A Universal Temperature-Dependence of Mutational Fitness Effects

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ABSTRACT

The biochemical properties underpinning the genotype-phenotype map can exert major influence over evolutionary rates and trajectories. Yet, the constraints set by these molecular features are often neglected within eco-evolutionary frameworks. Here, by applying a biophysical model of protein evolution, we demonstrate that rising global temperatures are expected to intensify natural selection genome-wide by increasing the effects of sequence variation on protein phenotypes. Our model further suggests that thermal adaptation will not alleviate this effect; warm and cold adapted species are expected to show the same temperature-dependent increase in the strength of selection. We tested these predictions using lines of seed beetle evolved at ancestral or warm temperature for 70-85 generations. According to predictions, fitness effects of induced mutations were stronger at high temperature for both ancestral and warm-adapted lines. We then calculated 98 estimates from the literature, comparing selection on newly induced mutations in stressful and benign environments across a diverse set of ectothermic organisms, ranging from viruses and unicellular bacteria and fungi, to multicellular plants and animals. We first show that environmental stress *per se* does not increase the strength of selection on new mutations. However, as predicted from the biophysical model, increased temperature does. These results bear witness to and extend the universal temperature dependence of biological rates and have important implications for global patterns of genetic diversity and the rate and repeatability of evolution under environmental change.

SIGNIFICANCE STATEMENT

Natural environments are constantly changing so organisms must also change in order to persist. Whether they can do so ultimately depends upon the reservoir of raw genetic material available for evolution, and the efficacy by which natural selection can discriminate among this variation to secure the survival of the fittest. Here we integrate theory from the fields of ecology, genetics and biophysics and combine mathematical modelling and data from organisms across the tree of life, to show that rising global temperatures will universally increase natural selection on DNA sequence variation in cold-blooded organisms. This finding has broad implications for our understanding of biodiversity patterns and suggests that evolution will proceed at an ever accelerating pace under climate warming.

INTRODUCTION

The efficacy of natural selection impacts on a range of evolutionary processes, including rates of adaptation, the maintenance of genetic variation (Lande 1975, Turelli 1984) and extinction risk (Kimura 1968, Burger & Lynch 1995). However, surprisingly little is known about whether certain types of environments generally impose stronger selection pressures than others (Chevin et al. 2010, Merilä & Hendry 2014, Kokko et al. 2017). In Sewell Wright's (1932) original fitness landscape metaphor the strength of selection can be viewed as the steepness of the gradient linking adaptive peaks and valleys across allele frequency space. This once static view of the fitness landscape has been superseded by a dynamic complex multiscape, in which the fitness surface itself responds to both environmental and mutational input (Dietrich & Skipper Jr. 2012, Pigliucci 2012, Kokko et al. 2017). Mapping of the molecular basis of developmental constraints and environmental sensitivity is therefore of paramount importance to understanding why certain evolutionary trajectories are favoured over others (Maynard Smith 1970, Weinreich et al. 2006, Tenaillon et al. 2012, Harms & Thornton 2013, Storz 2016), and how evolution can be repeatable despite mutation being an inherently random process (Nei 2013, de Visser & Krug 2014, Houle 2017, Lenski 2017, Lässig et al. 2017).

General theory and recent empirical evidence (Husby et al. 2011, Caruso et al. 2017) suggest that the strength of directional selection on key ecological traits underlying local adaptation increases in stressful environments due to the discrepancy between mean population phenotype and the new environmental optimum. However, the fitness consequences associated with this mismatch can be relatively small compared to the population's total genetic load. This segregating reservoir of genetic variation is expected to have a

fundamental influence on species' persistence and adaptability (Lande & Shannon 1996, Agrawal & Whitlock 2012), but how the environment impacts on its influence remains poorly understood (Martin & Lenormand 2006, Agrawal & Whitlock 2010, Chevin et al. 2010). For example, it is often argued that fitness effects of sequence variation are magnified in novel environments due to a lack of selection for phenotypic robustness under the new environmental conditions (de Visser et al. 2003, Landry et al. 2007, Lehner et al. 2010, Paaby & Rockman 2014, Siegal & Lie 2014). Yet, others have argued that novel or stressful environments are bound to have idiosyncratic effects on the strength of selection on alternative genetic variants (Martin & Lenormand 2006, Agrawal & Whitlock 2010). These conflicting predictions suggest that only by mapping the molecular basis of how environments mould the effects of allelic variation is it possible to fully understand the potential for and limits to adaptation in changing environments.

Here we demonstrate how considerations of underlying biophysical constraints on protein function can lead to insights about how climate change and regional temperatures predictably affect the strength of selection on sequence variation in ectothermic organisms. The laws of thermodynamics pose a fundamental constraint on protein folding and enzymatic reactions (Hochacka & Somero 2002, Elias et al. 2015, Sikosek & Chan 2015, Echave & Wilke 2017) resulting in a universal temperature-dependence of biological rates scaling from organismal (Huey & Kingsolver 1989, Gillooly et al. 2002, Brown et al. 2004, Dell et al. 2011) to ecosystem level (Enquist et al. 2003, Schramski et al. 2015). By applying a biophysical model of protein evolution we demonstrate from first principles, i) how elevated temperatures cause an exponential increase in mutational fitness effects over the biologically relevant temperature range and ii) how the evolution of increased protein

stability is predicted to offer robustness to both temperature and mutational perturbations, but iii) that the temperature-dependence of mutational fitness effects remains unaffected by such compensatory adaptation.

Our model thus suggests that climate warming will cause a universal increase in genome-wide selection in cold blooded organisms. We test these predictions by first measuring selection on randomly induced mutations at benign and high temperature in replicate experimental evolution lines of seed beetle adapted to either ancestral or warm temperature. Second, we collate and analyse 98 estimates from the literature on selection coefficients against new mutations in benign vs. stressful environments in a diverse set of unicellular and multicellular organisms. Our experimental data and meta-analysis demonstrate that environmental stress *per se* does not affect the mean strength of selection, but provide unequivocal support for our theoretical prediction that elevated temperature leads to a universal increase in genome-wide selection. These results have implications for global patterns of genetic diversity and suggest that evolution will proceed at an ever accelerating rate under continued climate warming.

RESULTS

Enzyme kinetics theory predicts temperature-dependence of mutational effects

Fitness of ectothermic organisms shows a well-characterised relationship with temperature that closely mirrors the thermodynamic performance of a hypothetical rate-limiting enzyme (Huey & Kingsolver 1989; Angilletta 2009; Fig 1B). Enzymatic reaction rate, r, increases exponentially with temperature, T (in Kelvin), according to the Arrhenius factor:

$$r = r_0 \, e^{H^{\#}/RT} \qquad \text{(Eq. 1)}$$

where $H^{\#}$ is the enthalpy of activation energy (kcal mol^{-1} K^{-1}) of the enzymatic reaction, R is the universal gas constant (0.002 kcal mol^{-1}) and r_0 is a rate-specific constant (Fig. 1A). The inevitable decline in biological rate at high temperatures, in contrast, is attributed to an increase in entropy which expands the distribution of possible protein configurations, reducing the proportion of enzyme in active folded state (Hochacka & Somero 2002, Echave & Wilke 2017, Fig. 1A). This temperature-dependence can be described as a function of the Gibbs free energy of folding, ΔG:

$$\text{Pr(active)} = 1/\left(1 + e^{\Delta G/RT}\right). \qquad \text{(Eq. 2)}$$

with the Gibbs energy itself being a function of environmental temperature: $\Delta G(T) = \Delta G\left(T_{Ref}\right) + (\mathrm{T} - T_{Ref})\Delta S$, where T_{Ref} is the reference temperature and ΔS is the change in entropy between folded and unfolded proteins (ca. $+0.25$ kcal mol^{-1} K^{-1}; Chen & Shakhnovich 2010). Since natural selection leads to inherently stable protein configurations (within limits set by effective population size: Goldstein 2011) most de novo mutations act to destabilise protein structure by increasing the free energy of folding (DePristo et al. 2005, Drummond & Wilke 2008, Sikosek & Chan 2015, Echave & Wilke 2017). Hence, mutation and increased temperature have synergistic effects on folding. The net effect of a mutation on the free energy of folding, $\Delta\Delta G$, has been estimated empirically to be ca. 1 kcal mol^{-1} (SD = 1.7: Zeldovich et al. 2007, Tokuriki & Tawfik 2009) and to be more or less independent of the original ΔG value (mean ΔG ~ -7 kcal mol^{-1} at 25°C; Chen & Shakhnovich 2009). Following

Chen and Shakhnovich (2010), the consequences of de novo mutation on ectotherm fitness at environmental temperature *T* can thus be predicted by combining Eq. 1 & 2 to estimate Malthusian fitness for a wildtype, $\boldsymbol{\omega}$, and mutant, $\boldsymbol{\omega}^*$, genotype with Γ rate-determining proteins (Fig. 1C):

$$\omega(\Delta G, T) = r_0 \frac{e^{H^{\#}/RT}}{\prod_{i=1}^{\Gamma}\left(1+e^{\Delta G_i/RT}\right)}. \qquad \text{(Eq. 3a)}$$

$$\omega^*(\Delta G^*, T) = r_0 \frac{e^{H^{\#}/RT}}{\prod_{i=1}^{\Gamma}\left(1+e^{\Delta G_i^*/RT}\right)}. \qquad \text{(Eq. 3b)}$$

We can estimate the mean selection coefficient $\boldsymbol{s}$ against de novo mutations across temperature *T* as: $\boldsymbol{s}_T = 1 - \boldsymbol{\omega}_T^*/\boldsymbol{\omega}_T$. Combining and simplifying Eqs. 3a & b gives:

$$\boldsymbol{s}(T) = 1 - \prod_{i=1}^{\Gamma} \frac{1+e^{\left(\Delta G_{i T_{ref}} + \left(T - T_{ref}\right)\Delta S_i\right)/RT}}{1+e^{\left(\Delta G^*_{i T_{ref}} + \left(T - T_{ref}\right)\Delta S_i\right)/RT}} \qquad \text{(Eq. 4)}$$

This model yields three key predictions: First, the strength of selection increases with temperature as a predictable consequence of enzyme-kinetic constraints on protein folding (Figure 1C). Second, the evolution of increased enzyme stability (more negative $\Delta G_{T_{ref}}$) predicted in hot climates (Angilletta 2009) can offset the effects of entropy, leading to proteins that are more robust also to mutational perturbation (Fig. 1C). Third, cold- and warm-adapted genotypes are expected to show the same relative increase in the strength of selection with temperature (Fig. 1D) in their respective native thermal environment (T_{ref}).

