulation above), meaning $E[s] < 0$. Thus, a decline in fitness components under MA is evidence that mutations have deleterious effects on average. Many, but not all MA experiments show this pattern; in some cases, there is little or no fitness decline ($\Delta M \cong 0$), even as significant genetic variance accrues among MA lines ($\Delta V > 0$). We think that the fact that this most basic measurement of mutational effects is not consistent deserves explanation, even as approaches to estimating the full DFE become more advanced.

One explanation for $\Delta M \cong 0$ that can probably be dismissed is that no mutations have accumulated, regardless of fitness effect ($U_{total} = 0$). Many MA studies now involve genome sequencing, confirming the presence of mutations. In general, MA experiments have been designed with a sufficient number of lines and generations that at least a modest number of mutations is expected, based on the genome size and likely mutation rate of the study organism. Another hypothesis is that mutations could be largely neutral, or do not affect the traits in question. However, increases in genetic variance under MA ($\Delta V > 0$) indicate

that at least some of the mutations must have measurable effects under the relevant assay conditions—the MA studies where $\Delta M \cong 0$ (see below) generally detect significant ΔV. Significant ΔV also implies sufficient statistical power to detect modest changes in mean fitness. Therefore, the most likely explanation for $\Delta M \cong 0$ is that the average effect of mutations on the trait is close to zero ($E[s] \cong 0$), implying that beneficial and deleterious mutations have similar net impact on the trait. This runs counter to the idea that most mutations are deleterious and, in our view, this variation in outcomes lacks a consensus explanation.

In this perspective we summarize cases of MA experiments that show no evidence of fitness decline and discuss possible general explanations. We propose that inconsistency in the outcomes of MA experiments is an interesting pattern in its own right, because it may stem from genuine differences in mutation or "fitness landscapes" among genotypes, species, and environments.

Studies with $\Delta M \cong 0$

While many of the earliest studies using the MA method were conducted in the model fruit fly *Drosophila melanogaster*, none of these studies found results other than a fitness decline (Mukai 1964; Fry et al. 1999; Halligan and Keightley 2009). It should be noted that there are methodological difficulties with measuring MA line fitness in fruit flies: unlike many of the other organisms that have been studied under MA, in flies it has usually been impractical to effectively cryo-preserve the MA "ancestor" for later fitness comparisons (but see Pletcher et al. 1998). Instead, large populations are maintained as controls, which could potentially adapt and cause fitness decline to be over-estimated. However, deleterious mutations will also appear and segregate in control populations, creating a bias in the opposite direction. Attempts to account for evolution in controls or avoid the need for them have still concludes that fitness decline has occurred (Fry 2004; Sharp and Agrawal 2018). The results of MA in *Drosophila* are therefore quite consistent, but this consistency does not appear to hold in other organisms.

MA studies conducted in other animals—namely the model roundworm *Caenorhabditis elegans* and crustacean *Daphnia pulex*—also generally show significant declines in fitness, but with exceptions. In an early MA experiment, it was found that there was no significant decrease in two fitness metrics, reproductive output and lifespan, despite significant increases in variance (Keightley and Caballero 1997). The authors attributed this to a "deleterious mutation rate at least 100 times smaller than previously assumed" based on the framework of the previous *D. melanogaster* studies. Later MA studies in *C. elegans* found declines in fitness traits following MA, but two studies conducted by one group found no decline in lifespan following MA (Vassileva et al. 2000) and even noted one case of significantly improved lifespan after MA (Vassileva and Lynch 1999). In *D. pulex*, an MA study involving multiple genetic backgrounds found that while most showed a decline in fitness comparable to the results of other *D. pulex* MA experiments, a few genetic backgrounds actually outcompeted their ancestors in a competitive fitness assay (Schaak et al. 2014).

