Review

# Environmental duress and epistasis: how does stress affect the strength of selection on new mutations?

**Aneil F. Agrawal[1] and Michael C. Whitlock[2]**

[1] Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON, Canada M5S 3B2
[2] Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

**To an evolutionary geneticist, the most important property of a new mutation is its effect on fitness. Stress is a reduction in fitness that can also alter the selection on new mutations. Although the effects of environmental and genetic stresses are typically studied separately, it is useful to consider them from the same perspective. Here we evaluate the common perception that stress increases selection. We consider various conceptual paradigms for thinking about selection and stress, and then review the empirical data. We reject the notion that stress typically increases selection. Instead, we find that different types of stresses affect selection differently, though the underlying mechanisms are, as yet, unclear in most cases.**

## Selection and stress

The effects of stress on selection matter for a wide variety of reasons. Stress may change the direction of selection on some mutations, with obvious ramifications for the evolutionary fates of those alleles. More typically, stress will alter the strength of selection. If selection becomes stronger (and therefore more effective) in stressful conditions, then the ability of organisms to adapt to changing environments or to new environments is greater than we might expect from studies typically conducted under benign conditions. If stressful environments increase selection, then predictions of mutation load, inbreeding depression, or the genetic variance in fitness may be skewed. An unfit genetic background can be considered a genetic stress. The effects of genetic stress on mutations at other loci (i.e. epistasis) make large differences to the predictions of various evolutionary processes, such as the evolution of sex [1,2] and the evolution of inbreeding depression [3]. If we can predict changes in the nature of selection as a function of measureable properties of the environment and previous mutations, we could make evolutionary genetics a more predictive science.

Following other authors [4,5], we define a 'stressful' context (genetic or environmental) as one in which the absolute fitness of the wild-type is reduced relative its absolute fitness in some other reference context. Changes in the environment such as a reduction in food abundance, an increase in natural enemies, or exposure to extreme temperatures or problematic chemicals (e.g. salt, heavy metals) are stressful for most organisms. A genetic background containing deleterious alleles can be considered to be a stressful genetic context relative to a mutation-free background. For genetic and environmental stresses, we would like to know how stress alters selection on new mutations. We will conclude that genetic and environmental stresses are conceptually similar and that there is little evidence that either type of stress typically increases selection.

In this contribution, we will use $s$ to refer to the selection coefficient against a deleterious allele. More precisely $s$ is the proportional reduction in fitness caused by the presence of that allele. The studies we collate are mixed between haploid and diploid organisms. In the latter, the effects of the allele are measured in the homozygous or heterozygous state, depending on the study. We will use $s$ to refer to the effects of these mutations however they are expressed in a given study.

Most non-neutral mutations are deleterious [6], but stress can change the fraction of mutations that are deleterious, neutral, and beneficial ($p_{del}$, $p_{neu}$, and $p_{ben}$, respectively). This is an issue of major importance but one for which the data are quite limited (but see [7,8]). Many studies that we consider examine the aggregate effects of multiple unknown mutations, for example, by examining the fitness of lines exposed to mutagenesis. Data from such studies can be used to ask how stress changes mean selection over all mutations, $\Delta E[s]$, and, in some cases, the variance in selection, $\Delta V[s]$, but not the finer-scale issues stated above (i.e. $\Delta p_{ben}$, $\Delta p_{neu}$). Even studies that examine selection on individual mutations typically have limited power to distinguish between changes in the abundance in each class ($p_{del}$, $p_{neu}$, and $p_{ben}$) versus the changes in the average effect size in each class. This is because of the difficulty of statistically differentiating among weakly deleterious, neutral, and weakly beneficial effects. Thus, for our review, we will focus on how stress changes the mean and variance ($\Delta E[s]$ and $\Delta V[s]$), while recognizing that measures of stress-induced changes in the proportion of beneficial mutations ($\Delta p_{ben}$) are a critical issue for future studies. If $p_{ben} \ll p_{del}$ in benign and stressful environments, then $\Delta E[s]$ and $\Delta V[s]$ can be thought of as resulting primarily from changes in the magnitude of selection against deleterious alleles.

## Why might stress increase the strength of selection?

Numerous authors have remarked on the conventional wisdom that selection is stronger under stress [9–12].

*Corresponding author:* Agrawal, A.F. (a.agrawal@utoronto.ca).