It seems likely that some mutations will in practice show unconditionally deleterious effects, as could be expected for major effect mutations or those that have a scaling effect on fitness, either directly by increasing the enthalpy of activation energy ($H^{\#}, Eq\ 1$), or indirectly by effectively limiting enzyme or substrate concentration within the living cell (Cornish-Bowden 2002). We can assess the potential impact of unconditional mutations by modifying equation 1 as follows for the mutant $(1-\theta)\omega^{*}(\Delta G, T)$, where θ represents the net proportional loss in catalytic rate due to unconditionally deleterious mutations. Figure 1D demonstrates how even a modest unconditional reduction in fitness on the order of 1% masks the temperature-dependence of mutational effects at cold temperatures while the disproportionate effects of protein (mis)folding at warmer temperatures override unconditional mutational effects so that the temperature-dependence remains pronounced over this thermal range.

Our predictions arise from two fundamental and well-established principles: i) that enzymes show reversible inactivation at high temperatures (Hochacka & Somero 2002), and ii) that de novo mutations act universally to destabilize protein structure (DePristo et al. 2005, Drummond & Wilke 2008, Sikosek & Chan 2015, Echave & Wilke 2017). Our qualitative results are also robust to the particular mathematical formulation of the enzyme-kinetic model, an assertion confirmed by extending this analysis to the various alternative models recently reviewed by DeLong et al. (2017) (results available upon request).

Deleterious fitness effects of mutations are consistently stronger at high temperature in seed beetles adapted to contrasting thermal regimes

To test the predictions outlined above, we measured the fitness effects of induced mutations at 30°C and 36°C in replicate lines of the seed beetle *Callosobruchus maculatus*, evolved at benign 30°C (ancestral lines) or stressful 36°C (warm-adapted lines) for more than 70 generations (overview in SI Fig. 1.1). A temperature of 36°C generally decreases offspring production relative to 30°C in these lines (X^2 = 62.5, df = 1, P < 0.001), but to lesser extent in warm-adapted lines (interaction: X^2 = 7.35, df = 1, P = 0.007; Fig. 2A). To characterize thermal adaptation further and relate it to the assumptions of the biophysical model (Fig. 1A), we quantified thermal performance curves for two traits that should reflect variation in the rate of catalysis and protein stability, respectively (Hochachka & Somero 2002); juvenile development rate and survival. In line with general expectations based on thermodynamic theory (Fig. 1A), temperature generally decreased juvenile survival (X^2 = 76.0, df= 3, P < 0.001) and increases development rate (X^2 = 1723, df= 3, P < 0.001). Divergence between ancestral and warm-adapted lines in the temperature-dependence of these two traits was weak (survival: X^2 = 5.43, df= 3, P = 0.14, Fig. 2B; development: X^2 = 6.71, df= 3, P = 0.082, Fig. 2C). Instead, ancestral lines show consistently faster development (X^2 = 27.2, df= 1, P < 0.001, Fig 2B) but lower survival (X^2 = 3.74, df= 1, P = 0.053, Fig. 2C). These results are qualitatively consistent with the general assumptions of the biophysical model of protein kinetics (compare: Fig. 1A & B with Fig. 2B & C), suggesting that the warm-adapted lines have evolved lower rates of catalysis (*r*, Eq. 1) but stable proteins with more negative activation energies (ΔG: Eq. 2).

To measure mutational fitness effects we induced mutations genome-wide by ionizing radiation in F0 males of all lines. Males were then mated to females that subsequently were randomized to lay eggs at either 30 or 36°C. By comparing the number of F1 and F2 offspring produced in these lineages relative to that in corresponding (non-irradiated) control lineages (SI Fig. 1.2), we could quantify the cumulative fitness effect of the mutations (i.e. mutation load) as: $\boldsymbol{\Delta\omega = 1\text{-}\ \omega_{IRR}/\omega_{CTRL}}$, and compare it across the two assay temperatures in ancestral and warm-adapted lines. High temperature increased $\boldsymbol{\Delta\omega}$, assayed in both the F1 (X^2 = 13.0, df = 1, P < 0.001) and F2 generation (X^2 = 7.46, df = 1, P = 0.006). According to model predictions, these temperature effects were consistent across ancestral and warm-adapted lines (interaction: P_{F1} = 0.43, P_{F2} = 0.90). These findings lend support to the model predictions of temperature-dependent mutational fitness effects based on protein kinetics (compare Fig. 1C and Fig. 3) and, as both ancestral and warm-adapted genotypes showed similar responses, suggest that high temperature rather than thermal stress *per se*, caused the increase in selection.

Mutational fitness effects across benign and stressful environments in unicellular and multicellular organisms

To test model predictions further, we retrieved 98 estimates comparing the strength of selection on de novo mutations across benign and stressful abiotic environments from 27 studies and 11 organisms, spanning viruses and unicellular bacteria and fungi, to multicellular plants and animals. These studies measured fitness effects in form of Malthusian growth rate, survival, or reproduction in mutants accrued by mutation accumulation protocols, mutagenesis, or targeted insertions/deletions, relative to wild-type controls (SI Table 2.1). Hence, selection coefficients against accumulated mutations could be

estimated as: $\Delta\boldsymbol{\omega}_i = 1-\boldsymbol{\omega}_i^*/\boldsymbol{\omega}_i$, where $\boldsymbol{\omega}_i^*$ and $\boldsymbol{\omega}_i$ is the fitness in environment *i* of the mutant and wildtype respectively. An estimate controlling for between-study variation was retrieved by taking the log-ratio of the selection coefficient at the stressful relative to corresponding benign environment in each study: $\text{Log}_e[\boldsymbol{\Delta\omega}_{\text{stress}}/\boldsymbol{\Delta\omega}_{\text{benign}}]$, with a ratio above (below) 0 indicating stronger (weaker) selection against mutations under environmental stress. We analysed log-ratios using a Bayesian mixed effects model incorporating study ID and organism as random effects. This analysis confirmed predictions from fitness landscape theory (Martin & Lenormand 2006; Agrawal & Whitlock 2010) suggesting that selection against de novo mutation does not generally seem to be greater in stressful abiotic conditions: the 95% credible intervals for the mean ratio across all data overlapped 0 (log-ratio $\neq$ 0; P_{MCMC} = 0.094, Fig 4). As follows, the result also implies that mutational robustness is generally not more efficient in ancestral versus novel environments.

A universal temperature dependence of mutational fitness effects

We analysed the 38 estimates derived at high and low temperature stress separately from the 60 estimates derived at various other stressful environments (of which increased salinity, other chemical stressors, and food stress, were most common: SI Table 2.1). This revealed that selection on de novo mutation increases at high temperature stress (log-ratio $\leq$ 0; P_{MCMC} < 0.001), whereas there was no increase in selection at low temperature stress (log-ratio $\leq$ 0; P_{MCMC} = 0.94) or for the other forms of stress pooled (log-ratio $\leq$ 0; P_{MCMC} = 0.20). Moreover, high temperature led to a larger increase in selection relative to the other two categories (P_{MCMC} < 0.001; Fig. 4). These results do not change when using restricted maximum likelihood (SI Table 2.2a) or when estimates were removed from analysis based on if they were deemed as outliers, were considered less suitable given experimental methodology, or

when excluding the four estimates from this study (SI Table 2.2b). Analysing a reduced number of studies for which we could extract measures of mutational variance, we also show that this alternative measure of mutational effects follows the same pattern as that for the cumulative mutation load (SI 2.3).

Using the 38 paired selection estimates at contrasting temperatures we partitioned the effect of i) stress *per se*; quantified as the mean decrease in relative fitness between the benign and stressful temperature, and ii) that of the temperature shift itself; quantified as the linear (1^{st} polynomial coefficient) and non-linear (2^{nd} polynomial coefficient) effect of that magnitude and direction of the temperature shift: T_{stress} - T_{benign}. The strength of selection was not significantly related to stress ($P_{MCMC} > 0.4$). However, a shift towards warmer assay temperature *per se* caused a substantial linear increase in deleterious mutational fitness effects (b = 0.069, CI: 0.034-0.11, $P_{MCMC} < 0.001$, Fig 5B). There was also a weak non-linear effect of temperature (b = 0.007, CI: 0.0003-0.013, $P_{MCMC} = 0.034$, Fig 5A), similar to that predicted to result from unconditional mutational effects (compare Fig. 1D and Fig. 5). These results thus confirm that, as predicted by the biophysical model of enzyme kinetics, selection against de novo mutations increases universally with temperature in ectotherms.

The fitness load at mutation selection balance is predicted to equal the genomic deleterious mutation rate, but be unrelated to the mean deleterious effect of mutation (Haldane 1937, Agrawal & Whitlock 2012). The long term consequences of the revealed relationship under climate warming will therefore depend on if the predicted effects of temperature on protein folding will change the relative abundance of nearly neutral to strongly deleterious alleles

(Berhstein et al. 2006, Siegal & Leu 2014). Thus, while we have explicitly modelled increases in mutational effects, these may in the eyes of natural selection also materialize as increases in the mean number of effectively deleterious mutations. In SI 2.4 we show that the scaling relationship between the mutational variance and mean mutational effect implies that increases in both the number of (conditionally) expressed mutations as well as increases in their average fitness effect are underlying the detected increase in $\Delta\boldsymbol{\omega}$ under temperature stress, further demonstrating that our model provides an accurate account of the underlying mechanistic basis for temperature-dependent mutational fitness effects.