It is among plants that the most notable cases of MA studies without significant fitness decline can be found. Indeed, a debate around the notion that mutations are predominantly deleterious was ignited by a study in the model plant *Arabidopsis thaliana* (Shaw et al. 2000; Shaw et al. 2002; Keightley and Lynch 2003). Shaw et al. (2000) examined at three life history traits: number of seeds per fruit, fruit number, and reproductive mass after 17 generations of MA. No significant decline was noted for any of the traits despite a significant increase in among-line variance for all traits. A prior study in the same organism (Schulz et al. 1999) with more lines but fewer generations of MA detected a decline in fitness of about the same magnitude as in this later study, but it was found to be statistically significant although variance was not reported. From their data, Shaw et al. (2000) concluded that about half of all mutations that occurred in their study were beneficial. Later studies of these lines extended MA to further generations (Rutter et al. 2010; Rutter et al. 2012) and in a variety of environmental conditions ranging from field plantings across geographic ranges to greenhouse studies and over different seasons (Roles et al. 2016; Rutter et al 2018). Across this wide range of conditions, the results consistently showed little or no fitness decline, and evidence for substantial accumulation of beneficial mutations. Other studies in *A. thaliana* shows that mutations are dependent on environmental context by transplanting MA lines from vast gradients along the species' range (Weng et al. 2021). In another plant study, 300 lines of the wild radish, *Raphanus raphanistrum* underwent 9 or 10 generations of MA in the field or the greenhouse, respectively (Roles and Connor 2008). No significant decline in fitness was detected, but the authors attributed this partially to the relatively few MA generations and possible selection pressure on the seeds during seed choice. In the same paper the authors found a decline in fitness compared with ancestors in less permissive growing conditions. MacKenzie et al. (2005) found no evidence of changes in the mean or variance of *A. thaliana* MA lines subjected to MA along with UV-B radiation, a common mutagen in plant MA studies.

Finally, there has also been mixed evidence of fitness decline in MA experiments with microbes. While "microbes" represent a far larger and less continuous slice of the tree of life than animals or land plants, we believe it is fitting to discuss them as a group because of their common laboratory culture and assay methodologies. The organisms most commonly studied under MA methods are yeasts, particularly the model *Saccharomyces cerevisiae*. In *S. cerevisiae* there have been experiments where mean fitness has declined following MA (e.g., Wloch et al. 2001; Joseph and Hall 2004; Dickinson 2008) but a series of experiments showed a much stronger signature of beneficial mutations than expected (Joseph and Hall 2004; Hall et al. 2008; Hall and Joseph 2010). These studies found that a large fraction of MA lines did not show significant deviation from ancestral fitness in traits like diploid growth rate, sporulation efficiency and haploid growth rate, with some lines showing significantly improved growth. A later MA study of comparable size and duration was conducted on both haploids and diploids from a single genetic background, finding significant decline in fitness in diploids but not haploids (Sharp et al. 2018). Other yeast studies have found a similar lack of fitness declines or improvements in a large number of their MA lines (Zeyl and DeVisser, 2001). An MA study in green alga *Chlamydomonas reinhardtii* from multiple genetic backgrounds (Kraemer et al. 2017) found that, following ~1000 generations of MA, four genetic backgrounds showed fitness decline and one did not.

This was suggested to be because said background was already very slow growing prior to MA, perhaps being evidence of poor adaptation to the laboratory and assay conditions (Kraemer et al. 2017; Bondel et al. 2021). Later, recombinant lines were generated by crossing six MA lines with their ancestors, generating 1526 recombinant lines, as a way of inferring the DFE; of the mutations with a fitness effect of 1% or more, one sixth were found to be beneficial (Bondel et al. 2019). Later modeling of the DFE of new mutations in *C. reinhardtii* MA lines has suggested a highly leptokurtic distribution and "approximately equal proportions" of growth rate increasing and growth rate reducing mutations (Böndel et al. 2021). Among bacteria, multiple studies have found significant fitness decline under MA in *Escherichia coli* (Kibota and Lynch 1996; Loewe et al. 2003; Funchain et al. 2000; Trindade et al. 2010), as well as *Pseudomonas aeruginosa* (Heilbron et al. 2014). The results of an MA experiment with *Burkholderia cenocepacia* were more mixed, with some lines showing significantly increased fitness in one environment (Dillon and Cooper 2016). In summary, MA in microbes mostly results in fitness decline, but exceptions can be found in multiple species.

