Journal of Biology

BioMed Central

Open Access

Research article

# Environmental stresses can alleviate the average deleterious effect of mutations

Roy Kishony and Stanislas Leibler

Address: Laboratory of Living Matter, Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

Correspondence: Stanislas Leibler.

Published: 29 May 2003

*Journal of Biology* 2003, **2:**14

The electronic version of this article is the complete one and can be found online at http://jbiol.com/content/2/2/14

Received: 13 December 2002
Revised: 17 April 2003
Accepted: 2 May 2003

© 2003 Kishony and Leibler, licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

## Abstract

**Background:** Fundamental questions in evolutionary genetics, including the possible advantage of sexual reproduction, depend critically on the effects of deleterious mutations on fitness. Limited existing experimental evidence suggests that, on average, such effects tend to be aggravated under environmental stresses, consistent with the perception that stress diminishes the organism's ability to tolerate deleterious mutations. Here, we ask whether there are also stresses with the opposite influence, under which the organism becomes more tolerant to mutations.

**Results:** We developed a technique, based on bioluminescence, which allows accurate automated measurements of bacterial growth rates at very low cell densities. Using this system, we measured growth rates of *Escherichia coli* mutants under a diverse set of environmental stresses. In contrast to the perception that stress always reduces the organism's ability to tolerate mutations, our measurements identified stresses that do the opposite - that is, despite decreasing wild-type growth, they alleviate on average the effect of deleterious mutations.

**Conclusions:** Our results show a qualitative difference between various environmental stresses ranging from alleviation to aggravation of the average effect of mutations. We further show how the existence of stresses that are biased towards alleviation of the effects of mutations may imply the existence of average epistatic interactions between mutations. The results thus offer a connection between the two main factors controlling the effects of deleterious mutations: environmental conditions and epistatic interactions.

## Background

Efficient purging of deleterious mutations arising in a population is essential for the prolonged survival of the population. Consequently, the characteristics of deleterious mutations are of critical importance for major open questions in evolutionary genetics, including the advantage of sexual reproduction, maintenance of genetic variability and extinction of small populations [1-3]. In general, the effect

of each deleterious mutation on fitness may depend on environmental conditions and could be alleviated (become less deleterious), be unchanged, or be aggravated (become more deleterious) under environmental stress (Figure 1). Existing experimental evidence shows, however, that the average mutation effect - the average effect taken over a large set of random mutations - is generally aggravated or unchanged, but not alleviated, under environmental stress [4-13]. Such a bias towards aggravation of the effects of mutations by stress suggests that the organism's ability to compensate for deleterious mutations is reduced under stress. In contrast to this perception, the results of quantitative growth rate measurements of *Escherichia coli* mutants, which are presented here, identify a variety of environmental stresses whose influence on deleterious mutations is strongly biased towards the alleviation of mutation effects.

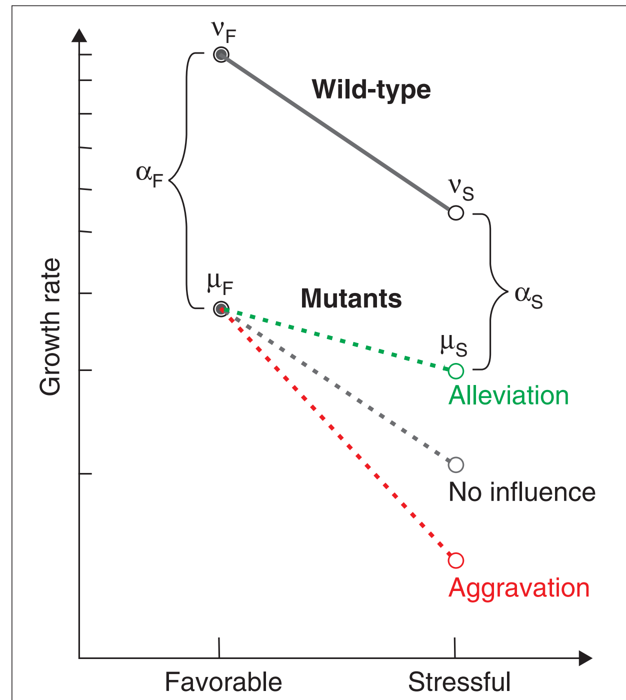


**Figure 1**
The possible influences of environmental stresses on the effects of mutations on fitness. Shown are schematic reaction norms of a wild-type strain (solid line) and three different mutants (dashed lines). The wild-type growth rates in favorable and stressful conditions are represented by $\nu_F$ and $\nu_S$, respectively. The growth rates of each specific mutant in these environments is represented by $\mu_F$ and $\mu_S$, respectively. The effects of mutations in favorable and stressful environments are illustrated; they are defined as $\alpha_F \equiv \log(\nu_F/\mu_F)$ and $\alpha_S \equiv \log(\nu_S/\mu_S)$, respectively. The effect of a specific mutation could be alleviated ($\alpha_S < \alpha_F$, green), unchanged ($\alpha_S = \alpha_F$, black) or aggravated ($\alpha_S > \alpha_F$, red) under stressful conditions. The average mutation effects under favorable and stressful conditions $\bar{\alpha}_F$ and $\bar{\alpha}_S$, are calculated by averaging $\alpha_F$ and $\alpha_S$ over a set of random mutations. We define a stress as alleviating (or aggravating) mutation effects if the average mutation effect is decreased, $\bar{\alpha}_S < \bar{\alpha}_F$ (or increased, $\bar{\alpha}_S > \bar{\alpha}_F$) by the stress.

## Results

Our results are based on a sensitive assay for the quantitative measurement of bacterial growth rates. The assay is designed in a 96-well plate format and is based on photon counting of light emitted from a constitutively expressed luciferase reporter. The main advantage of this technique is its high sensitivity and wide dynamic range, which allows detection of as few as 100-1,000 cells per well up to approximately $10^7$ cells per well (see Figure 2 and Figure S1 at the end of this article). Such sensitivity exceeds by more than a thousand-fold the lower detection limit of commonly used optical density measurements and allows accurate measurements of several orders of magnitude of early exponential growth. The resulting accuracy of the measurement is about 5%. Also important is the ability to measure the growth of small populations, which greatly reduces the incidence of compensatory mutations [14].