DISCUSSION

Early work has revealed that specific mutations can show strong temperature sensitivity, but how temperature systematically affects selection on polygenic variation across the genome, and therefore fitness and adaptive potential of whole organisms, has not been demonstrated. We have shown that temperature increases selection genome-wide by magnifying allelic effects on protein folding. Our analyses further suggest that the evolution of protein stability in response to hot climates can indirectly confer mutational robustness, but that the temperature-dependent increase in the strength of selection will be independent of the organism's thermal optimum, and hence, universal across ectothermic taxa. These result are in contrast to the non-significant effect of environmental stress *per se* on mutational fitness effects, suggesting that mutational robustness is generally not greater in benign relative to stressful environments (Martin & Lenormand 2006, Agrawal & Whitlock 2010).

The observed temperature dependence of mutational fitness effects builds a scenario in which contemporary climate warming will lead to globally accelerating rates of evolution and molecular signatures of genome-wide convergence in taxa inhabiting similar thermal environments. In support of this claim, Sabath et al. (2013) showed that growth temperature across thermophilic bacteria tend to be negatively correlated to the non-synonymous to synonymous nucleotide substitution-rate (dN/dS-ratio), suggesting stronger purifying selection in the most pronounced thermophiles. Effects could possibly extend beyond nucleotide diversity to other aspects of genome architecture. For example, Drake (2009) showed that two thermophilic microbes have substantially lower mutation rates than their seven mesophilic relatives, implying that increased fitness consequences of mutation at hot temperature can select for decreased genome-wide mutation rate. Following the same reasoning, increased mutational effects in warm climates could select for increased mutational robustness (Van Nimwegen et al. 1999, Wagner 2005, Jones et al. 2014). However, since the strength of selection on both mutation rate and mutational robustness should be proportional to the deleterious mutation rate, it remains an open question whether the increase in the efficacy of selection is strong enough to result in improved genome integrity in species with medium to small effective population sizes (Lynch 2007). Alternatively, mutational robustness could result indirectly from selection for genome features leading to increased environmental robustness (de Visser et al. 2003, Landry et al. 2007, Lehner 2010, Siegal & Leu 2014), in line with predictions from the biophysical model of enzyme kinetics suggesting that increased protein stability is beneficial at warm temperature and confers increased robustness to de novo mutation.

The increased efficacy of selection at warm temperature is predicted to influence regional patterns of standing genetic variation and future evolutionary potentials. Previous studies have highlighted a range of possible consequences of temperature on evolutionary potential in tropical versus temperature regions, including faster generation times (Gillooly et al. 2002), higher maximal growth rates (Walters et al. 2012) and higher mutation rates (Allen et al. 2006) in the former. Our results imply that also the efficacy of selection may be greater in the warmer tropical regions, which together with the aforementioned factors predict more rapid evolution and diversification, in line with the generally greater levels of biodiversity in this area. However, implications for species persistence under climate change will crucially depend on demographic parameters such as reproductive rates and effective population size (Burger & Lynch 1995, Kokko e al. 2017) and greater selection in tropical areas may even result in increased extinction rates if evolutionary potential is limited (Huey & Kingsolver 1993, Deutsch et al. 2008, Hoffmann & Sgro 2010, Walters et al. 2012). Such a scenario could be envisioned if temperature-mediated selection has led to a greater erosion of genetic variation in ecologically relevant traits, such as reported for thermal tolerance limits in tropical *Drosophila* species (Kellerman et al. 2009).

Environmental tolerance has classically been conceptualized and modelled by a Gaussian function mapping organismal fitness to an environmental gradient (e.g. Levins 1968, Bürger & Lynch 1995). In this framework stress is not generally expected to increase the mean strength of purifying selection against de novo mutation (Martin & Lenormand 2006), a prediction supported by our estimates of selection under forms of environmental stress other than hot temperature (Fig. 4). This framework assumes that mutational effects on, or standing genetic variation in, the phenotypic traits under selection remain constant across

environments. The applied biophysical model differs fundamentally from this assumption in that mutational effects on the phenotypes under selection, in form of misfolded protein, are assumed to increase exponentially with temperature. While supported by a number of targeted protein studies (Drummond & Wilke 2008, Tokuriki & Tawfik 2009, Elias et al. 2014, Sikosek & Chan 2015, Echave & Wilke 2017), it remains less clear how the effects on protein folding map to the level of morphological and life history traits, which have previously been used with varying outcome to study phenotypic effects of environmental stress (Hoffmann & Merilä 1999, Husby et al. 2011, Berger et al. 2013, Rowinski & Rogell et al. 2017). Another open question is how the unveiled universal temperature dependence interacts with other features expected to influence the distribution of fitness effects of segregating genetic variants, such as phenotypic complexity (Orr 2000, Wagner et al. 2008) and effective population size (Charlesworth 2009, Kokko et al. 2017). These questions will be crucial to answer in order to understand regional and taxonomic patterns of genetic diversity and predict evolutionary trajectories under environmental change.

Methods:

Temperature-dependent fitness effects of de novo mutations in seed beetles

Study Populations

Callosobruchus maculatus is a cosmopolitan capital breeder. Adult beetles do not require food or water to reproduce at high rates, starting from the day of adult eclosion (Fox 1993). The juvenile phase is completed in approximately three weeks, and egg to adult survival is above 90% at benign 30°C (Martinossi-Allibert et al. 2017). The lines were derived from an outbred population created by mixing beetles collected at three nearby sites in Nigeria (Fricke & Arnqvist 2007). This population was reared at 30°C on black eyed beans (*Vigna*

unguiculata), and maintained at large population size for >90 generations prior to experimental evolution. Replicate lines were kept at 30°C (ancestral lines) or exposed to gradually increasing temperatures from 30°C to stressful 36°C for 18 generations (i.e. 0.3°C/generation) and then kept at 36°C (warm-adapted lines). Population size was kept at 200 individuals for the first 18 generations and then increased to 500 individuals. In this study we compared three replicate lines of each regime.

Thermal reaction norms for juvenile survival and development rate

Previous studies have revealed significant differentiation in key life history traits between the regimes (Rogell et al. 2014, Berger et al. 2017). Here we quantified reaction norms for juvenile survival and development rate across five temperatures (23, 29, 35, 36 & 38°C) following 100 generations of experimental evolution. Prior to the assaying all six lines were raised at 30°C for two generations to ascertain that differences between evolution regimes were due to genetic effects. Newly emerged second generation adults were allowed to mate and lay eggs for 24h on new *V. unguiculata* seeds that were subsequently randomized to each assay temperature in 90mm diameter petri-dishes with ca. 100 seeds per dish with each carrying no more than 4 eggs to make sure larval food was provided ad libitum. Two dishes were set up per temperature for each line. In total we scored egg-to-adult survival for 2755 offspring. Data were analysed with survival (dead/alive) as the binomial response using generalized linear mixed effects models the lme4 package (Bates et al. 2011) for R. Temperature and selection regime as well as their interaction were included as fixed effects, and line identity crossed by temperature was added as random effect.

Temperature dependent mutational fitness effects

We compared fitness effects of induced mutations at 30°C and 36°C for each line of the two evolution regimes. At the onset of our experiments in 2015 and 2016, the populations had been maintained for 70 and 85 generations, respectively. A graphical depiction of the design can be found in SI 1. All six lines were maintained at 36°C for two generations of acclimation. The emerging virgin adult offspring of the second generation were used as the focal F0 individuals of the experiment.

We induced mutations by exposing the F0 males to gamma radiation at a dose of 20 Grey (20 min treatment). Gamma radiation causes double and single stranded breaks in the DNA, which in turn induces DNA repair mechanisms (Friedberg et al. 2006). Such breaks occur naturally during recombination, and in yeast to humans alike, point mutations arise due to errors during their repair (Friedberg et al. 2006). Newly emerged (0-24h old) virgin males were isolated into 0.3ml ventilated Eppendorf tubes and randomly assigned to either be placed inside a Gamma Cell-40 radiation source (irradiated), or on top of the machine for the endurance of the treatment (controls). After two hours at room temperature post-irradiation males were emptied of ejaculate and mature sperm by mating with females (that later were discarded) on heating plates. The males were subsequently moved back to the climate cabinet at 36°C to mature a new ejaculate. This procedure discarded the first ejaculate that will have contained damaged seminal fluid proteins in the irradiated males (Daly 2012), causing unwanted paternal effects in offspring. Irradiation did not have a mean effect on male longevity in this experiment, nor did it affect the relative ranking in male longevity among the studied populations (Berger et al. 2017), suggesting that paternal effects owing to the irradiation treatment (other than the mutations carried in the sperm)

were small. After another 24h, males were mated with virgin females from their own population. The mated females were immediately placed on beans presented ad libitum and randomized to a climate cabinet set to either 30°C or 36°C (50% RH) and allowed to lay their lifetime storage of F1 eggs.

To measure mutational effects in the F2 generation, we applied a Middle Class Neighborhood breeding design to nullify selection on all but the unconditionally lethal mutations amongst F1 juveniles (Shabalina et al. 1997). This approach allowed us to quantify the cumulative deleterious fitness effect of all but the unconditionally lethal mutations induced in F0 males (i.e. mutation load, $\Delta\boldsymbol{\omega}$) by comparing the production of F2 adults in irradiated lineages, relative to the number of adults descending from F0 controls: $\Delta\boldsymbol{\omega} = 1-\boldsymbol{\omega}_{\mathrm{IRR}}/\boldsymbol{\omega}_{\mathrm{CTRL}}$ (SI 1). We also calculated $\Delta\boldsymbol{\omega}$ from F1 adult counts, acknowledging that this estimate may include non-trivial paternal effects from the irradiation treatment. However, results based on F1 and F2 estimates were consistent (Fig 3).