Potential Explanations

As shown above there are clearly many cases where the MA approach seems to result in a lack of fitness decline, and even fitness improvements. In discussing potential explanations, we should first consider limitations of the MA method itself. Theoretically, a new mutant allele will have about the same probability of fixation as that of a neutral allele when its effect on fitness is smaller than the inverse of the effective population size (Lynch and Walsh 1998; Lynch et al. 1998). In practice, this means that if N_e is small (e.g., due to population bottlenecks) a mutation would have to be very impactful on fitness to be subject to any effective positive or negative selection (but note that most new alleles will be lost by chance even when selection is very effective). While the true distribution of the fitness effects of mutations is not known with much certainty, analyses often point to a highly skewed distribution, where weak effects are common and strong effects are rare (Eyre-Walker and Keightley 2007; Fig. 1). Under these circumstances, where most mutations have small effects, the MA strategy can be expected to be successful.

Nevertheless, mutations with large fitness effects do exist, and experiments that combine sequencing with fitness assays have found that mutations with large effects can significantly skew the overall fitness of an MA line even if they are very rare (Schultz and Lynch 1997; Heilbron et al. 2014). Whether mutations of large effect will be acted upon by selection depends on how effectively N_e can be reduced, which varies among study systems. In most MA studies on animals, only a single individual or pair is allowed to contribute to the next generation, ensuring that N_e is small. In plants, single seeds can be used to propagate each MA line, but because plants do not have a segregated set of germlines cells, they are subject to clonal selection in the meristem (Klekowski and Kazarinova-Fukshansky 1984; Otto and Orive 1995, Otto and Hastings 1998; Schoen and Schultz 2019; Cruzan et al 2022), which may prevent some harmful mutations from being passed to the next generation. Similarly, more gene expression takes place in the male gametes of plants relative to animals, potentially exposing recessive mutations to selection (Mulachy et al. 1996; Otto et al. 2015). MA studies that include sequencing have some opportunity to address these

potential sources of bias by examining the distribution of mutations within genes and across the genome (e.g., Zhu et al. 2014; Sharp and Agrawal 2016; Sharp et al. 2018; Liu and Zhang 2019; Weng et al. 2019).

Microbes suffer from a similar problem of clonal selection. Because microbial generation times are short, MA typically involves streaking to single colonies repeatedly. Multiple generations of growth between bottlenecks means that N_e is not as low as it is in animal systems, and so selection could influence the fixation of new mutations. Statistical methods have been developed to account for selection when estimating the DFE in microbial MA experiments, greatly reducing the inferred frequency of beneficial mutations from both simulations and empirical datasets (Mahilkar et al. 2021; Wahl and Agashe 2022). These findings suggest that at least some of the variation in outcomes among microbial MA experiments is likely the result of differences in practical aspects of these experiments, such as transfer times and colony sizes.

One method to experimentally address how differences in N_e affect the outcomes of MA is to conduct the procedure using a range of effective population sizes (e.g., Estes et al. 2004; Katju et al. 2014; Katju et al. 2018; Luijckx et al. 2018). These experiments have shown that as N_e increases the likelihood of seeing a significant fitness decline decreases. For example, Katju et al. (2014) studied hermaphrodite nematode MA lines with various bottleneck sizes. Lines with $N_e = 1$ showed significantly decreased productivity and survivorship after 409 generations, but lines with $N_e = 10$ and $N_e = 100$ did not. All population size treatments showed significant increases in among-line variance for both traits, with the exception of survivorship in the $N_e = 100$ treatment. The simplest interpretation of these results is that deleterious alleles with moderate effects behaved neutrally in the smallest populations resulting in the observed decline of mean fitness, but selection counteracted this in the larger populations. Increased genetic variance in the larger populations implies that some mutations became fixed and produced changes in the fitness of those lines. This reinforces the idea that even with some level of selection acting, deleterious mutations are still able to reach fixation although at a lower rate (Schultz and Lynch 1997). The influence of population size on the rates of fixation for deleterious versus beneficial mutations is hard to predict, but these studies find little or no evidence of fitness improvement under large population sizes, suggesting beneficial mutations were mild or rare.