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There are several reasons that may lead us to expect that fitness differences may increase in more stressful contexts. First, organisms may have margins of safety in physiological functions with excess capacity for these functions under normal conditions. A typical individual may therefore not be affected by minor genetic problems (i.e., mutations) under otherwise good conditions, but under worse conditions genetic problems may further stress its capacity.

Second, biological organisms are buffered from assault by various physiological responses and/or repair systems (e.g. healing by regeneration or repair, immune response, heat-shock chaperones, DNA repair systems). Some mutations may affect these genetic or somatic repair mechanisms. Such mutations affecting repair functions on their own may show little fitness effects but, if the organism is subjected to a further environmental or genetic problem, the effects of that problem may be more manifest in the absence of repair. If these systems are not fully functioning (e.g. repair capacity is reduced with age), the consequences of a subsequent injury can be severe. By extension, a stress that compromises such buffering systems will result in stronger selection against any additional mutation. This scenario seems plausible for some situations, but it seems unlikely that all (or even most) stresses result in compromised buffering systems.

Third, some stressful environments affect the ecological relationships between individuals in a way that can exacerbate the fitness differences between those individuals. If a deleterious mutation affects the rate at which an organism gathers resources, relatively healthy individuals can gather resources or grow faster than the less healthy, leaving few resources behind. This idea has been discussed explicitly in the context of the effect of density on the variance in fitness in plant populations [13,14]. The 'dominance and suppression' model of plant growth postulates that small differences in initial size become exaggerated over time as larger plants deprive smaller ones of access to light and nutrients. A similar situation happens in a typical fly culture. As eggs hatch and the larvae develop, food is consumed and waste products produced. High-quality individuals with elevated growth rates reduce the availability of food and contaminate the medium, making the environment a harsher place for slower-developing individuals [15,16]. Under this ecological model, limiting resources coupled with scramble competition and/or degradation of the shared environment means that slightly more fit individuals have access to many more resources and so effectively experience better environments than slightly less fit individuals. As a result, realized fitness may differ far more in high-density conditions than in low-density conditions. By this mechanism, less fit individuals experience a worse environment than more fit individuals as a result of the changes in the environment caused by competition.

Finally, previous selection in benign environments (in nature or during experimental protocols) will reduce the frequency of alleles that are particularly bad in the benign environment. Stressful environments are often also novel environments, which have not often been experienced during the evolutionary history of the population. If so, the worst alleles in the normal, benign environment have been eliminated or reduced to low frequency by selection, but a similar process would not have removed the worst alleles in the novel environment. As a result, we might expect that standing genetic variation for fitness would be greater in a novel environment [17,18].

The logic in the previous paragraph applies only to standing genetic variation, but not to the effects of new mutations. Our focus in this contribution is on the effects of stress on new mutations, so we will not discuss this further except to note that this creates a potential bias in investigating the effects of new mutations because of unavoidable selection in some experimental protocols. There is good reason to expect the patterns for standing variation to differ from those for new mutations, so we must be particularly alert to the nature of the genetic variation being studied when reading the literature.

## Stress should have variable effects on selection

We have outlined above some reasons underlying the common intuition that stress increases selection, but we have also noted that these scenarios are not universal. There are other conceptual frameworks that lead one to believe that stresses may increase or decrease selection. One such framework is the 'fitness-as-flux' perspective in which fitness is viewed as being proportional to the flux through some pathway. Theoretical analyses of metabolic pathways and biological networks show that a genetic stress (the first mutation) can increase or decrease selection on a subsequent mutation (i.e. negative or positive epistasis) depending on the structure of the network and the relative positions of the two mutations within these pathways [19–22]. Depending on the position of the initial mutation within the pathway, average selection on subsequent mutations could be stronger or weaker than in the absence of the genetic stress (i.e. the initial mutation). That is, it is difficult to make a universal prediction about the sign of average epistasis between a focal mutation and subsequent mutations.

An environmental stress may alter which pathways in a network are the most important in determining fitness, thereby making selection stronger on some genes but weaker on others. The net effect of stress on new mutations may depend on the mutational target size of each affected step. That is, do the pathways that experience increased selection under stress involve more genes than the pathways that are most strongly selected under benign conditions?