To estimate the effects of high temperature on mutational fitness effects in the two genetic backgrounds, we used Restricted Maximum Likelihood (REML) linear mixed effects models testing for interactions between radiation treatment, assay temperature and evolution regime. As mutation load is quantified as offspring production in irradiated lineages *relative* to corresponding controls, offspring counts were log-transformed before REML analysis.

Meta-analysis of selection on de novo mutation in good and bad environments

Using raw data, tables or figures, we collated data from studies that had measured fitness effects of de novo mutations in at least two environments, of which one had been labelled

stressful relative to the other by the researchers of the study. In all but two cases analysed this labelling was correct in the sense that fitness estimates, based either on survival, reproductive output or population growth rate, were lower in the environment labelled as stressful. In the remaining two cases, the temperature assigned as stressful did not have an effect on the nematode *Caenorhabditis briggsae* (Baer et al. 2006); these estimates were therefore excluded when analysing effects of environmental stress on selection (Fig. 4), but included when analysing the effect of temperature (Fig 5). The studies measured effects of mutations accrued by mutation accumulation, mutagenesis, or targeted insertions/deletions, relative to wild-type controls. We found a few cases that were excluded from analysis since it seemed likely that the protocol used to accrue mutations (mutation accumulation at population sizes >2) may have failed to remove selection, biasing subsequent comparisons of mutational fitness effects across environments. In total we retrieved 98 paired estimates of selection from 27 studies and 11 organisms, spanning unicellular viruses and bacteria to multicellular plants and animals (summary in SI 2).

An estimate controlling for between-study variation was calculated by taking the log-ratio of the cumulative fitness effect of the induced mutations at stressful relative to corresponding benign conditions in each study: $LOG_e[\boldsymbol{\Delta\omega}_{stress}/\boldsymbol{\Delta\omega}_{benign}]$, where $\boldsymbol{\Delta\omega = 1 - \omega_{IRR}/\omega_{CTRL}}$. Hence, a ratio above (below) 0 indicates stronger (weaker) selection against mutations under stress. We used both REML and Bayesian linear mixed effects models (available in the MCMCglmm package (Hadfield 2010) for R) to estimate if log-ratios differed from 0 for three levels of environmental stress: cold temperature, warm temperature, and other types of stress (Table SI 3A), as well as for the total effect of stress averaged across all studies. We also tested if log-ratios differed between the three types of abiotic stress. All models included stress-type,

mutation induction protocol and fitness estimate as main effects, although effects of the latter two were never significant. We included study organism and study ID as random effects. The MCMC resampling ran for 1.000.000 iterations, preceded by 500.000 burn-in iterations that were discarded. Every 1000^{th} iteration was stored, resulting in 1000 independent posterior estimates from each model. We used weak and unbiased priors for the random effects.

Using the 38 estimates that compared the strength of selection across temperatures, we partitioned the effect of i) temperature stress; quantified as the mean relative drop in fitness between the benign and stressful temperature, and ii) that of temperature itself; quantified as the linear (1^{st} polynomial coefficient) and non-linear (2^{nd} polynomial coefficient) effect of the magnitude and direction of the temperature shift: T_{stress} - T_{benign}. We included stress and temperature as the two fixed effect covariates, and study organism and study ID as random effects.

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Figure Legends:

Fig. 1. Predicted consequences of mutation (ΔΔG =+1, dashed lines) on A) enzyme kinetics, B) biological rate and C) the strength of selection for a cold-adapted (blue; ΔG = -6, $H^{\#}$ = 19.3, Γ = 100, T_{OPT} = 20 ^{o}C, θ = 0.01) vs. a hot-adapted genotype (red; ΔG = -8, $H^{\#}$ = 20.0, Γ = 20, T_{OPT} = 35 ^{o}C, θ = 0.01). Cold- and hot-adapted genotypes show D) the same increase in the strength of selection for a given temperature increase relative to a standardised benign reference temperature, defined here as $T_{REF} = T_{OPT}$ -10^{o}C. The temperature dependence of selection is predicted to D) decrease with unconditional mutational effects (solid line: θ = 0, medium dash θ = 0.01 and short dash θ = 0.1) and increase with the level of thermal specialisation (here ΔΔG(T) = 0.25; for ΔΔG(T) = 0.5 see supplementary).

Fig 2: Level of adaptation to simulated climate warming measured as (A) adult offspring production at 30 and 36°C, and thermal reaction norms for (B) juvenile survival and (C) development rate (means ± 95% confidence limits). Blue and red symbols denote ancestral and warm-adapted lines, respectively. Although there are clear signs of GxE for offspring production (P = 0.007), reaction norms for survival and development rate show no clear differences in temperature dependence among the three replicate ancestral and warm-adapted lines. Instead, ancestral lines show generally faster development (P < 0.001) but lower survival (P = 0.053) across temperatures.

Fig. 3: Mutation load (**Δω**) (mean ± 95% confidence limits) measured for (A) F1 juvenile survival and (B) F2 adult offspring production, at the two assay temperatures. There was an overall strong and significant increase in **Δω** at hot temperature. This effect was similar

across the three ancestral (blue) and three warm-adapted (red) lines, in both the F1 ($P < 0.001$) and F2 generation ($P = 0.006$).

Fig. 4: Meta-analysis of the effect of abiotic stress on the mean strength of selection against de novo mutations analysed by log-ratios (Bayesian posterior modes ± 95% credible intervals): $\boldsymbol{\Delta\omega}_{stress}/\boldsymbol{\Delta\omega}_{benign}>0$ correspond to stronger selection under environmental stress. The 96 paired estimates of $\boldsymbol{\Delta\omega}$ show that selection is not greater in stressful environments overall ($P = 0.09$) and highly variable across the 27 studies. However, estimates of $\boldsymbol{\Delta\omega}$ at high temperature are greater than their paired estimates at benign temperature ($P<0.001$). The box shows the eleven species, covering all major groups of the tree of life, included in the analysis. See main text and Supplementary 3 for further details.

Fig. 5: Temperature-dependent mutational fitness effects. In (**A**) the strength of selection on de novo mutations as a function of the direction and magnitude of the temperature shift between the benign and stressful temperature across the 14 studies analysed. In (**B**) the same relationship for the seven species analysed, controlled for study ID, the method used to induce mutations and the non-linear effect of temperature. Selection generally increases with temperature ($P_{MCMC} < 0.001$) whereas stress per se (quantified as the reduction in relative fitness between the benign and stressful temperature) did not affect the strength of selection ($P_{MCMC} > 0.2$).