We first built a library containing 65 random mutations generated by chemical mutagenesis, along with 12 copies of the parental strain as controls. Importantly, we avoided as far as possible any selection against slow-growing mutants during the library construction procedures. The library was screened for growth under various environmental conditions and the growth rate of each mutant culture was defined as the reciprocal of the doubling time of the population during exponential growth.

It should be noted that our assay is designed to measure absolute growth rates of the mutants in isolation, rather than their relative fitness in competition. Such an absolute measurement is important for some of the analyses presented (in particular the analysis relevant to Figure 4, below). In general, since actual fitness depends on many factors - such as the particular environment, the specific competitors or the population densities - it is always being defined only in an operational way. In our case, the growth-rate measurements should be considered simply as direct measurements of a fitness-related trait.

Environmental stresses are defined as conditions leading to a reduction of fitness in a population [15,16]. The environmental stresses we tested, which are listed in Table 1, can be divided into two main classes - stresses that target specific cellular pathways and stresses with broad cellular impact. The first class includes the bacteriostatic antibiotics chloramphenicol and trimethoprim, which specifically target translation and folic acid biosynthesis, respectively. The second class includes low pH, low temperature, high osmolarity and

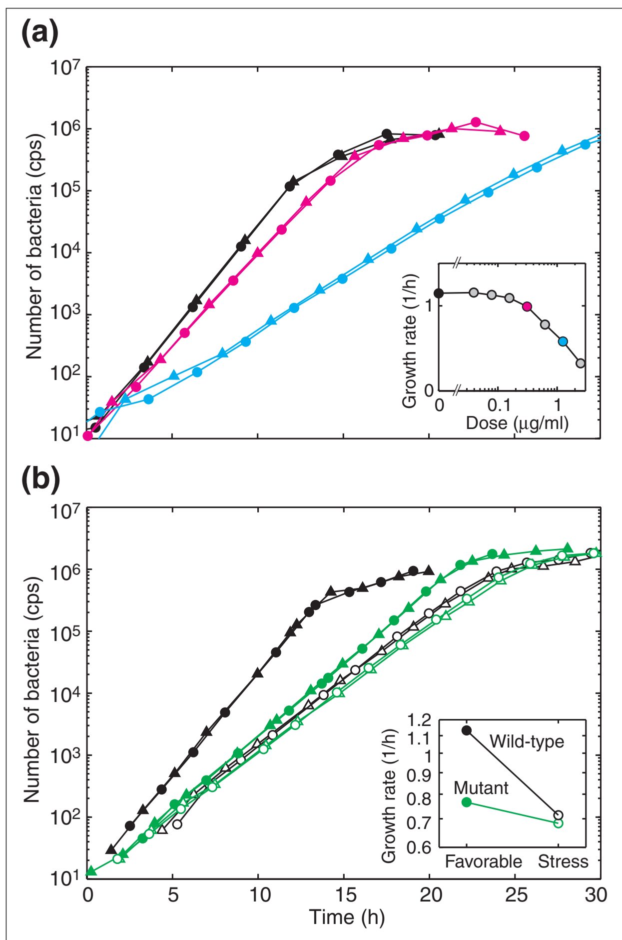


**Figure 2**
Examples of growth curves in various conditions. For each case, two independent measurements (triangles and circles) are shown, demonstrating the reproducibility of the measurement. The origin of the time axis corresponds to 10 counts per second (cps). **(a)** Influence of chloramphenicol stress on the parental strain. Growth in a favorable environment (black), and supplemented with 0.2 μg/ml (magenta) and 1.2 μg/ml (cyan) chloramphenicol are shown. Inset: the growth rate, determined from these and similar data, against chloramphenicol concentration. **(b)** One mutant of the library (green) compared to the parental strain (black) in the favorable environment (solid symbols) and under chloramphenicol stress (open symbols). Inset: the growth rates of the parental strain and the mutant in the two environments. The data indicate a strong alleviation of the effect of this specific mutation under chloramphenicol stress.

the reducing reagent dithiothreitol, which are stresses with wider impacts (the reducing reagent dithiothreitol may have general impacts on protein disulfide bonds as well as more specific impacts on modules involved in maintaining redox balance [17]). Growth of the mutants under these stresses was compared to their growth in a standard favorable medium. Additionally, the standard favorable medium itself was tested as a possible stress compared to an even more favorable medium created by supplementing it with conditioned medium [18] from a 2-day-old culture of the parental strain (the standard medium in this context is designated as 'unsupplemented' stress). For each stress, a particular strength was chosen that reduces the parental strain growth rate considerably but does not completely suppress growth (see a dose-curve example in Figure 2a); the chosen stress strengths are listed in Table 1.

In total, several thousand growth curves were measured. Typically, at least two replicates of each mutant were grown in each of the environmental conditions. An example of the growth curve of one mutant from the library compared to the parental strain, in the favorable environment and under chloramphenicol stress, is shown in Figure 2b.

The influence of each of the stresses on the average mutation effect of the library of mutants is given in Table 1. The results of the chloramphenicol and acidic stresses are illustrated in Figure 3, while the complete dataset is given in Figures S2 and S3, at the end of this article. As expected, within a measurement error of 5%, the absolute growth rates of the parental strain and most of the mutant strains are reduced by the stress. This is reflected in Figure 3a,b by the position of the mutants' points below the main diagonal, which is the geometric locus of mutants whose absolute growth rates are not affected by the stress. More important, however, is the position of the mutant points with respect to the equal-effect line (see the schematic illustration in Figure 3c). This line is defined as the geometric locus of mutants whose growth rates relative to the parental strain in the same environment are not altered by the stress. Thus, mutant points on this line represent mutation whose effects are not changed under stress; points above this line represent mutations whose effects are alleviated by the stress and points below the line correspond to aggravated mutation effects. In the cases of the stresses chloramphenicol, trimethoprim, low temperature and dithiothreitol, most of the mutations lie above the equal-effect line: that is, their effects are alleviated by the stress. We can thus conclude that, on average, these stresses alleviate the phenotypic effects of mutations on growth. The average mutation effects and confidence levels for a difference between stressful and favorable conditions are given in Table 1 and strongly support a bias towards decreased mutation effects under these stresses. The distribution of the distance of mutations from the equal-effect line is shown in Figures 3d and S3. For the stresses discussed above, the distributions are biased towards positive values, corresponding to mutations whose effects are alleviated under these stresses.