Perhaps the best-known framework for thinking about selection is the fitness landscape metaphor [23–25], which provides a map between phenotype and fitness (Figure 1). From the landscape perspective, we expect heterogeneous responses to stress. An environmental change may cause the landscape to change in three (non-mutually exclusive) ways: (i) change in the location of the optimum; (ii) change in the height of the optimum; and (iii) change in the shape (curvature) around the optimum. In contrast to the intuition that selection increases mean selection, Martin and Lenormand [5] showed that there is no change in the average selection against new mutations ($\Delta E[s] = 0$) if environmental stress causes a change in the location of the optimum. However, other forms of change in the land-

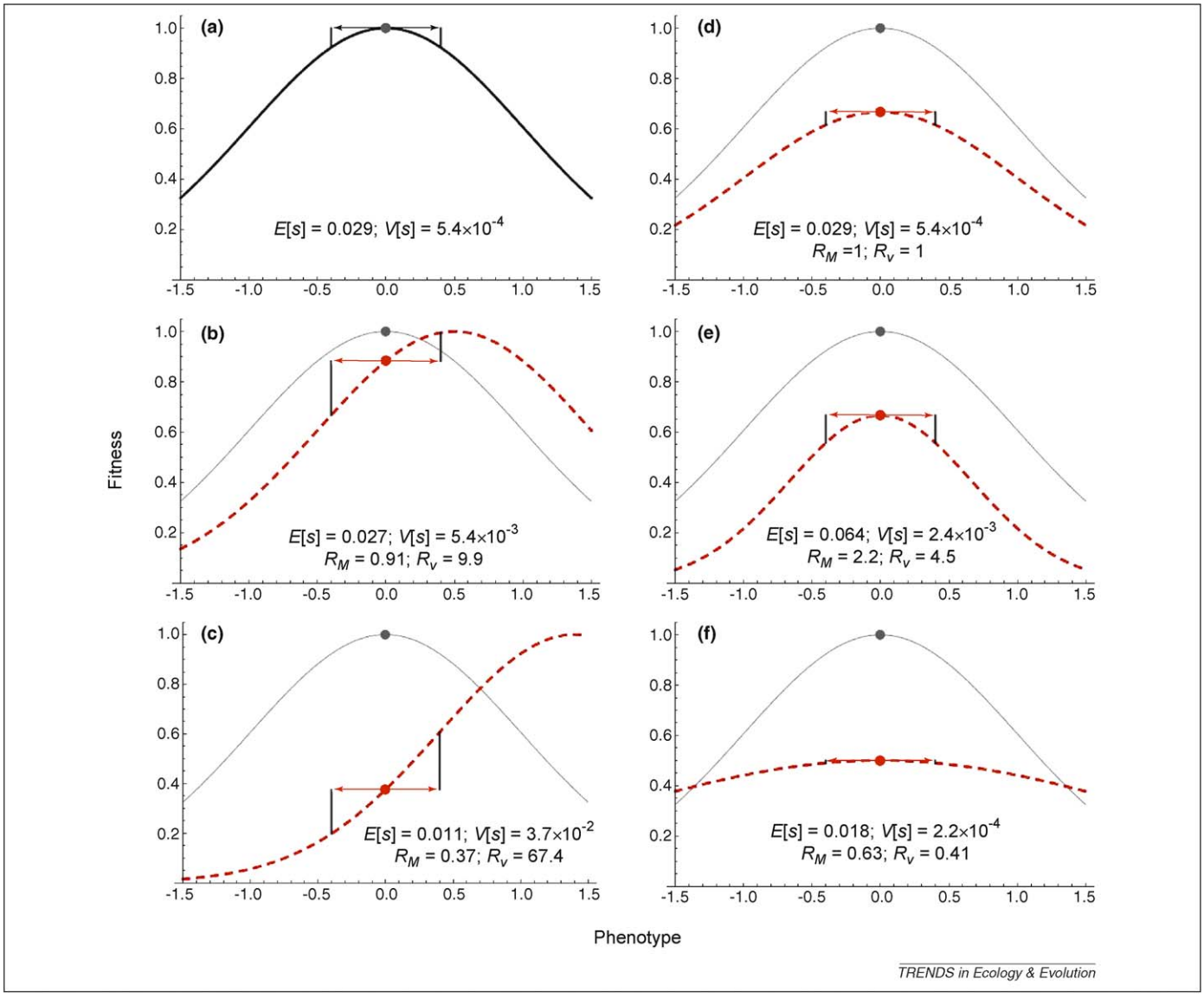


**Figure 1**. Fitness landscapes and stress. In each panel, fitness is shown as function of the phenotype. Mutations are envisioned as deviations (arrows) from the reference genotype (point). **(a)** In the benign environment, the population has the optimal phenotype and has high fitness. In the remaining panels, environmental stress is represented as a change in the landscape (red dashed line) that results in a reduction in absolute fitness; these figures represent only a sample of an infinite number of ways stress could be represented in the landscape framework. In **(b)**, stress is envisioned as a small shift in the optimum. This is the scenario emphasized by Martin & Lenormand [5] in their landmark study using landscapes to predict changes in selection under stress. In **(c)**, there is a larger shift in the optimum. In **(d–f)**, stress is an environmental change that reduces the maximal fitness but does not change the optimum. In **(d)**, fitness of all phenotypes is reduced by a constant proportion. In **(e)** the surface is shrunken to maintain a similar shape. In **(f)**, the surface has been flattened. Using a three-dimensional version of each of these scenarios, we calculated the mean and variance of selection ($E$[s] and $V$[s]) on new mutations that were assumed to be normally distributed perturbations to the reference phenotype. A three-dimensional version was selected because two very different recent approaches have suggested that the effective number of dimensions is quite small [24,69]. For each stressful environment, we have calculated the ratio of the mean and variance in the stressful environment relative to that in the benign environment; $R_M = E[s_{stress}]/E[s_{benign}]$ and $R_V = V[s_{stress}]/V[s_{benign}]$. Some types of stress cause little or no change in mean selection **(b, d)** where as others cause substantial increases **(e)** or decreases **(c, f)**. Similarly, different types of stresses can increase **(b, c, e)** or decrease **(f)** the variance or have no affect at all **(d)**.

scape can have positive or negative effects on the mean of $s$ (Figure 1).