Figure 1

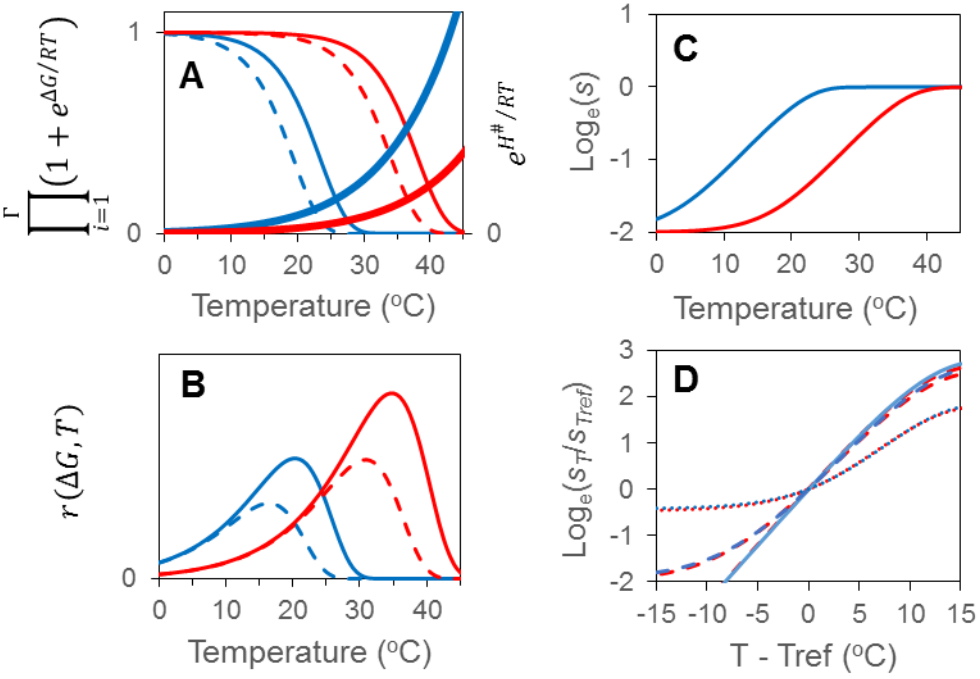


Figure 2

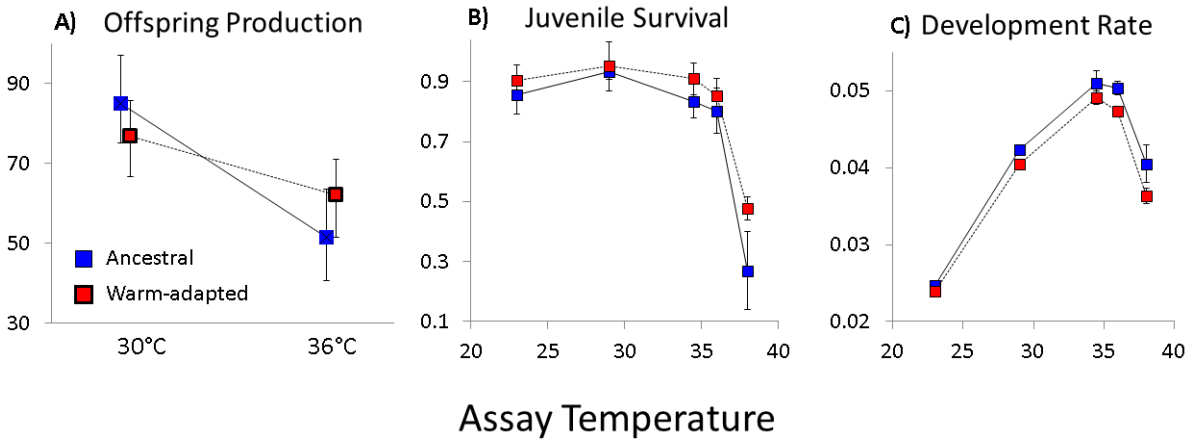


Figure 3

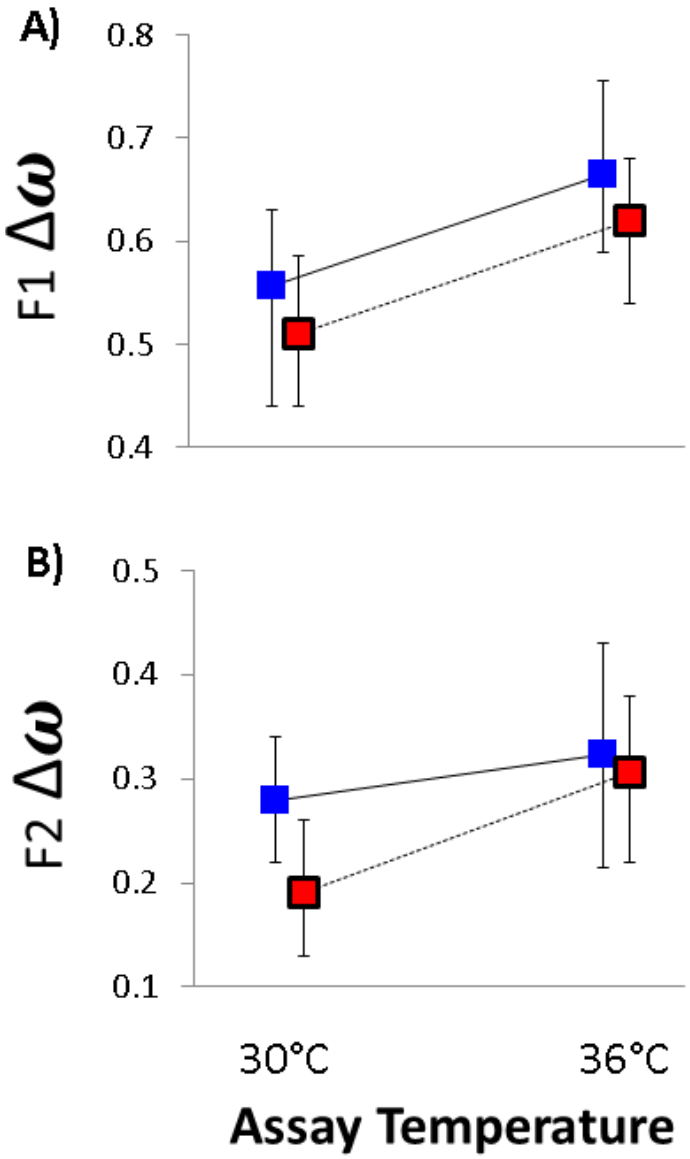


Figure 4

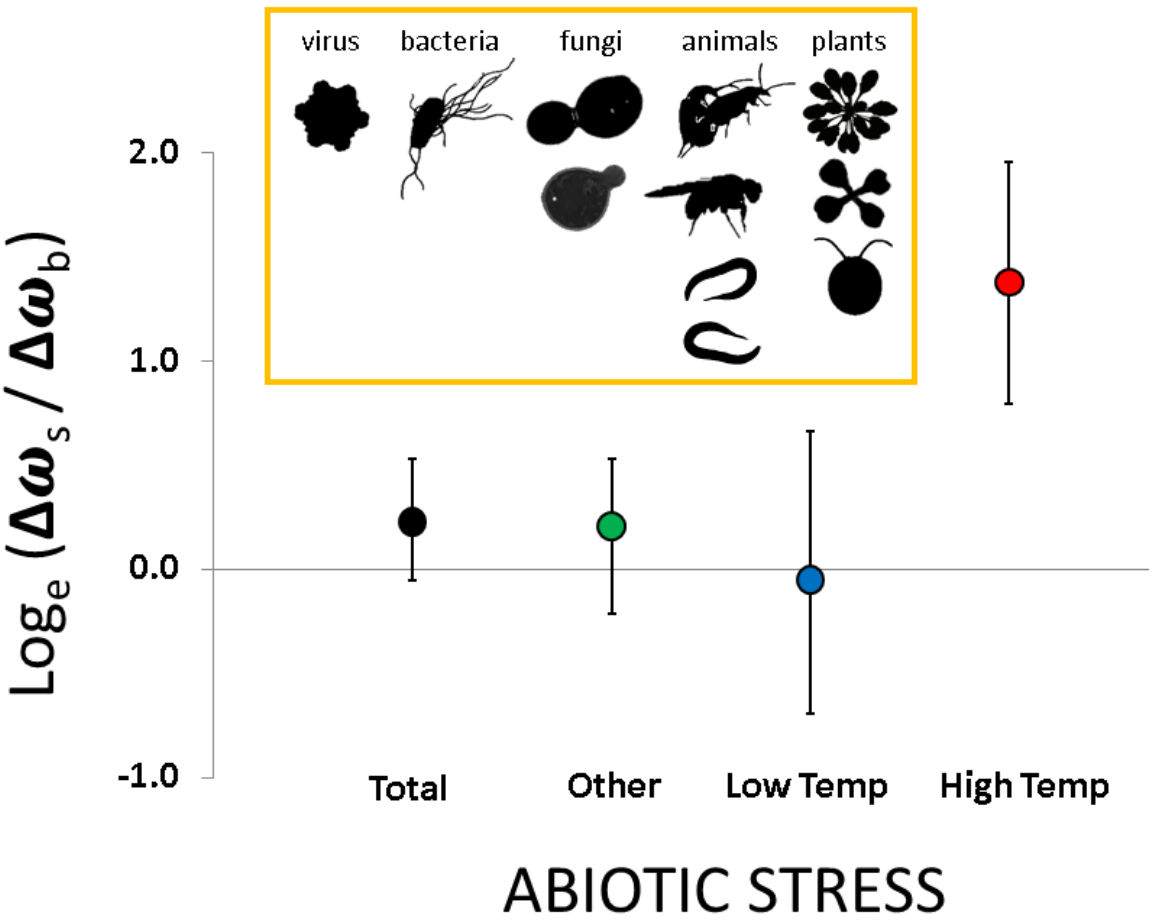
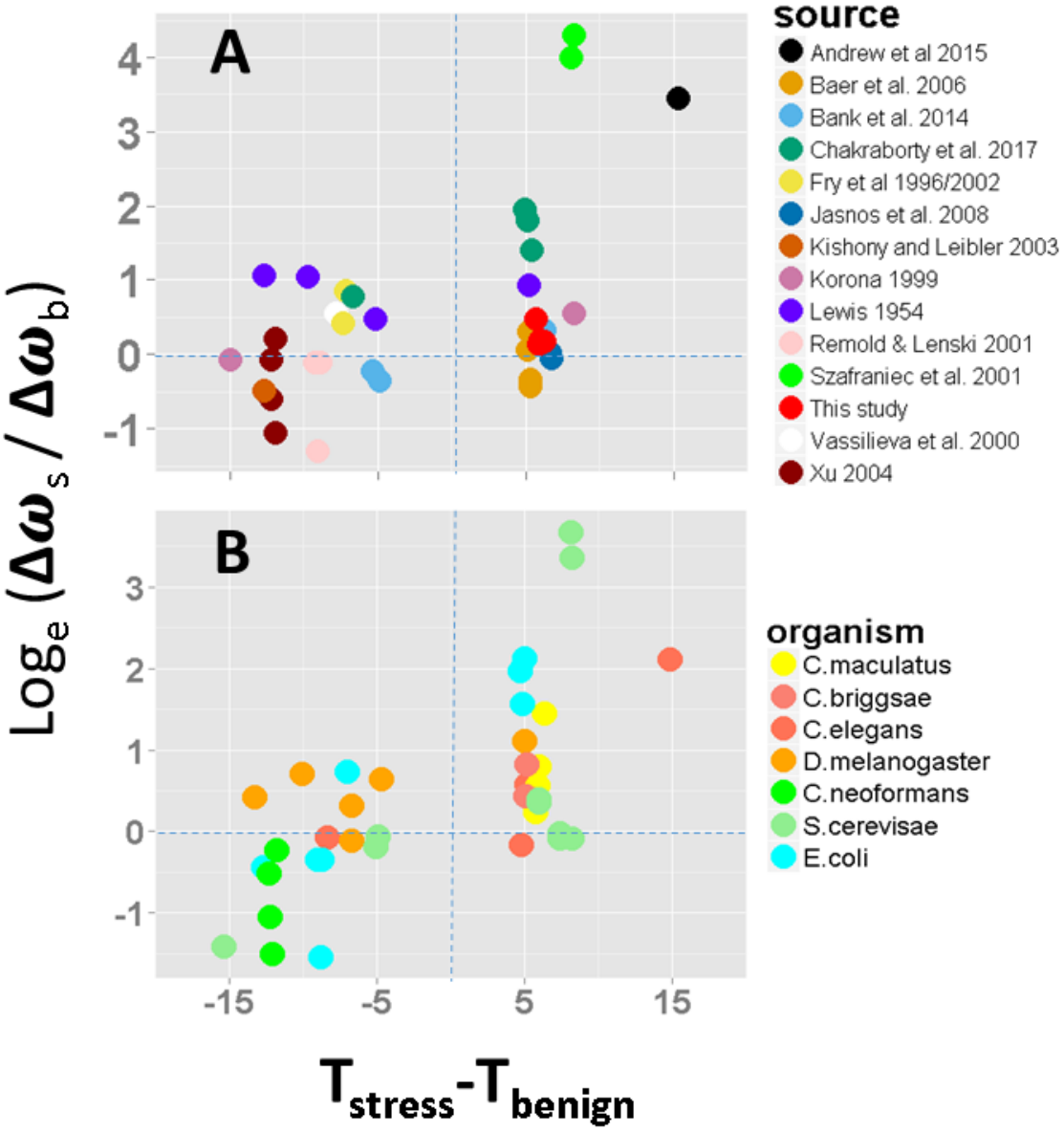