The results of the acidic stress, on the other hand, are qualitatively different, showing a small but significant ($p < 0.01$)

**Table 1**

**The stresses tested, and their influence on average relative mutation effects**

| Stress | Strength | $\eta$ | $\bar{\alpha}_F$* | $\bar{\alpha}_S$ | Lethal† | Bias‡ | $p$§ |
|---|---|---|---|---|---|---|---|
| Acidic stress | pH 5¶ | 0.19 | 0.27 | 0.30 | 0.11 | -0.18 | < 0.01 |
| Unsupplemented¥ | 30% old supernatant | 0.31 | 0.27 | 0.26 | 0 | 0.02 | NS |
| High osmolarity | 600 mM NaCl | 0.43 | 0.28 | 0.25 | 0.05 | 0.05 | NS |
| Dithiothreitol | 1.6 mg/ml | 0.30 | 0.29 | 0.21 | 0.05 | 0.26 | < 0.01 |
| Trimethoprim | 0.4 μg/ml | 0.53 | 0.28 | 0.10 | 0.05 | 0.33 | < 0.0001 |
| Chloramphenicol | 1 μg/ml | 0.43 | 0.28 | 0.15 | 0 | 0.30 | < 0.001 |
| Low temperature | 17°C | 1.77 | 0.27 | 0.15 | 0.05 | 0.07 | < 0.03 |

$\eta = \log(\nu_F / \nu_S)$ representing the reduction of the parental strain's growth rate by the stress. The average relative mutation effects $\bar{\alpha}_F$ and $\bar{\alpha}_S$ are defined in Figure 1 and are calculated here as median values of the mutant library. *Measurements of mutant growth rates in the favorable environment were repeated in parallel with each of the stress measurements. †'Lethal' indicates the fraction of mutants showing growth in the favorable media but no growth under stress after one week. ‡Bias $\equiv (\bar{\alpha}_F - \bar{\alpha}_S)/\eta$ represents a bias towards alleviation of the mutations' effects under the stress. §The $p$ value is from a paired Student's t-test for the difference between mutation effects under stress and under favorable conditions; NS, not significant ($p > 0.05$). ¶Acid stress is 0.25 mM sorbic acid and 16 mM citric acid. ¥The standard favorable environment is defined as 'unsupplemented' stress and is compared to an even more favorable environment constructed by supplementing it with 30% supernatant of an old culture (see text for further details).

aggravation of the effects of mutations. As shown in Figure 3d, the distribution of distances from the equal-effect line is now more centered and shifted slightly towards the negative region. Note also that a relatively large number of mutations become lethal under acidic stress. For the high osmolarity stress and the unsupplemented stress, mutations occur equally on both sides of the equal-effect line (Figure S3), indicating a neutral or non-significant influence of these stresses on the average mutation effect.

## Discussion

Explaining the observed qualitative diversity of the average impacts of stress on mutations, ranging continuously from alleviation to aggravation of average mutation phenotypic effects, is beyond the scope of this paper. We briefly discuss, however, some possible mechanisms that could be evoked to explain the existence of stresses that alleviate the average mutation effect. First, certain stresses - in particular the bacteriostatic antibiotics chloramphenicol and trimethoprim - may target a specific functional module in the bacterium, thus generating a rate-limiting step for growth. The data on the effects of these stresses may, to some extent, be interpreted in terms of an extremely idealized picture in which cell growth results from the combined functionalities of many parallel modules [19]. Assuming that proliferation rate is determined by the 'slowest module' and that the mutation and the stress target different modules, the mutant growth rate under the stress should be $\mu_S = \min[\mu_F, \nu_S]$, where $\mu_F$ is the growth rate of the mutant in favorable conditions and $\nu_S$ is the parental strain growth rate under the stress (Figure 1). This necessarily implies that the effect of the mutation on the relative growth rate is decreased under the stress ($\alpha_S < \alpha_F$). A similar argument stating that the "genetic potential of organisms is not reached under poor nutrition" was also made as a possible explanation for evidence of reduced heritability of natural populations seen under certain stressful conditions [20]. Second, it is known that certain bactericidal antibiotics, such as penicillin, confer an advantage on non-growing mutants [21,22]. In sub-lethal concentrations, which allow slow growth of the parental strain, these reagents could potentially reduce the deleterious effect of mutations on relative growth rates. This does not seem to be the mechanism behind the results described here, however. One reason is that there would have to have been a positive correlation between the reduction in relative growth rate and the level of buffering by the stress, while the results indicated in Figure 3b do not show such a correlation. Third, chemicals such as chloramphenicol and dithiothreitol may cause increased error rates of translation and protein folding, respectively. The effects of mutations could then be obscured by the already high error rates imposed by the stress.