The landscape model can also be used to examine the effects of genetic stress. Martin et al. [26] showed that the landscape model predicts that epistasis will be common but that positive and negative interactions occur, so the average epistasis is close to zero. In this model, mutations are represented as random vectors that displace the carrier from the position of the wild-type in phenotypic space, thus altering its fitness. The direction of the second mutation is random with respect to the direction of the first, so their fitness effects when combined can be greater or less than expected based on their individual effects.

This latter concept may be useful beyond the landscape model and apply to environmental or genetic stresses. The effects of a new mutation are 'random' with respect to whichever aspect of organismal function is being strained by a genetic or environmental stress. As a result, there may be no effect of stress on average even though the stress will certainly interact with the effects of some mutations. If we are considering a subset of mutations that are non-random with respect to the stress then it will be easier to make predictions. For example, genes involved in osmoregulation will be under strong selection under salt stress. Similarly, selection will be stronger on a mutation if it affects the same part of a metabolic pathway that already harbors

a mutation [21,27]. Another example is dominance. Two mutant alleles at the same locus clearly affect the same function and thus tend to have synergistic effects on fitness. In contrast to the very mixed data about average epistasis (see below), there is strong evidence that deleterious alleles tend to be recessive [28–30].

## Empirical tests of stress on mean selection

Based on many divergent predictions about how stressful contexts might affect the strength and pattern of selection, we reviewed the literature on the effects of environmental or genetic stress on the strength of selection.

We have compiled the results of many studies that compare the fitness effects of mutations in benign and more stressful contexts. First, let us examine the effects of deleterious mutations as a function of the quality of the environment. We have divided studies on this topic into two groups. The first group of 31 studies (presented in Table 1) examines environmental stressors such as suboptimal temperature, chemical toxins, or low food quality.

**Table 1. Studies measuring selection using a non-density form of stress. The percentage changes in mean selection are intended as heuristics. In most cases, they are calculated from mean fitness estimates of mutants and wild-types reported in the original articles (ignoring measurement error) because estimates of average *s* were typically not provided. Where appropriate, growth rate estimates were converted to fitness based on the equation $W = e^{rt}$ and using $t = \ln(2)/r_{wild\text{-}type}$ to scale to generation time. An asterisk (*) indicates that stress was shown to reduce fitness. A double asterisk (**) indicates a statistically significant effect of stress on selection. 'MA' denotes a mutation accumulation experiment; 'MCN' denotes a middle class neighborhood experiment. [1] In this study, selection was significantly stronger in 45 of 216 cases, whereas it was significantly weaker in only 14 cases. [2] Negligible effect of mutations in the benign environment. [3] Mutations selected on basis of having fitness effect in standard conditions (YPD). [4] Temperature effect is more pronounced in *C. elegans* than in *C. briggsae*.**