Figure 5



Supplementary Information 1

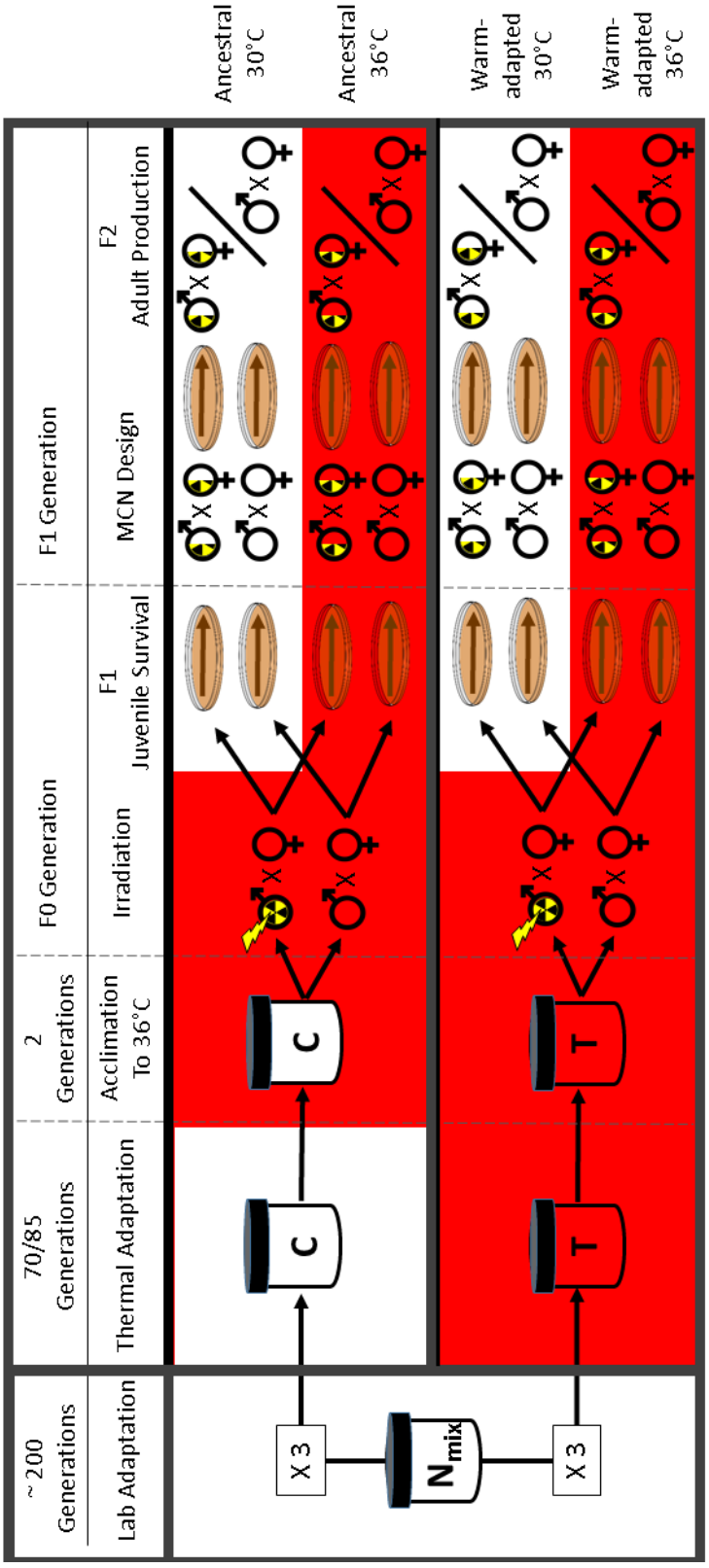


Fig SI 1.1: Broad schematic overview of the experimental design used to induce and measure mutations in Callosobruchus maculatus (see *Methods* for details).

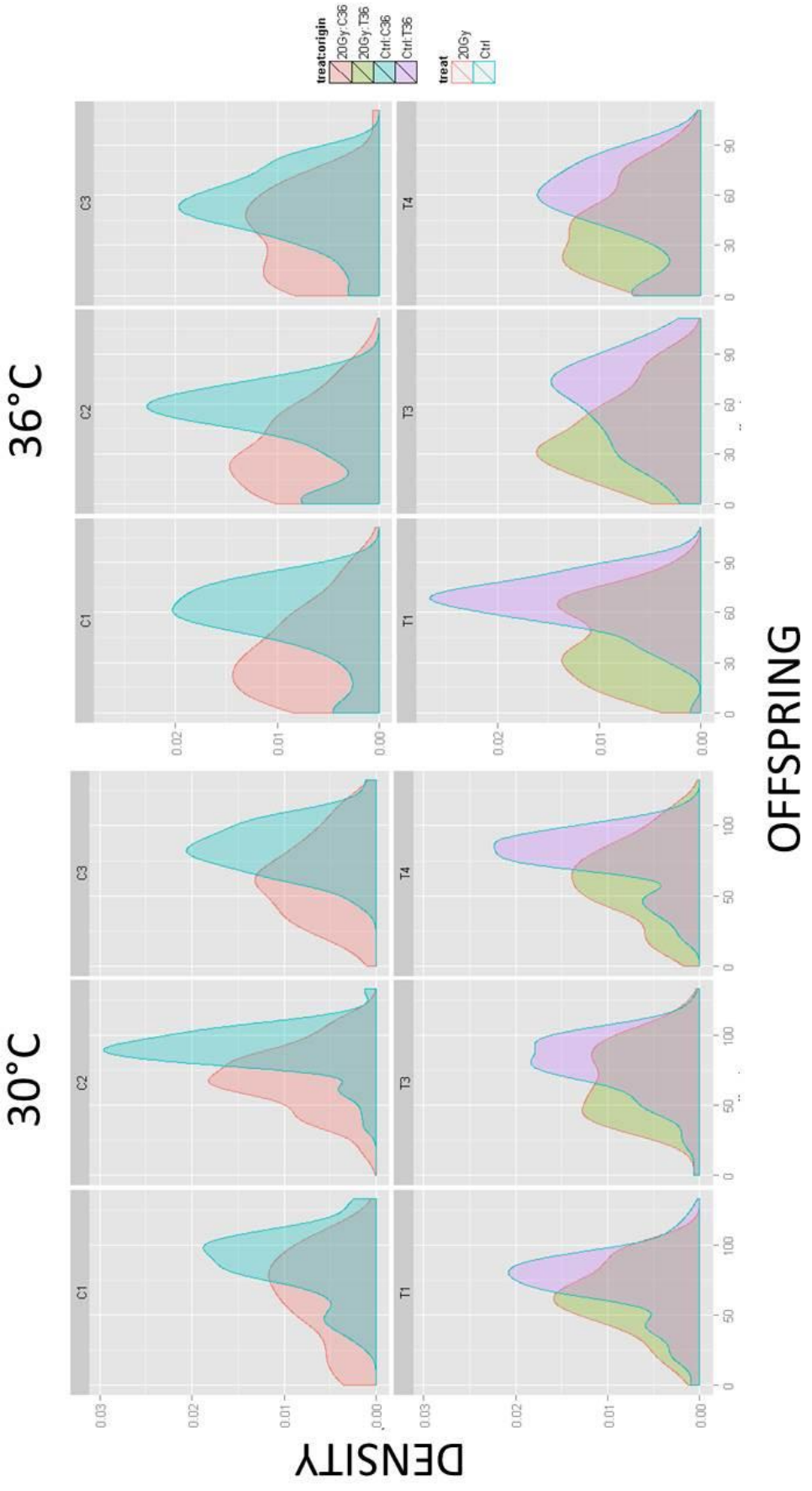


Fig SI 1.2: Density distribution of lifetime F2 offspring production in the irradiated and control lineages for each line and temperature (C = ancestral lines, T = warm-adapted lines).

Supplementary Information 2: Meta-Analysis of Mutational Effects

Table SI 2.1: Summary of the 98 estimates comparing selection on de novo mutation in benign and stressful environments, from 27 published studies on 11 species.

Andrew et al. 2015, compared the top and bottom 5 MA lines in terms of fitness across environments to assess mutational effects. However, since ranking was done in the benign environment, the cross-environment comparison is potentially biased (Halligan & Keightley 2009). Here we therefore averaged the 10 MA lines and compared them to available proper controls to estimate selection. For Dandage et al. (2017) selection was calculated from estimates of presence/absence (implying survival) rather than absolute measures of growth rate since (non-synonymous) mutants only had proper controls (synonymous mutants) in each environment for this measure. In Szafraniec et al. (2001) one measure of the ratio $LOG(\Delta\omega_{stress}/\Delta\omega_{benign})$ was extremely large because selection at the benign temperature was ~0. Therefore, this ratio was set equal to the paired estimate from the same study (=4.31). This is thus a conservative estimate of the increase in selection at hot temperature. Stress in terms of fitness in the stressful relative to the benign environment (ω_s/ω_b) was calculated from the studies except in two studies when it was derived from other studies (these estimates denoted [a] and [b] respectively).