Regardless of mechanism, we propose that the existence of stresses that reduce the average effect of mutations has direct implications for the form of epistatic interactions between deleterious mutations (Figure 4). Epistasis, in the 'population genetic sense', means that the combined effect of mutations is larger ('synergistic epistasis') or smaller ('diminishing return epistasis') than the simple product of their individual effects [23]. The average nature of epistasis is crucial for various issues in evolutionary biology, including

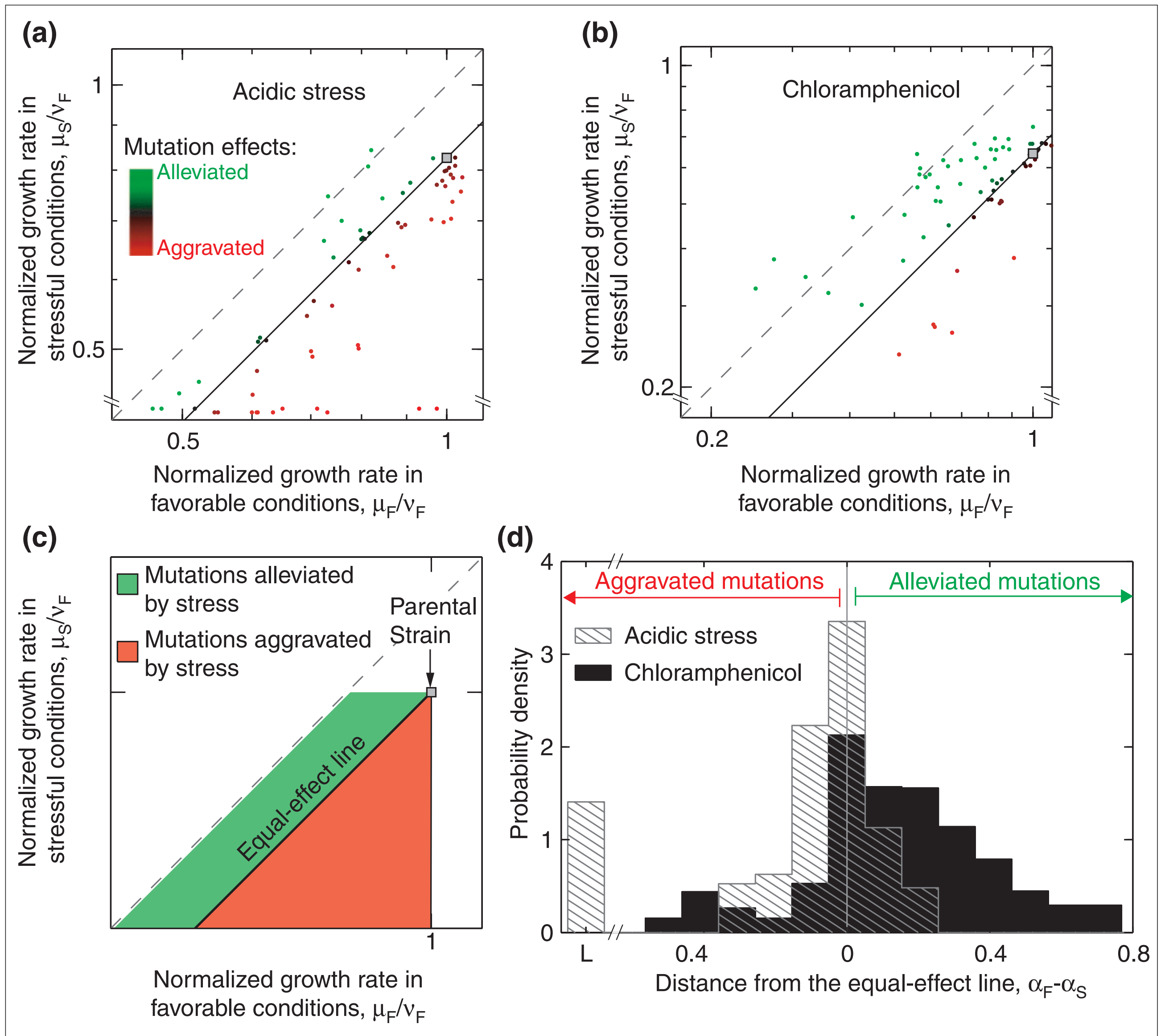


**Figure 3**
The qualitative difference between stresses in their influence on the effect of mutations. **(a,b)** The growth rates of the individual mutants (dots) and the parental strain (square) under (a) acidic stress and (b) chloramphenicol stress, compared to their growth in the favorable environment. The acidic stress is seen to aggravate the effect of most mutations, while the chloramphenicol stress alleviates their effects. **(c)** Schematic representation of the possible impacts of stress on mutations. The main diagonal represents the geometric locus of mutants whose absolute growth rates are not affected by the stress ($\mu_S = \mu_F$). The equal-effect line represents the geometric locus of mutants whose relative growth rates are not altered by the stress ($\mu_S/\nu_S = \mu_F/\nu_F$, or $\alpha_S = \alpha_F$). Mutations above (or below) this line, shown in green (or red) are alleviated (or aggravated) under stress. **(d)** The distribution of distances of mutations from the equal-effect line. The area below the lines is normalized to 1. Lethality or very slow growth under the stress is represented by 'L' on the *x* axis. Positive (or negative) distance corresponds to mutations alleviated (or aggravated) under the stress.

the advantage of sexual reproduction [23-28]. Thus far, direct attempts to test for the average nature of epistasis have shown null results [29,30], while positive evidence [31,32] remains controversial [3,23,29,33]. Figure 4 shows a hypothetical extrapolation of the averaged growth rates measured under favorable conditions and under the mutation-alleviating stress trimethoprim. The measurement error bars are small enough to strongly support ($p < 0.0001$) a

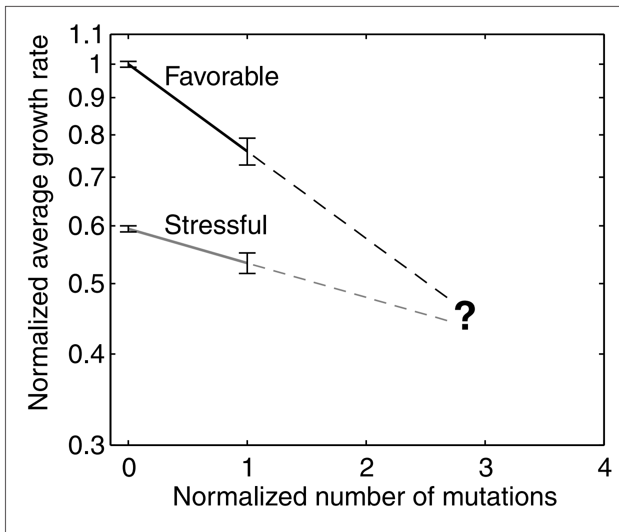


**Figure 4**
The existence of stresses that alleviate average mutation effects could imply that there is average epistasis between mutations. Average absolute growth rates of the parental strain (with no mutations) and of the mutant library (defined as having an average of 1 unit of mutation per mutant in the library) are shown under favorable conditions (black) and under trimethoprim stress (gray). Linear extrapolation (dashed) of the data, assuming an absence of epistasis, would lead to intersection of the lines. Such an intersection seems unrealistic, however, as it would imply an increase of the average absolute growth rate under stress. To avoid intersection at least one of the lines must bend, which would reflect the existence of average epistatic interactions between mutations. Note that the fact that our library may contain a variable number of mutations per genome does not affect the argument presented above.