| Organism | Mutant type | Fitness measure | Stress | Change in selection with stress | Source |
|---|---|---|---|---|---|
| *E. coli* | 65 lines created by mutagenesis | Growth rate (non- competitive) | Acid* | Stronger (+9%) | [31] |
| | | | High osmolarity* | Weaker (–9%) | |
| | | | Dithiothreitol* (reducing agent) | Weaker** (–23%) | |
| | | | Trimethoprim* (antibiotic) | Weaker** (–59%) | |
| | | | Chloramphenicol* (antibiotic) | Weaker** (–41%) | |
| | | | Cold temperature* | Weaker** (–39%) | |
| | | | Standard media* (relative to enhanced medium) | Weaker (–3%) | |
| *E. coli* | 26 insertion mutations | Growth rate relative to competitor | New food source (maltose rather than glucose) | Stronger** (NA) | [50] |
| | | | Low temperature | Stronger | |
| *E. coli* | 216 lines each containing 1, 2 or 3 insertions | Growth rate relative to competitor | Intracellular parasite* | Stronger**,[1] (+77%) | [41] |
| *S. cerevisae* (haploid) | Mutation accumulation via mis-match repair | Maximum growth rate (non-competitive) | Low temperature* | Weaker (–7%) | [52] |
| | | | High temperature* | Stronger (+74%) | |
| | | | Reduced food quality* (minimal media) | Weaker (–24%) | |
| | | | Reduced food quality* (YGP) | Stronger (+7%) | |
| *S. cerevisae* (diploid) | Mutation accumulation via mis-match repair | Maximum growth rate (non-competitive) | Reduced food quality* (minimal media) | Weaker (–31%) | |
| *S. cerevisae* (diploid) | Mutation accumulation via mis-match repair | Growth rate (non-competitive) | High temperature* | Stronger[2] (+7358%) | [53] |
| *S. cerevisae* (haploid) | 526 single deletion lines and 263 double deletion lines[3] | Growth rate | High temperature* | Stronger (+2%) | [9] |
| | | | Reduced food quality* (minimal media) | Weaker** (–39%) | |
| | | | Caffeine* | Weaker** (-20%) | |
| | | | Salinity* | Weaker** (–53%) | |
| *R. raphanistrum* | Two populations that accumulated mutations via MCN design for 9–10 generations | Total seeds produced | Field (relative to greenhouse) | Stronger (+183%) | [54] |

**Table 1 (*Continued*)**

| Organism | Mutant type | Fitness measure | Stress | Change in selection with stress | Source |
|---|---|---|---|---|---|
| *C. elegans* | 73 lines tested after 163 and 214 generations of MA | Intrinsic rate of increase | Low temperature* | Stronger** (+75%) | [55] |
| *C. elegans* | 68 lines tested after ~200 generations of MA for each of two progenitor strains | Lifetime reproduction | Low temperature* | Mixed (–16%, +27%) | [56] |
| *C. briggsae* | As above | Lifetime reproduction | Low temperature[4] | Stronger (+3%, +30%) | |
| *D. melanogaster* | Single tumor-inducing mutation | Survival to third instar | Temperature | Stronger (+117% for cold, +95% for hot) | [57] |
| *D. melanogaster* | Two MA lines compared with control line | Competitive productivity assay (fecundity, survival assay) | Food quality* | Stronger** (NA) | [10] |
| *D. melanogaster* | 30–38 lines tested after 27–35 generations of MA for each of two progenitor strains | Viability relative to competitor | Low temperature | Stronger (+300%, +28%) | [58] |
| | | | Ethanol | Stronger (+270%, +22%) | |
| *D. melanogaster* | EMS mutagenesis | Productivity (fecundity and viability) relative to competitor | Food quality | Weaker (–22%) | [59] |
| *D. melanogaster* | 20 visible mutations | Egg-to-adult viability | Food quality* | Stronger (+28%) | [42] |
| *D. melanogaster* | 8 visible mutations | Egg-to-adult viability | Bacterial infection | Stronger** (13%) | [60] |

These studies present a similar environmental challenge to all individuals in the stressed treatment within a study. As a result, these studies emphasize the intrinsic mechanisms of interaction between the mutation and the environment, with fewer effects caused by ecological competitive interactions between individuals. The second group of eight studies (reported in Table 2) used high density as the environmental stressor. For this group of studies, the ecological mechanism of density-dependent competition should be stronger because at high density those individuals that can first access resources may do disproportionately well and leave their slower companions in a different, resource-depleted environment.