Organism	Method	Measure	stressor	T_{stress}	T_{benign}	w_s/w_b	$Log(\Delta w_s/\Delta w_b)$	$Log(V_s/V_b)$	source
A. thaliana	MA	growth rate	other				0	1.63	Rutter et al. 2010.
A. thaliana	MA	reproduction	other				0	0	Chang & Shaw 2003
A. thaliana	MA	reproduction	other				0	0.16	Chang & Shaw 2003
A. thaliana	MA	reproduction	other				0	0	Chang & Shaw 2003
A. thaliana	MA	reproduction	other				0	0	Kavanaugh & Shaw 2005
A. thaliana	MA	reproduction	other				0	0	Kavanaugh & Shaw 2005
C. briggsae	MA	reproduction	none	25	20	0.96	0.06	-0.42	Baer et al. 2006
C. briggsae	MA	reproduction	none	25	20	0.95	-0.33	-0.01	Baer et al. 2006
C. elegans	MA	reproduction	high	25	20	0.57	0.32	-0.12	Baer et al. 2006
C. elegans	MA	reproduction	high	25	20	0.57	-0.42	-0.07	Baer et al. 2006
C. elegans	MA	growth rate	low	12	20	0.30	0.56	3.50	Vassilieva et al. 2000
C. elegans	MA	reproduction	other				0.05	0.20	Andrew et al 2015
C. elegans	MA	reproduction	other				0.10	0.49	Andrew et al 2015
C. elegans	MA	survival	high	35	20	0.4	3.44	4.65	Andrew et al 2015
C. elegans	MA	survival	other				-0.25	-0.84	Andrew et al 2015
C. elegans	MA	survival	other				3.02	0.90	Andrew et al 2015
C. maculatus	mut.gen	reproduction	high	36	30	0.74	0.48	-0.31	This study
C. maculatus	mut.gen	reproduction	high	36	30	0.59	0.14	-0.07	This study
C. maculatus	mut.gen	survival	high	36	30	0.75	0.20		This study
C. maculatus	mut.gen	survival	high	36	30	0.59	0.17		This study
C. neoformans	MA	growth rate	other			0.47	0.91	-1.44	Xu et al. 2004
C. neoformans	MA	growth rate	other			0.47	0.40	0.02	Xu et al. 2004
C. neoformans	MA	growth rate	other			0.47	0.46	0.42	Xu et al. 2004
C. neoformans	MA	growth rate	other			0.47	0.12	-2.92	Xu et al. 2004
C. neoformans	MA	growth rate	low				-0.60		Xu et al. 2004
C. neoformans	MA	growth rate	low	25	37		0.21		Xu et al. 2004
C. neoformans	MA	growth rate	low	25	37		-1.06	0.37	Xu et al. 2004
C. neoformans	MA	growth rate	low	25	37		-0.07	-2.00	Xu et al. 2004

Table SI 2.1 (Continued)

Organism	Method	Measure	stressor	T_{stress}	T_{benign}	w_s/w_b	$Log(\Delta w_s/\Delta w_b)$	$Log(V_s/V_b)$	source
C. reinhardtii	MA	growth rate	other				1.63		Kraemer et al. 2016
C. reinhardtii	MA	growth rate	other				0.64		Kraemer et al. 2016
C. reinhardtii	MA	growth rate	other				0.56		Kraemer et al. 2016
C. reinhardtii	MA	growth rate	other				0.41		Kraemer et al. 2016
D. melanogaster	ins/del	reproduction	other				0.13		MacLellan et al. 2012
D. melanogaster	ins/del	reproduction	other				-0.08		MacLellan et al. 2012
D. melanogaster	ins/del	survival	other				0.34	0.11	Wang et al. 2009
D. melanogaster	ins/del	survival	other				0.29	0.57	Wang et al. 2009
D. melanogaster	ins/del	survival	other				0.25		Wang et al. 2009
D. melanogaster	ins/del	survival	other				0.39		Wang et al. 2014
D. melanogaster	ins/del	survival	other				0.38		Wang et al. 2014
D. melanogaster	ins/del	survival	other				-0.15		Wang et al. 2014
D. melanogaster	ins/del	survival	other				-0.35		Wang et al. 2014
D. melanogaster	ins/del	survival	other				0.12		Young et al. 2009
D. melanogaster	MA	survival	low	18	25	0.94[a]	0.42	2.83	Fry and Heinsohn 2002
D. melanogaster	MA	survival	other				0.31	1.86	Fry and Heinsohn 2002
D. melanogaster	MA	survival	low	18	25	0.94[a]	0.86	-0.62	Fry et al. 1996
D. melanogaster	MA	survival	other				0.00	0.00	Fry et al. 1996
D. melanogaster	MA	survival	other				-0.19	0.07	Fry et al. 1996
D. melanogaster	mut.gen	reproduction	other				-0.25	-1.42	Yang et al. 2001
D. melanogaster	mut.gen	survival	high	30	25	0.75[a]	0.94		Lewis 1954
D. melanogaster	mut.gen	survival	low	20	25	0.96[a]	0.48		Lewis 1954
D. melanogaster	mut.gen	survival	low	15	25	0.75[a]	1.04		Lewis 1954
D. melanogaster	mut.gen	survival	low	12	25	0.63[a]	1.06		Lewis 1954
E. coli	ins/del	growth rate	other				0.57		Cooper et al. 2005
E. coli	ins/del	growth rate	low	28	37	0.75[b]	-1.30	-0.23	Remold & Lenski 2001
E. coli	ins/del	growth rate	low	28	37	0.75[b]	-0.11	-0.31	Remold & Lenski 2001
E. coli	ins/del	growth rate	low	28	37	0.75[b]	-0.11	-0.56	Remold & Lenski 2001
E. coli	ins/del	growth rate	other				2.15	6.56	Remold & Lenski 2001
E. coli	ins/del	growth rate	other				1.39	5.16	Remold & Lenski 2001
E. coli	ins/del	growth rate	other				3.69	2.88	Remold & Lenski 2001
E. coli	mut.gen	growth rate	low	17	30	0.35[b]	-0.49	0.38	Kishony and Leibler 2003
E. coli	mut.gen	growth rate	other				-0.53	-0.16	Kishony and Leibler 2003
E. coli	mut.gen	growth rate	other				-0.03	0.05	Kishony and Leibler 2003
E. coli	mut.gen	growth rate	other				-0.09	0.39	Kishony and Leibler 2003
E. coli	mut.gen	growth rate	other				-0.26	0.45	Kishony and Leibler 2003
E. coli	mut.gen	growth rate	other				0.09	0.47	Kishony and Leibler 2003
E. coli	mut.gen	growth rate	other				-0.89		Kishony and Leibler 2003
E. coli	mut.gen	survival	high	42	37	0.83[b]	1.96		Chakraborty et al. 2017
E. coli	mut.gen	survival	high	42	37	0.83[b]	1.41		Chakraborty et al. 2017
E. coli	mut.gen	survival	high	42	37	0.83[b]	1.81		Chakraborty et al. 2017
E. coli	mut.gen	survival	low	30	37	0.83[b]	0.77		Chakraborty et al. 2017
E. coli	mut.gen	survival	other				0.33		Chakraborty et al. 2017
E. coli	mut.gen	survival	other				-0.23		Chakraborty et al. 2017
E. coli	mut.gen	survival	other				-0.71		Chakraborty et al. 2017
E. coli	mut.gen	survival	other				-0.87		Chakraborty et al. 2017
Phage φX174	ins/del	growth rate	other				0.60		Vale et al. 2012
R. raphanistrum	MA	reproduction	other				1.04		Roles and Conner 2008
S. cerevisae	MA	growth rate	high	38	30	0.54	4.31	1.60	Szafraniec et al. 2001
S. cerevisae	MA	growth rate	high	38	30	0.73	4.31	9.74	Szafraniec et al. 2001
S. cerevisae	MA	growth rate	other				-0.37	1.28	Korona 1999
S. cerevisae	MA	growth rate	high	38	30	0.64	0.55	3.65	Korona 1999
S. cerevisae	MA	growth rate	low	15	30	0.22	-0.07	1.54	Korona 1999
S. cerevisae	MA	growth rate	other				0.07	0.04	Korona 1999
S. cerevisae	MA	growth rate	other				-0.27	0.31	Korona 1999

Table SI 2.1 (Continued)

Organism	Method	Measure	stressor	T_{stress}	T_{benign}	w_s/w_b	$Log(\Delta w_s/\Delta w_b)$	$Log(V_s/V_b)$	source
S. cerevisae	ins/del	growth rate	high	37	30	0.80	0.02	0.13	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	high	37	30	0.81	-0.05	0.45	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	other				-0.52	-0.80	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	other				-0.78	-0.53	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	other				-0.51	-0.41	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	other				-0.22	0.01	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	other				-0.70	0.14	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	other				-0.24	0.25	Jasnos et al. 2008
S. cerevisae	ins/del	growth rate	high	36	30	0.83	0.34	0.35	Bank et al. 2014
S. cerevisae	ins/del	growth rate	high	36	30	0.73	0.29	0.45	Bank et al. 2014
S. cerevisae	ins/del	growth rate	low	25	30	0.63	-0.22	-0.88	Bank et al. 2014
S. cerevisae	ins/del	growth rate	low	25	30	0.67	-0.36	-0.73	Bank et al. 2014
S. cerevisae	ins/del	growth rate	other				-1.07	-2.30	Bank et al. 2014
S. cerevisae	ins/del	growth rate	other				-1.20	-2.16	Bank et al. 2014
S. cerevisae	ins/del	growth rate	other				-1.25	-2.06	Bank et al. 2014

***MA* refers to mutation accumulation, *mut.gen* to mutagenesis (inducing mutations through radioactivity, UV-radiation or chemical mutagens), and *ins/del* refers to insertions or deletions. Fitness measures were provided through estimates of reproduction (e.g. female egg/offspring production), survival (e.g. egg-to-adult survival) or growth rate (propagation through clonal growth; Malthusian fitness). Estimates of relative fitness at benign and stressful temperature for D. melanogaster and E.coli were derived from Schou et al. 2017([a]) and Bronikowski et al. 2001 ([b]) respectively. For further information, see main text.**

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Table SI 2.2a: Full REML mixed effect model on changes in $Log_e(\Delta\omega_{stress}/ \Delta\omega_{benign})$.