smaller slope of the trimethoprim-stress line than the favorable-condition line. Without epistasis, the lines would be straight and would have to intersect (the 'bias' parameter in Table 1 measures the reciprocal of the distance to the intersection; trimethoprim, shown Figure 4, has the strongest bias, but the claim of intersection of the lines can also be made for all the stresses that alleviate average mutation effects). Such an intersection seems unrealistic, however, because it would imply that, on average, the stress increases the absolute growth rate of bacteria carrying enough random mutations. To avoid intersection, at least one of the lines has to curve, or, in other words, average epistatic interaction between mutations must occur. The above argument thus allows us to make an inference about average genotype-by-genotype interactions from sufficiently precise genotype-by-environment data.

## Conclusions

Our results show that organisms may actually become more tolerant to genetic perturbations when put under certain environmental stresses. This intriguing result implies a connection between the two main factors controlling the deleterious effects of mutations: environmental conditions and epistatic interactions (for additional support see [34]). Such a connection may allow a unification of environmental and mutational theories for the advantage of sexual reproduction [2,24,35]. While the current study was aimed at the statistical characteristics of random mutations, the same approach and experimental techniques can also be applied to libraries of known and marked mutants, which should give further insight into the modular structure of the organism [29,36,37]. Finally, double and triple mutants constructed from such libraries may make it possible to test our prediction for the existence of epistasis and its dependence on environmental conditions.

## Materials and methods

### Strains and media

*E. coli* K12 strain DL41 ($\lambda^-$, metA28)[38] was obtained from the *E. coli* Genetic Stock Center, CGSC# 7177. Plasmid pCS16 (SC101 ori, a luxCDABE operon and a Kan$^R$ marker) was obtained from M. Surette. The luciferase promoter in pCS16 was *Bam*HI-excised and a synthetic lambda promoter [39] was ligated instead to form pCS-λ. The parental strain of the current study is the constitutively bright DL41 strain bearing pCS-λ.

The standard favorable medium (FM) is a M63 minimal medium [40], supplemented with 0.2% glucose, 0.01% casamino acids, 0.5 μg/ml thiamine, 33 μg/ml methionine and 40 μg/ml kanamycin. Growth temperature was 30°C unless otherwise indicated. Stressful environments were formed by supplementing FM as indicated in Table 1.

### Mutant library construction

The parental strain culture was mutagenized by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) according to standard methods [41]. The mutagen dose used (7.5 μg/ml NTG for 10 minutes) corresponds to a relatively low number of mutations per genome (rifampicin resistance frequency of $3 \times 10^{-5}$). It should be noted that the exact number of mutations per genome may vary between the mutants, but none of the arguments made in the current study assume, in any way, a specific constant number of mutations per mutant (see in particular the legend to Figure 4). After mutagenesis, cells were allowed to recover in LB for only 2 hours to avoid considerable selection against slow-growing mutants. Cells were then plated for single colonies on FM agar plates and incubated at 30°C. At five time points (21, 24, 34, 50 and 73 hours after plating), newly arising colonies were counted (there were 1,268, 58, 29, 18 and 6, respectively) and colonies (7, 35, 20, 13 and 3, respectively) were randomly picked and re-streaked on FE agar

plates. Each re-streaked plate was placed at 4°C when small visible colonies first appeared. Once all re-streaked mutants formed visible colonies, they were picked into separate wells on a 96-well microtiter plate containing 100 μl FM per well. Twelve parental strain controls, which went through the same procedure with no mutagen, were also included in the library. The library microtiter plate was then used as a master plate from which the library was replicated to initiate the growth rate assays. Frozen -80°C copies of the library were also made by replicating the master plate into M63 + 3.5% v/v DMSO.

The growth rates measured for the seven clones picked in the first time point were equal to the parental strain growth rate under all tested environments, and were therefore excluded from the statistical analysis. Mutants picked at the four later time points were assigned a statistical weight equal to the ratio of the total number of new colonies that appeared at a given time point divided by the number of colonies picked at that time point. This statistical weight was used to properly weight the growth-rate measurements for the statistical analysis shown in Figures 3d and S3 and Table 1.

### Growth curve assay

The 96-well plates (Costar 3792 black, round bottom) were filled with 100 μl per well of the tested media, inoculated with the library cells using a 96-pin replicator and tightly sealed with a clear adhesive tape (Perkin-Elmer 1450-461). For a given medium, at least two replications of several cell inoculations (typically three different inoculations aimed around 0.15, 3 and 25 cells per well) were made. Photon counting was done in Packard's TopCount NXT Microplate Scintillation and Luminescence Counter. The instrument was placed in a 30°C (or 17°C for the cold-temperature experiment) environmental room and the same temperature was also set in the instrument's reading chamber. Acquisition time was 2 seconds per well. A total of 10-20 microtiter plates were typically assayed in parallel using the instrument stacker. No shaking for aeration was performed. A calibration of counts per second (cps) in the detector to number of cells per well is 30 cells per cps during exponential growth of the parental strain in favorable conditions (see Figure S1).