For the non-density stressors, we find that mean selection is weaker in the stressful environment in 13 out of 31 studies (Table 1), including several of the largest studies [9,31]. This offers no support for the hypothesis that stress increases the strength of selection against new mutations on average. Moreover, the pattern of change in mean $s$ is highly heterogeneous; several studies report strong increases in selection with stress whereas others report large decreases in selection. There is a tendency in the data for single-celled organisms to show weaker selection under stressful conditions, and the opposite for multicellular organisms. However, the available data are insufficient to make a strong claim on this issue.

Although there is little consistency in the interaction between mutation and non-density environmental stresses, there is some evidence that ecological interactions between individuals of different genotypes usually increase the strength of selection against deleterious mutations. In studies conducted at varying density, seven of eight studies found increased selection at high density (Table 2). We conjecture that this increased frequency of stronger selection with high density may result from the ecological interactions described above, but this should be tested explicitly in future studies. With the set of available studies, it is not possible to rigorously statistically isolate any one factor as being a good predictor of whether stress will increase or decrease selection because taxa, mutation type, stressor and type of fitness assay are largely confounded.

Another source of related data comes from studies of inbreeding depression, which is a measure of the strength of selection against inbred genotypes relative to outbred genotypes. Armbruster and Reed [32] reported that 74% (39 of 53) of the cases that they reviewed showed higher inbreeding depression under stress. However, there are several reasons why inbreeding depression does not necessarily provide a good measure of how stress alters selection against new mutations. First, inbreeding depression is dependent upon standing variation. It is therefore expected to show a stronger relationship with stress if stressful environments are also novel environments. Indeed, many of the studies examined by Armbruster and Reed [32] involve stressful environments that are also novel environments. Second, inbreeding depression is more strongly dependent upon the degree of dominance $h$ than on the strength of selection $s$. Changes in $h$ rather than $s$ may underlie stress-induced changes in inbreeding depression. Third, inbreeding depression may be partly due to genes involving overdominance rather than due to partially recessive deleterious mutations, and such alleles are not typical of new mutations. Similar to what we

**Table 2. Tests of selection that used increased density as a stress. The study by Lewontin and Matsuo [61] used *Drosophila busckii*, all of the remainder used *D. melanogaster*. An asterisk indicates statistically significant effect of stress. [1] See [62] for comments on estimation. [2] Relative viability of mutants declines with density in 3 of 4 cases.**

| Mutant type | Fitness measure | Change in selection with stress (percent increase in selection) | Reference |
|---|---|---|---|
| Single tumor-inducing mutation | Survival to third instar | Stronger* (+10131%) | [57] |
| Two MA lines compared with control line | Competitive productivity assay (fecundity, survival assay) | Stronger* (NA) | [10] |
| 30–38 lines tested after 27–35 generations of MA for each of two progenitor strains | Viability relative to competitor | Mixed (–66%, +113%) | [58] |
| Population that accumulated mutations via MCN design for 30 generations | Productivity relative to competitor | Stronger[1] (+1102%) | [63] |
| EMS mutagenesis | Productivity relative to competitor | Stronger (+20%) | [59] |
| 4 visible mutations (unclear if tested on common background) | Larval viability | Stronger[2] (NA) | [61] |
| One visible mutation (*e*, unclear if tested on common background) | Egg-to-adult viability | Stronger* (+1380%) | [64] |

discuss here for new mutations, almost all of the studies examined by Armbruster and Reed [32] that clearly manipulated the competitive context find stronger inbreeding under competitive conditions (e.g. [33–37], but see [38]).