The above explanations center on selection as a source of bias during MA, inflating the impact of rare beneficial mutations and reducing the impact of deleterious mutations. An alternative explanation for the variation in fitness decline among MA experiments is that substantial beneficial genetic variation is available, but only in some circumstances. In particular, it is worth considering that different genotypes can have different histories of adaptation to any particular environment—genotypes that are initially well-adapted to the testing conditions for fitness may be more likely to show fitness decline under MA than genotypes that are poorly adapted to those conditions (Orr, 2006; Stearns and Fenster 2016). A useful metaphor for this prediction is the “fitness landscape”, where a well-adapted genotype is located at or near the peak and less well adapted genotypes are farther from the peak (Fig. 2). In a genotype at the peak of such a landscape, a step in any direction would represent a descent: a movement towards a less fit genotype; consequently, mutations

in well-adapted lines will tend to decrease fitness. By contrast, in genotypes further from the peak we might expect beneficial mutations to be more common, though the aggregate effect of multiple mutations is more difficult to predict, e.g., in the context of Fisher's Geometric Model (Martin and Lenormand 2006; 2008; Tenaillon 2014; Martin and Lenormand 2015). Fitness landscapes in the wild may be complex and variable, perhaps creating many opportunities for beneficial mutations. Nevertheless, we can simply consider whether any given genotype has had an opportunity to adapt to given fitness assay conditions prior to MA.

While having huge practical benefits, seed storage and cryopreservation may limit the opportunities of laboratory organisms to become well-adapted to laboratory assay conditions. As noted above, *Drosophila* are rarely cryo-preserved, and outbred strains have typically been maintained in relatively large laboratory populations for many generations. We might therefore expect strong adaptation to fitness assay conditions in this system, particularly in terms of early-life traits (e.g., Sgro and Partridge 2000; 2001). In general, experimental evolution studies maintaining large populations show that various model organisms can improve their fitness under standard lab conditions (e.g., Frankham and Loebel 1992; Lenski et al. 2001; Knoppel et al. 2018; Johnson et al. 2021). Strains used in MA experiments that were previously maintained in small populations or under very different conditions might be expected to have access to mutations that improve fitness in a novel assay environment because of their lesser degree of adaptation (Bondel et al. 2021). Additionally, model organisms are often genetically modified, e.g., to control reproduction or create selectable markers, and may have had little opportunity for the evolution of compensatory changes.

There are a few direct tests of how adaptive history influences the fitness effects of spontaneous mutations, along with several types of indirect evidence. Stearns and Fenster (2016) performed EMS mutagenesis on *A. thaliana* strains and showed a negative correlation between founder fitness and the relative fitness of the post-treatment lines. Although mutagenesis creates a different spectrum of mutation than spontaneous mutation, this serves as support for the idea that the "adaptedness" of any MA line founder can influence the fitness consequences of MA. If mutations are more likely to be beneficial in less evolutionarily optimized genotypes, then we would expect an eventual reduction in the rate of fitness decline under MA, as the effects of new beneficial and deleterious mutations become balanced. Silander et al. (2017) observed just such an effect in a bacteriophage where the decline in fitness plateaued. Work that is conscious of the effect of genetic diversity on the fitness effects of mutations has increased, including studies on the fitness effects of MA in different genetic backgrounds (e.g., Schaak et al. 2014; Kraemer et al. 2016), which demonstrate that these effects are variable. An MA study of haploid and diploid *S. cerevisiae* from the same genetic background (Sharp et al. 2018) found that mean fitness declined in diploid lines but not haploid lines, contrary to the expectation that haploids should suffer more due to recessive deleterious mutations. It is possible that the particular genetic background used in this experiment was more adapted to growth in the diploid form, but not the haploid form. In nature this species spends most of its time in the diploid life stage and laboratory experiments with haploid populations have previously found evidence that diploidy is beneficial (e.g., Gerstein and Otto 2011; Voordeckers et

al. 2015; Venkataram et al. 2016; Fisher et al. 2018; reviewed in Gerstein and Sharp 2020). The mutational spectrum differed between haploid and diploid MA lines in the Sharp et al. (2018) study, which somewhat confounds the comparison of their mean fitness. Additionally, selection in the haploid form against recessive alleles and the lack of masking of lethal alleles may have contributed to the result that was observed—in other studies of *S. cerevisiae*, sporulated MA lines showed no decline in fitness if recessive lethals were ignored (Joseph and Hall 2004; Hall et al. 2008). However, the haploid lines from Sharp et al. (2018) accumulated more mutations per base pair than diploids, and there were no indications of differential selection on mutations in haploids and diploids based on the molecular data.