### Growth-rate determination

Growth rates were determined by a linear fit of the log of the counts per second against time during exponential growth. A background of 20 cps was subtracted from the raw data. Crosstalk coefficient from neighboring wells was evaluated (nearest neighbors, $10^{-4}$; nearest-nearest neighbors, $0.3 \times 10^{-4}$; and all other wells, $10^{-6}$). Data points with significant crosstalk (more than 10% of the well signal) were excluded. Guidelines for determining the time interval to which the linear fit was applied were: first, to assure high signal-to-background and to give the cells enough time to reach pure exponential growth, only readings higher than 100 cps were considered; second, only data points at least one order of magnitude below stationary phase were considered; third, for each clone the lowest initial cell inoculation which gave rise to a growing culture was used. Usually these guidelines left two to three orders of magnitude of pure exponential growth for which a linear fit (M-estimate fit) was performed. Within- and between-plate variation in growth rates of the parental strain were evaluated. Growth rates of replicates on different plates in the same well position were usually within 1-2% of each other. Variation between different wells within the same plate was about 5%. Half of this variance was systematically correlated with the position of the well on the plate (presumably due to a small temperature gradient) and was corrected for. After these corrections, the total (within and between plates) measurement variation of the growth rates was about 5%. The measured growth rate was validated for a few cases by plating cultures for single colonies at several time points. They were found accurate within the measurement error of 5%.

## Acknowledgements

Special thanks to M.G. Surette for kindly providing plasmid pCS16, to A.W. Murray for important comments and to M. Elowitz and R. Chait for proofreading the manuscript. We thank the following for helpful discussions: B.L. Bassler, D. Fisher, D. Kahne, P. Model, M. Russel, T.J. Silhavy, M.G. Surette and all the members of our lab. This work was partially supported by the National Institutes of Health and the Human Frontiers Science Program.

## References

1. Barton NH, Charlesworth B: **Why sex and recombination?** *Science* 1998, **281:**1986-1990.
2. Kondrashov AS, Shpak M: **On the origin of species by means of assortative mating.** *Proc R Soc Lond B Biol Sci* 1998, **265:**2273-2278.
3. Lynch M, Blanchard J, Houle D, Kibota T, Schultz S, Vassilieva L, Willis J: **Perspective: Spontaneous deleterious mutation.** *Evolution Int J Org Evolution* 1999, **53:**645-663.
4. Kondrashov AS, Houle D: **Genotype-environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*.** *Proc R Soc Lond B Biol Sci* 1994, **258:**221-227.
5. Vassilieva LL, Hook AM, Lynch M: **The fitness effects of spontaneous mutations in *Caenorhabditis elegans*.** *Evolution Int J Org Evolution* 2000, **54:**1234-1246.
6. Shabalina SA, Yampolsky LY, Kondrashov AS: **Rapid decline of fitness in panmictic populations of *Drosophila melanogaster* maintained under relaxed natural selection.** *Proc Natl Acad Sci USA* 1997, **94:**13034-13039.
7. Korona R: **Genetic load of the yeast *Saccharomyces cerevisiae* under diverse environmental conditions.** *Evolution Int J Org Evolution* 1999, **53:**1966-1971.
8. Szafraniec K, Borts RH, Korona R: **Environmental stress and mutational load in diploid strains of the yeast *Saccharomyces cerevisiae*.** *Proc Natl Acad Sci USA* 2001, **98:**1107-1112.
9. Fry JD, Heinsohn SL, Mackay TFC: **The contribution of new mutations to genotype-environment interaction for fitness in *Drosophila melanogaster*.** *Evolution* 1996, **50:**2316-2327.
10. Yang HP, Tanikawa AY, Van Voorhies WA, Silva JC, Kondrashov AS: **Whole-genome effects of ethyl methanesulfonate-induced mutation on nine quantitative traits in outbred *Drosophila melanogaster*.** *Genetics* 2001, **157:**1257-1265.

11. Remold SK, Lenski RE: **Contribution of individual random mutations to genotype-by-environment interactions in *Escherichia coli*.** *Proc Natl Acad Sci USA* 2001, **98:**11388-11393.
12. Fry JD, Heinsohn SL: **Environment dependence of mutational parameters for viability in *Drosophila melanogaster*.** *Genetics* 2002, **161:**1155-1167.
13. Fernandez J, LopezFanjul C: **Spontaneous mutational genotype-environment interaction for fitness-related traits in *Drosophila melanogaster*.** *Evolution Int J Org Evolution* 1997, **51:**856-864.
14. Moore FBG, Rozen DE, Lenski RE: **Pervasive compensatory adaptation in *Escherichia coli*.** *Proc R Soc Lond B Biol Sci* 2000, **267:**515-522.
15. Hoffmann AA, Hercus MJ: **Environmental stress as an evolutionary force.** *Bioscience* 2000, **50:**217-226.
16. Lenski RE, Bennett AF: **Evolutionary response of *Escherichia coli* to thermal stress.** *Am Nat* 1993, **142:**S47-S64.
17. Gennis RB, Stewart V: **Respiration.** In *Escherichia coli and Salmonella: Cellular and Molecular Biology.* Edited by Neidhardt FC. Washington, DC: American Society for Microbiology; 1996:217-222.
18. Rozen DE, Lenski RE: **Long-term experimental evolution in *Escherichia coli*. VIII. Dynamics of a balanced polymorphism.** *Am Nat* 2000, **155:**24-35.
19. Hartwell LH, Hopfield JJ, Leibler S, Murray AW: **From molecular to modular cell biology.** *Nature* 1999, **402:**C47-C52.
20. Hoffmann AA, Merila J: **Heritable variation and evolution under favourable and unfavourable conditions.** *Trends Ecol Evol* 1999, **14:**96-101.
21. Davis BD: **Isolation of biochemically deficient mutants of bacteria by penicillin.** *J Am Chem Soc* 1948, **70:**4267-4267.
22. Lederberg J, Zinder N: **Concentration of biochemical mutants of bacteria with penicillin.** *J Am Chem Soc* 1948, **70:**4267-4268.
23. Wolf JB, Brodie ED III, Wade MJ: *Epistasis and the Evolutionary Process.* New York: Oxford University Press; 2000.
24. Rice WR: **Experimental tests of the adaptive significance of sexual recombination.** *Nat Rev Genet* 2002, **3:**241-251.
25. Kondrashov AS: **Deleterious mutations and the evolution of sexual reproduction.** *Nature* 1988, **336:**435-440.
26. Kondrashov AS: **Classification of hypotheses on the advantage of amphimixis.** *J Hered* 1993, **84:**372-387.
27. Maynard Smith J: *The Evolution of Sex.* Cambridge, MA: Cambridge University Press; 1978.
28. Williams GC: *Sex and Evolution.* Princeton: Princeton University Press; 1975.
29. Elena SF, Lenski RE: **Test of synergistic interactions among deleterious mutations in bacteria.** *Nature* 1997, **390:**395-398.
30. Peters AD, Keightley PD: **A test for epistasis among induced mutations in *Caenorhabditis elegans*.** *Genetics* 2000, **156:**1635-1647.
31. Mukai T: **Genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interaction of spontaneous mutant polygenes controlling viability.** *Genetics* 1969, **61:**749-761.
32. de Visser JAGM, Hoekstra RF, van den Ende H: **An experimental test for synergistic epistasis and its application in *Chlamydomonas*.** *Genetics* 1997, **145:**815-819.
33. Keightley PD: **Nature of deleterious mutation load in *Drosophila*.** *Genetics* 1996, **144:**1993-1999.
34. You LC, Yin J: **Dependence of epistasis on environment and mutation severity as revealed by *in silico* mutagenesis of phage T7.** *Genetics* 2002, **160:**1273-1281.
35. Bell G: *The Masterpiece of Nature: The Evolution and Genetics of Sexuality.* Los Angeles: University of California Press; 1982.
36. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, *et al*: **Functional profiling of the *Saccharomyces cerevisiae* genome.** *Nature* 2002, **418:**387-391.
37. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, *et al*: **Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis.** *Science* 1999, **285:**901-906.
38. Hendrickson WA, Horton JR, Lemaster DM: **Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (mad) - a vehicle for direct determination of 3-dimensional structure.** *EMBO J* 1990, **9:**1665-1672.
39. Lutz R, Bujard H: **Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements.** *Nucleic Acids Res* 1997, **25:**1203-1210.
40. Ausubel FM, Kingston RE, Moore DM, Seidman JG, Smith JA, Struhl K (Eds): *Short Protocols in Molecular Biology.* 2nd Edition. New York: John Wiley and Sons; 1992.
41. Miller JH: *A Short Course in Bacterial Genetics.* Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1992.