As with environmental stress, the effects of genetic stress on new mutations are highly heterogeneous. Sanjuan and Elena [39] reviewed the literature on epistasis. They found that some studies found significantly positive average epistasis, some studies found significantly negative average epistasis, and most found no significant epistasis. With the possible exception of viruses, average epistasis is usually close to zero even if it is statistically significantly different from zero. This is highlighted by the recent yeast study of Jasnos and Korona [40] that examined a large number of lines containing 0, 1, or 2 deletions. This study had considerable statistical power and could therefore detect significant positive average epistasis. Nonetheless, it is clear from their histogram of epistatic effects that mean epistasis in yeast is slightly positive but very close to zero and that there is high variance around this value. Martin et al. [26] found a similar result with respect to epistatic interactions in *Escherichia coli*. Overall, epistasis between deleterious genes can be strongly positive or strongly negative, but there is little evidence that the average epistatic effect is very different from zero. Moreover, claims of a relationship between genomic complexity and the nature of epistasis may be premature (see Box 1).

## Variation in the effects of stress on selection

Looking across studies of genetic and environmental stress, it is clear that the effects of stress on selection vary in several ways. Unsurprisingly, there are plenty of examples within studies in which a particular environmental stress increases selection on some genes but decreases it on others [31,41,42]. Likewise, within studies of epistasis, there are cases in which a given mutant background alleviates selection at some loci but increases it for others [43,44]. There is some evidence that a gene will have different types of epistatic interactions with functionally linked genes compared with unlinked genes [45]. To our knowledge, no one has quantitatively tested a large number of random mutations against a series of different mutant backgrounds to determine if some particular mutant backgrounds tend to increase selection on average and others tend to reduce it. In a qualitative assay, Tong et al. [46] tested each of 132 gene deletions against a set of 1007 other deletions. There was high variability among the 132 focal genes in the frequency with which they had strong negative interactions with other mutations. Moreover, it is easy to imagine that mutations affecting buffering systems such as *Hsp90* [47] would be likely to aggravate average selection to a greater extent than other types of genetic stresses.

One important scenario makes it clear that we often will expect heterogeneous responses to stress. By definition, if a population consists of the optimal genotype (i.e. the population is well matched to its environment), all mutations are deleterious. Any time a population is displaced away from the optimum because of environmental or genetic stress, there is an increased opportunity for beneficial mutations [23]. There is some good empirical evidence that the effects of beneficial mutations increase after fixation of deleterious mutations [48,49] or under environmental stress [50,51]. Thus, we might expect stress to increase the variance in selection coefficients among loci. Such a prediction is a formal outcome of the landscape model by Martin and Lenormand [5]. In their survey of nine mutation accumulation experiments in which fitness assays were undertaken in multiple environments, Martin and Lenormand [5] found that, though the effects of stress on the mean were quite mixed, there was a much more consistent pattern for the variance to be higher under stressful conditions.

Different environments and different taxa may show different levels of interaction between stress and the strength of selection. Among the studies of environmental stress (Tables 1 and 2), there are cases in which stress increases average selection and other cases in which stress decreases it. In many of these cases, the effect of stress is

**Box 1. Does epistasis correlate with genomic complexity?**

Sanjuan and Elena [39] reviewed 21 studies of epistasis for fitness. Five of these studies (one each from an RNA virus [65], *E. coli* [66], yeast [67], *Aspergillus* [43], and *Drosophila* [68]) had sufficient data to allow them to calculate the epistasis of pairs of deleterious mutations. When Sanjuan and Elena plotted the average epistasis of each species against the species genetic complexity, they found a tight linear relationship between the two. The most complex species (*Drosophila*) had negative epistasis (meaning that the combination of two deleterious alleles was on average even worse than expected), whereas the RNA virus had significantly positive epistasis (meaning that two bad alleles together was better than predicted from the single mutation effects). Support for such a relationship seemed to be reinforced by qualitative information from various other studies.

In the few years since this review, however, other data on the same taxa have been collected which sometimes give different results from those available earlier. For example, new data on *Drosophila melanogaster* on the epistatic interactions between deleterious dominant mutations show that the epistatic interactions tend to be positive, not negative, for this species [42], and a very large study of yeast showed slight antagonistic epistasis [40]. If these new data and insights are accounted for, the significance of the relationship between genomic complexity (as defined in [39]) and the sign of the average epistasis is much weakened ($P = 0.033$, Spearman's $\rho$ based on $n = 5$ species; Figure I). Given how uncertain and sensitive to experimental details most of the estimates of epistasis are (as revealed by the strong changes in estimates on the same taxa by these new studies), this result must be viewed with some caution. Moreover, viruses may behave differently from other organisms (due to their unique life-cycle and drastically simplified genome structure), and excluding the virus data from this analysis leaves no evidence for a broader relationship between complexity and epistasis ($P = 0.17$). More data are needed if this conjecture is to be rigorously tested.