More directly, a study in *D. melanogaster* disrupted 36 genes in strains that had previously adapted to two different stressful media environments (Wang et al. 2013). Each mutant strain was then tested in both the environment in which they adapted and in the alternative environment. The mean and variance of mutational effects depended on the assay environment, but not on adaptedness to the testing environment. A similar test used natural isolates of *A. thaliana* from across its native range. Weng et al. (2021) performed MA for 7–10 generations and then measured multiple traits at each natural site. MA lines showed greater reduction in fitness at "away" sites to which they had not been adapted than in their more familiar "home" sites.

At face value these studies do not seem to support the idea that diverse levels of prior adaptation can explain the variation in fitness decline observed in MA experiments. Instead, these experiments seem to reveal complex patterns, perhaps stemming from interactions between standing variation, new mutations, and environmental stresses. These studies also recapitulate other findings in their respective model organisms: little evidence for abundant beneficial mutations in *D. melanogaster* and a higher propensity towards it in *A. thaliana*. Given the relatively small set of mutations interrogated in the case of Wang et al. (2013), and the complex patterns of adaptation in the case of Weng et al. (2020), we suggest that it would be premature to discard the adaptedness hypothesis, and that more work on this question would be valuable.

Future Directions

It remains an open question whether one can truly predict the fitness outcomes of any given MA experiment. Many experiments show a decline in fitness, but this outcome is far from uniform and the reasons for that are still being actively investigated. To fully understand the range of outcomes of MA experiments we recommend three strategies.

First, it is important to consider the limitations and assumptions underlying MA studies in general and how those factors may come into play depending on the biological realities of the study organism. After the first studies suggested that fitness does not necessarily decline after MA (Vassilieva and Lynch 1999; Shaw et al. 2000; Shaw et al. 2002) some suggested that this could be due to the traits measured being under stabilizing selection rather than directional selection (Keightley and Lynch 2003; Shaw et al 2003). In such a case, any deviation from the mean, regardless of sign, would actually correspond to

a reduction in fitness. However, this suggestion has not been shown to be particularly relevant to the organisms they were directed at, *A. thaliana* and *C. elegans*, and the fitness metrics used in those studies continue to be used as fitness proxies. However, it is certainly possible for traits in a given organism to be under real or apparent stabilizing selection, and experimenters should carefully consider the life history of each study organism. Further, while MA is arguably the best available strategy for studying natural mutations, the resulting spectrum of mutations observed may still be influenced by selection to some extent, including within-individual selection (Otto and Orive 1995; Wei et al. 2019). We can think of within-individual selection as biasing the inheritance of new alleles in some organisms and not others, with the potential to contribute to variation in the outcomes of MA. Studies looking at mutation spectra following MA studies, however, have often found no evidence for somatic selection at least in *A. thaliana* (Monroe et al. 2022). An analogous challenge arises for studies of microorganisms, with colonies representing small populations with some opportunity for selection. It will be interesting to compare the effects of MA using the traditional approach versus the use of microfluidics to separate cells upon division (Kobel et al. 2010), maintaining truly small populations. Researchers should continue to develop and apply statistical methods to correct for potential biases due to selection (Mahilkar et al. 2021; Wahl and Agashe 2022).

Second, we would like to encourage experimenters to consider the specific adaptive history and genetic background of the founder lines and how that might affect the results and interpretation of MA. Microbes in particular spend much of the time in stasis, with little opportunity to adapt to the assay conditions to which they will later be subjected. While it may not always be feasible to give organisms time to adapt to their assay conditions, it remains important that results are interpreted in the light of this. More experiments that are designed to directly test the idea that adaptedness can impact the fitness effects of mutations would be particularly valuable. If beneficial mutations are indeed more likely for poorly adapted genotypes, MA should produce a less severe decline in fitness. Work like that of Silander et al. (2017) which sought to characterize the fitness effects of mutations in extremely unfit phenotypes, while difficult, could be performed in other organisms to perhaps get a finer understanding of how the fitness landscape behaves far away from the fitness optimum.