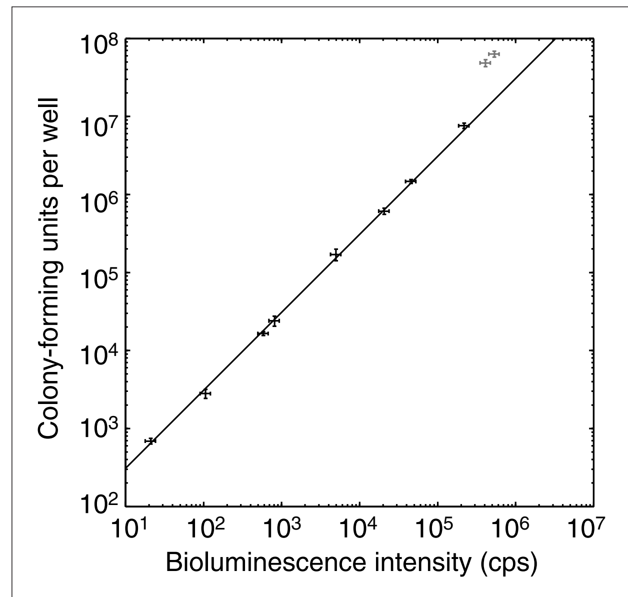


**Figure S1**
The relationship between the number of colony-forming units (CFUs) per well and counts per second (cps) of bioluminescence intensity. CFUs were measured by plating for single colonies at various time points during exponential growth (black) and at the end of exponential growth (gray). The linear fit corresponds to 30 CFUs per well per cps. This linear relationship holds throughout four orders of magnitude of exponential growth; it breaks only at high cell densities, when the population enters stationary phase.