One other caveat should apply to any interpretation of almost all of the available studies of epistasis with deleterious alleles. In all but a very few cases (e.g. [65,68]), the experimental designs of these studies allowed some opportunity for particularly bad genotypes to be removed or reduced in frequency by some natural selection. As a result, most studies of epistasis are biased towards finding estimates of the interactions between loci that are too positive.

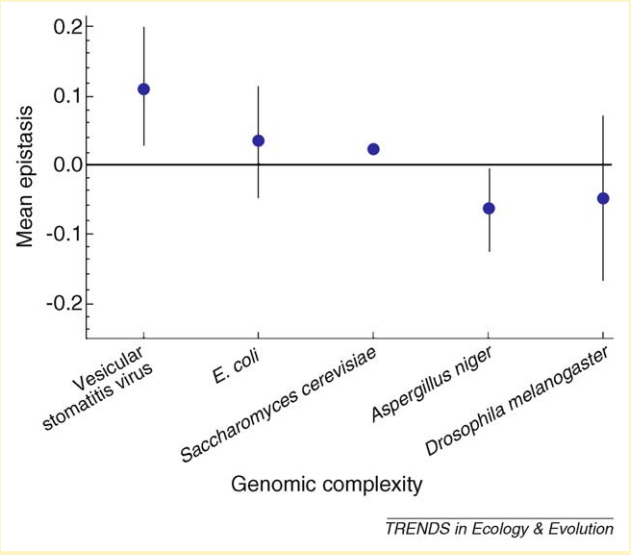


**Figure I**. Genomic complexity (as used in [39]) and the mean sign of epistasis are poorly correlated (if at all). The mean epistatic coefficient, expressed as the mean deviation from a multiplicative interaction between a pair of deleterious alleles, is near zero for a wide diversity of taxa. Error bars show the 95% confidence intervals of the mean for each species. The relationship between genomic complexity and the mean epistatic coefficient is significant (Spearman's correlation, $P = 0.033$). (Note that here we use the independent units of species in the analysis, rather than inappropriately treating the gene pairs as independent.) The point for yeast is a weighted average of 48 comparisons from [67] and 639 estimates from [40]. The point for *Drosophila* is a weighted average of six comparisons not involving beneficial mutations from [68] and eight estimates from [42].

statistically significant and so cannot be attributed to measurement error. That is, several studies do not seem to fit the theoretical prediction of Martin and Lenormand [5] that stress has no effect on the average strength of selection. Rather, some types of stresses appear to increase selection whereas other types of stress decrease it.

Even though it appears that some stresses alleviate average selection and others aggravate it, in most cases we do not understand why these effects occur. For example, why do chemical stresses often alleviate selection in bacteria and yeast (Table 1)? Developing hypotheses and testing them remains an important challenge for understanding different classes of stresses. Competitive stress seems to increase the difference between mutations. For this observation we have evidence of a pattern, a plausible mechanism by which it could work, and some evidence of its operation by way of experiments showing that individuals with a slight initial advantage tend to do disproportionately well under high density [13–16]. However, formal tests of this pattern and empirical testing of the mechanism using new mutations are lacking. From the genetic viewpoint, a plausible mechanism exists for how a genetic stress affecting a buffering system such as an *Hsp90* mutant background could increase selection, but in this case we are lacking the explicit fitness assays to show that it does so. Conversely, Jasnos et al. [9] found that random genetic stresses tended to alleviate selection as did environmental stresses, leading to the suggestion that both types of stresses operate via a common mechanism. Determination of this mechanism would greatly enhance our understanding of selection.

## Conclusions

The generic effects of environmental or genetic stress may be understood in similar ways, but the specific nature of the effects of stress must be investigated in each special case. There is neither a compelling theoretical reason nor empirical support for the common intuition that stress, broadly defined, makes selection stronger. In most cases, a given stress probably makes selection stronger on some mutations but weaker on others. Some stresses probably have no average effect on selection whereas other stresses may cause an average increase or an average decrease. One of the biggest challenges is identifying different classes of stresses that affect mean selection and elucidating the mechanisms by which these effects occur. Another major challenge is to move beyond average effects. This involves obtaining good estimates of selection on many individual genes in multiple environments. Such data will allow us to better study changes in the variance in selection and understand the extent to which selection changes in magnitude versus sign when under stress.

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