Additionally, organisms may have specific properties that affect the results we see from MA. In *S. cerevisiae*, for example, losses in fitness may be due to phenotypes created by aneuploidy (Joseph and Hall 2004; Hall et al. 2008, Sharp et al. 2018), a type of mutation we wouldn't expect to accumulate in many other model organisms; species likely differ in both the overall spectrum of mutation types and in the fraction of these mutations that are effectively neutral under MA. Another possibility is that the DFE itself could evolve to reduce the rate of mutation to deleterious alleles. Recent work in *A. thaliana* using data from MA lines, polymorphism and divergence finds that mutations are biased against areas under purifying selection and toward those under positive selection (Weng et al. 2019; Monroe et al. 2022). This astounding result lines up with previous work in the system showing epigenetic regulation of DNA repair mechanisms to preferentially protect coding regions from mutation (Belfield et al. 2018) and provides a convincing explanation for why studies of *A. thaliana* tend to find little decay in mean fitness under MA. Biases in DNA repair

activity and mutation have also been observed in tumors and human cell lines (Frigola et al. 2017, Supek and Lehner 2017, Huang et al. 2018), but the presence of such effects in organisms like bacteria is debated (Martinocera et al. 2012, Chen and Zhang 2013). Further studies combining molecular information on the (epi)genomics of DNA replication and repair coupled with fitness measures, along with theoretical model development, would help to establish the role of selection in determining the rate of deleterious mutation, and the causes of variation in these patterns among species. There also remains a need for further development of theoretical models addressing the DFE under non-equilibrium scenarios, particularly when environments fluctuate (e.g., Mustonen and Lassig 2009, Bataillon and Baley 2014).

Lastly, while MA can be informative about the aggregate effect of mutations on fitness, it provides much less insight into the impact of any specific mutation. To combat this, it is important to combine MA techniques with genomic sequencing and other methods that can allow a more fine-tuned analysis of individual mutations. The combination of fitness measures and genome sequencing for the same sets of MA lines, sometimes combined with crossing methods, is a promising way to understand genotype-phenotype connections (e.g., Shaw and Chang 2006; Rutter et al. 2012; Kraemer et al. 2017; Bondel et al. 2019; Sandell and Sharp 2021; Bondel et al. 2021). Mutation accumulation is a useful technique that has been applied for decades, and continues to be valuable in combination with other inference methods; these novel approaches hold promise for understanding the complex and sometimes unpredictable impacts of spontaneous mutations on fitness.

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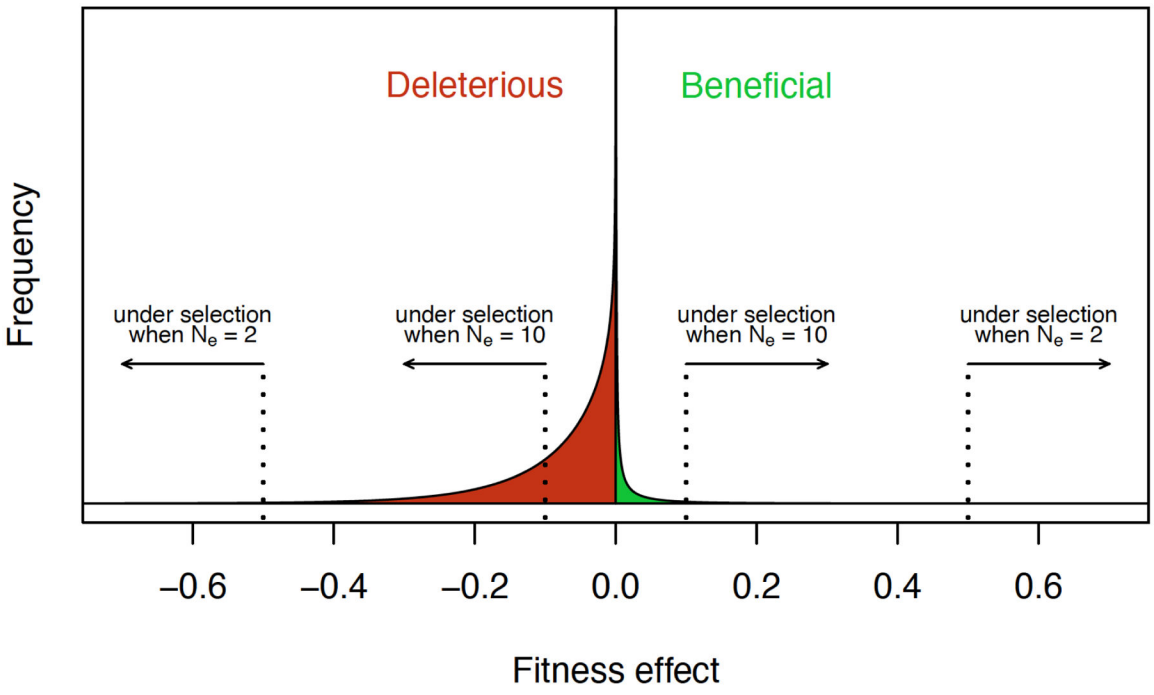