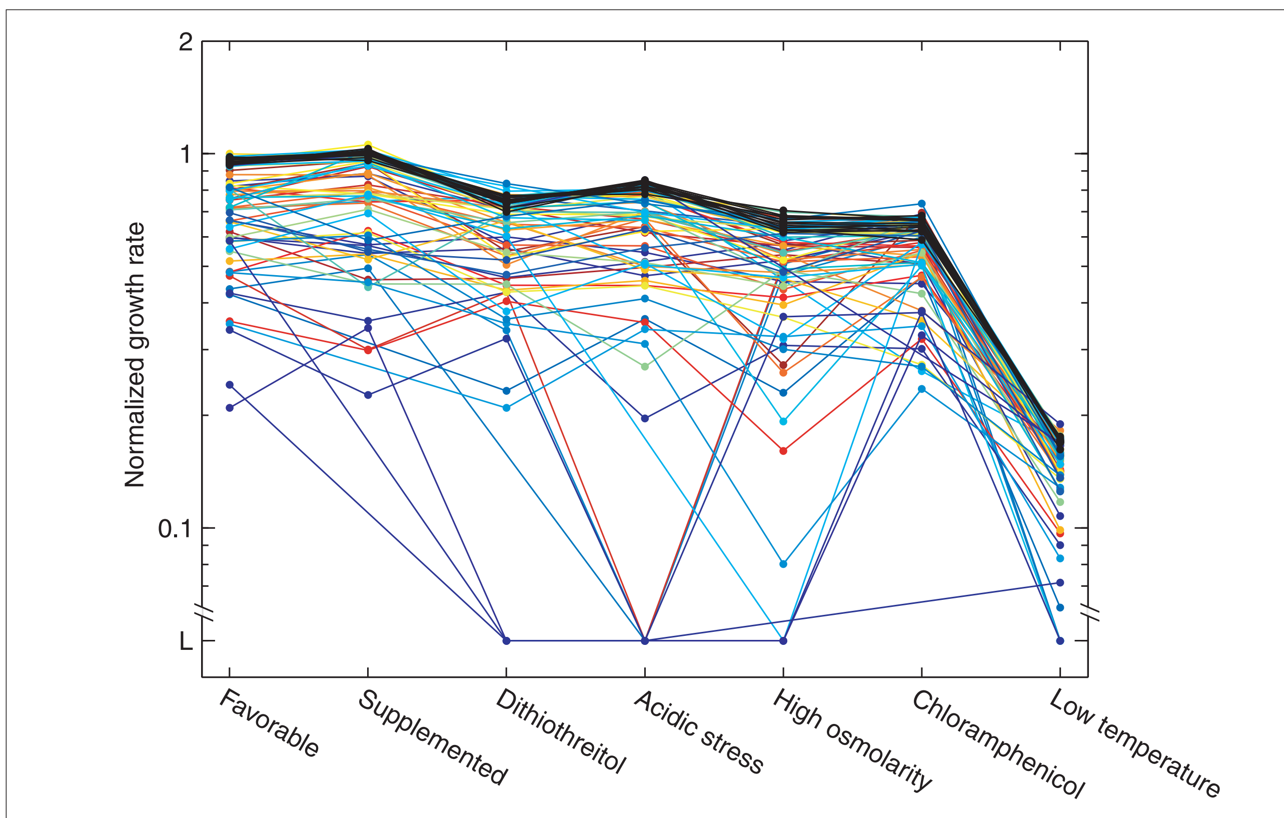


**Figure S2**
Reaction norms of the library mutants. Growth rates of the duplicated parental strain (black) and of the various mutants (color) are shown in the different environments tested (trimethoprim stress could not be shown here as it was measured with a slightly different set of mutants). Lethality or very slow growth under the stress is represented by 'L' on the *y* axis.

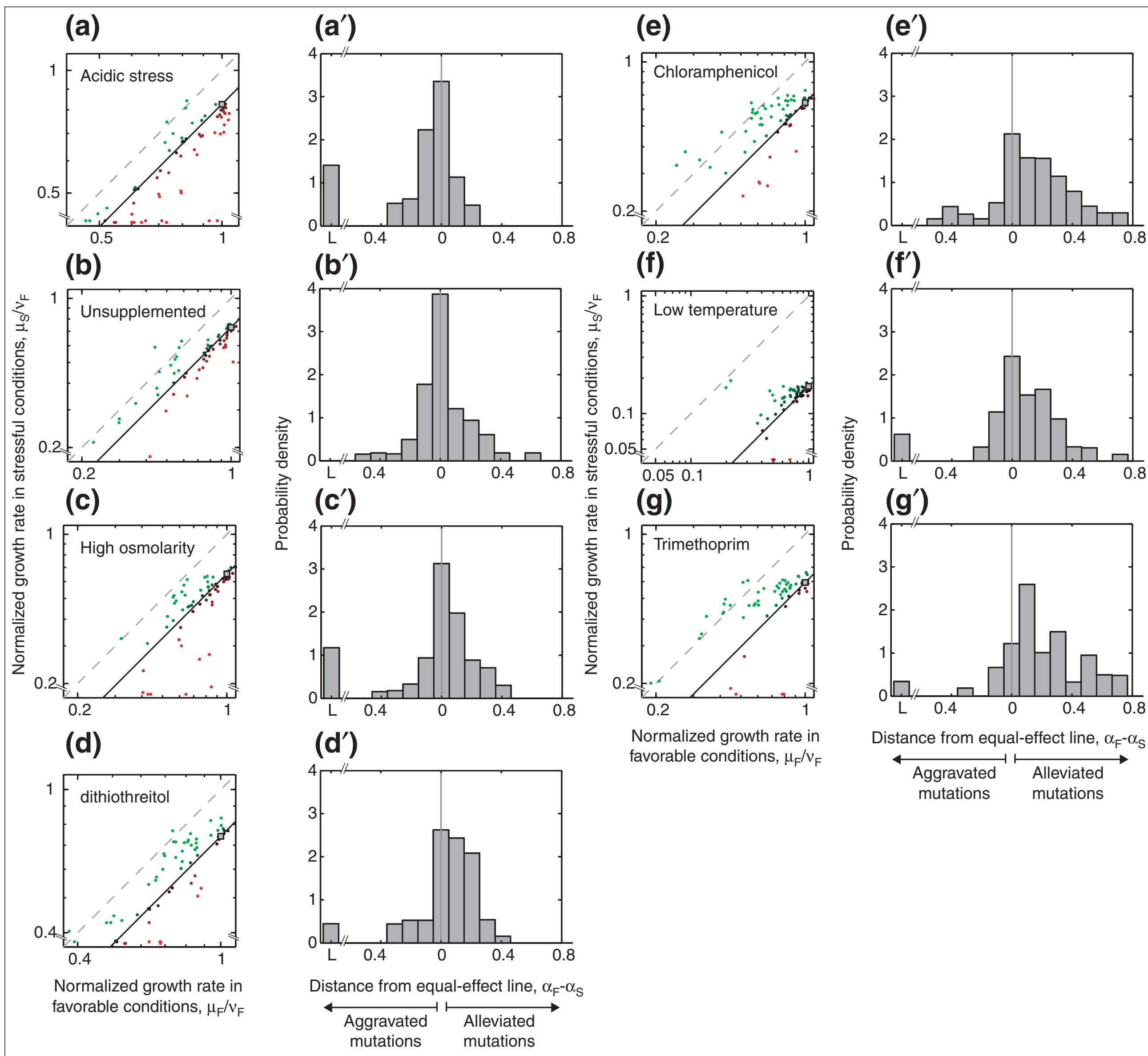


**Figure S3**

The impacts of different stresses on the effects of mutations on growth rates. **(a-g)** Growth rates of the individual mutants (dots) and the parental strain (gray square) under the different stresses, plotted against their growth in the favorable environment. The solid off-diagonal line describes the equal-effect line. Mutations above (or below) this line, shown in green (or red) are alleviated (or aggravated) under stress. **(a′-g′)** The distribution of distances of mutations from the equal-effect line. The area below the lines is normalized to 1. Lethality or very slow growth under the stress is represented by 'L' on the *x* axis. Positive (or negative) distance corresponds to mutations alleviated (or aggravated) under the stress.