Figure 1. Hypothetical distribution of mutational effects.

We often expect deleterious mutations (negative fitness effects) to appear more frequently than beneficial mutations (positive fitness effects). Available evidence suggests both types of mutations may have skewed distributions, where weak effects are common and strong effects are rare. Under MA, the effective population size (N_e) will determine which mutations will behave neutrally. As N_e increases, a larger fraction of mutations will be subject to effective selection, i.e., deleterious (beneficial) mutations would be less (more) likely to fix than the neutral expectation.

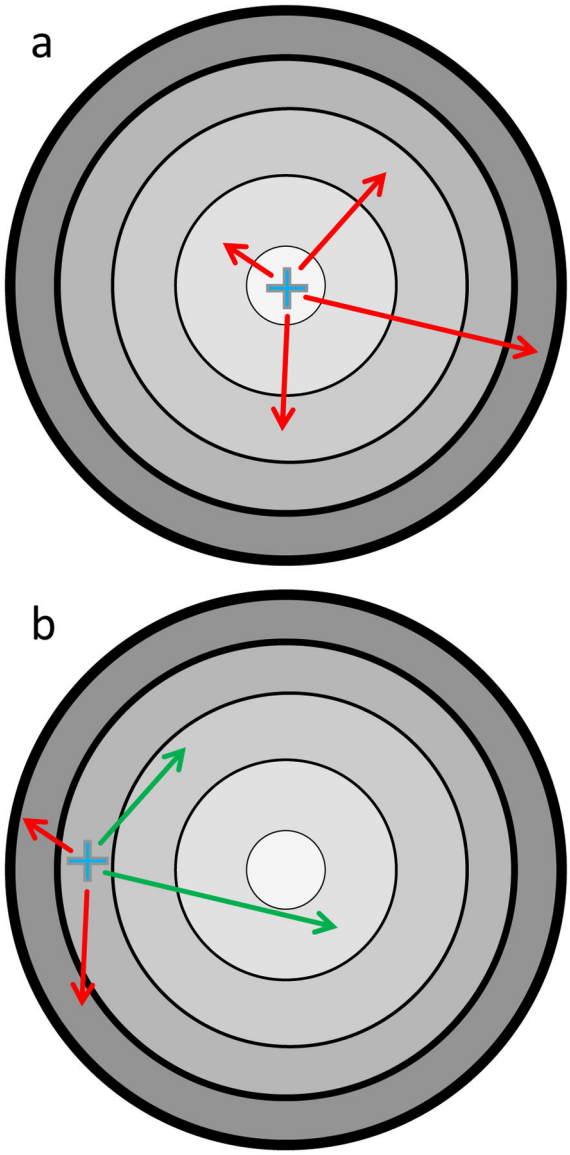

Figure 2. The fitness landscape concept.

Lighter shades indicate higher fitness. (a) In a well-adapted genotype (+ symbol) we expect the vast majority of mutations to be deleterious (red arrows). (b) In a poorly adapted genotype, the same genetic changes are more likely to be beneficial (green arrows).