Random effects:

Groups	Name	Variance	Std.Dev.
source	(Intercept)	0.4429	0.6655
organism	(Intercept)	0.0000	0.0000
Residual		0.5366	0.7325

Number of obs: 96, groups: source, 27; organism, 11

Fixed effects: (contrasts reported against model intercept: Mutational effects measured on growth rate at high temperature stress, for mutations introduced by insertions/deletions)
Fixed effects:

	Estimate	Std. Error	t value
Intercept (HighT:ins/del:growth rate)	1.2142	0.3807	3.189
LowT	-1.3192	0.3066	-4.303
Other	-1.1166	0.2510	-4.448
Method:MA	0.5133	0.3652	1.405
Method:mutagenesis	-0.1304	0.4611	-0.283
Measure:reproduction	-0.5927	0.3831	-1.547
Measure:survival	0.1576	0.3569	0.442

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)
stress	11.0072	2	79.339	6.041e-05 ***
method	1.2260	2	17.687	0.3172
measure	2.0718	2	11.788	0.1694

Table SI 2.2b: Anova tables for alternative analyses excluding studies based on suitability. As measurement errors were hard to come by consistently for the analyzed studies, we checked the robustness of our results by performing a set of alternative analyses excluding studies which inclusion could be questioned based on methodology (MA protocols applying weak selection specific to the MA-environment), the generality of the result (e.g. Banks et al. targeted mutations to the HSP90-gene, which a priori might be predicted to affect robustness under stress), or their influence on the analyses in terms of being extreme observations (e.g. Szafraniec et al. 2001 with log-ratios >3).

#Testing bias of methodology: Model without studies using mutation accumulation:

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)
stress	4.7963	2	47.132	0.01271 *
method	0.2137	1	6.492	0.65899
measure	0.1597	2	10.398	0.85445

#Testing influence of extreme values: Model without estimates of log-ratios >3.

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)
stress	7.0545	2	75.332	0.001555 **
method	0.9474	2	14.200	0.410960
measure	0.2680	2	24.376	0.767134

#Testing influence of HSP90 targeted mutations and recalculation of selection coefficients from Andrew et al. 2015: Model without Banks et al. 2014 and Andrew et al. 2015:

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)
stress	8.2156	2	67.359	0.0006416 ***
method	0.3917	2	15.957	0.6822160
measure	0.7251	2	8.134	0.5131333

#Excluding our own estimates: Without Berger et al. 2017.

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

	F	Df	Df.res	Pr(>F)
stress	11.3948	2	74.244	4.832e-05 ***
method	1.0243	2	16.246	0.3811
measure	2.4377	2	10.040	0.1371

Table SI 2.3: Estimates of mutational variance across environments in REML analysis

Mutational variance (ΔV) is typically quantified as the excess variance found among mutation accumulation lines or lineages exposed to mutagenesis (as in this study), relative to variance found among control strains. Alternatively, it is estimated from the among- MA line variance component in an ANOVA. Effects of stress on mutational effects were also analyzed in terms of ΔV, which is expected to follow the same patterns as the changes in the cumulative deleterious fitness effect (mutation load; $\Delta\boldsymbol{\omega}$). Indeed, when replacing the log-ratio of $\Delta\boldsymbol{\omega}$ across stressful and benign environments with the log-ratio of ΔV, we found the same qualitative pattern, with high temperature stress resulting in more mutational variance compared to cold temperature and other forms of stress. The same qualitative results were attained in Bayesian analysis (presented in Figure SI 2.3).

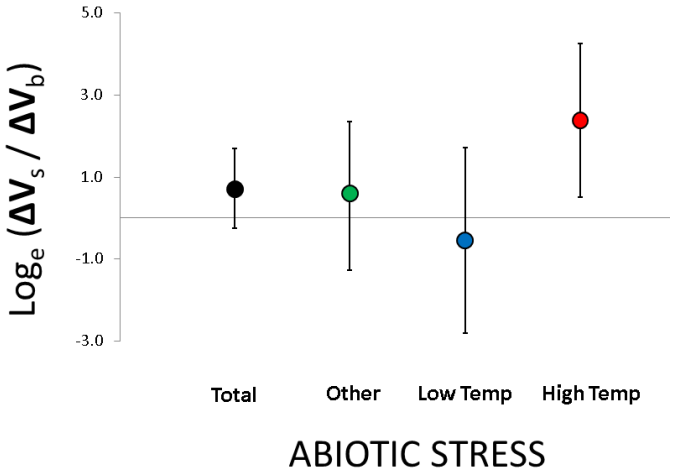
```
Random effects:
 Groups   Name        Variance Std.Dev.
 source   (Intercept) 1.408    1.186
 organism (Intercept) 0.000    0.000
 Residual             2.441    1.562
Number of obs: 64, groups:  source, 17; organism, 8

Fixed effects:
                    Estimate Std. Error t value
(Intercept)           1.9369     0.9140   2.119
stresslow            -2.6560     0.7456  -3.562
stressother          -1.6179     0.6150  -2.631
methodMA              1.9228     1.0146   1.895
methodmutagenesis     0.2924     1.2907   0.227
measurereproduction  -2.4125     0.9170  -2.631
measuresurvival      -1.1324     1.0503  -1.078

Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)

Response: logratio.V
             F Df Df.res   Pr(>F)
stress  5.3543  2 52.081 0.007679 **
method  1.8365  2  8.169 0.219452
measure 2.6469  2 13.984 0.105957
```

Fig SI 2.3: Effects of environmental stress and temperature on mutational variances (Bayesian mode ± 95% credible intervals).



SI 2.4: Estimating number vs effect of mutations via relationships between mutation load and mutational variance.

We explicitly modelled increases in mean mutational effects across temperature. Moreover, the analysed studies, including our own, induced a fixed number of mutations to be compared across environments. Nevertheless, environmentally induced changes in mutational effects may in the eyes of natural selection also materialize as increases in the mean number of effectively deleterious (and no longer quasi-neutral) mutations. Assuming that the number of accumulated mutations is approximated by a Poisson process, and that variance in effects of single mutations (coefficients of mutational variation: CV_m) are constant across environments, we can approximate changes in the number and effect of deleterious mutations across environments by comparing mutation load ($\Delta\boldsymbol{\omega}$) and mutational variance (ΔV) (Bateman 1959, Mukai 1964). If the observed increase in $\Delta\boldsymbol{\omega}$ at temperature stress is only due to the number of deleterious mutations, then changes in ΔV are predicted to be proportional to changes in $\Delta\boldsymbol{\omega}$ across environments. On the other hand, if increases in $\Delta\boldsymbol{\omega}$ were only due to increases in the average fitness effects, then $\Delta V \sim \Delta\boldsymbol{\omega}^2$ (Bateman 1959. Mukai 1964). Thus, changes in only the number of mutations should yield: $\text{Log}(\Delta V) \sim \text{Log}(\Delta\boldsymbol{\omega})$, whereas changes in only mutational effects should yield: $\text{Log}(\Delta V) \sim 2\text{Log}(\Delta\boldsymbol{\omega})$, and changes in both the number and average effects should yield logarithmic exponents between 1 and 2.

We tested these predictions by regressing $\text{Log}_e(\Delta V_{stress}/\Delta V_{benign})$ on $\text{Log}_e(\Delta\boldsymbol{\omega}_{stress}/\Delta\boldsymbol{\omega}_{benign})$ for each stress type by applying Standardized Major Axis regression using the smatr package (Warton et al. 2012) for R. We did not find any significant difference in this relationship between high and low temperature, so we pooled all estimates of temperature stress and compared the relationship to that found for other kinds of stress, resulting in 25 estimates for temperature stress and 38 estimates for other forms of stress. This showed that there was no significant difference in the relationship between the two types of stress (LR = 0.03, df = 1, P = 0.87: slope temperature = 1.73, slope other = 1.68, Fig S3.2c). We also performed an analysis excluding extreme observations, with an arbitrary cut-off set at log-ratios greater than 3, removing five observations. This gave the same qualitative result (difference in the relationship between stressors: LR = 0.49, df = 1, P = 0.49).

For the full dataset, the log-ratio of mutational variance increased with a factor of 1.70 (R^2 = 0.52, P < 0.001) which 95% CI (1.43-2.03) did not overlap 1, suggesting that increases in $\Delta\boldsymbol{\omega}$ under stress cannot be explained by increases in the number of deleterious mutations only. For the dataset with extreme observations removed, ΔV increased with $\Delta\boldsymbol{\omega}$ by a factor of 2.09 (95% CI: 1.67-2.63, R^2 = 0.31, P < 0.001). Together this suggests that increases in both the number of (conditionally) expressed mutations as well as their average fitness effect are likely to underlie the increase in $\Delta\boldsymbol{\omega}$ under temperature stress, and thus, that our model provides an accurate representation of the mechanistic basis for temperature-dependent mutational fitness effects

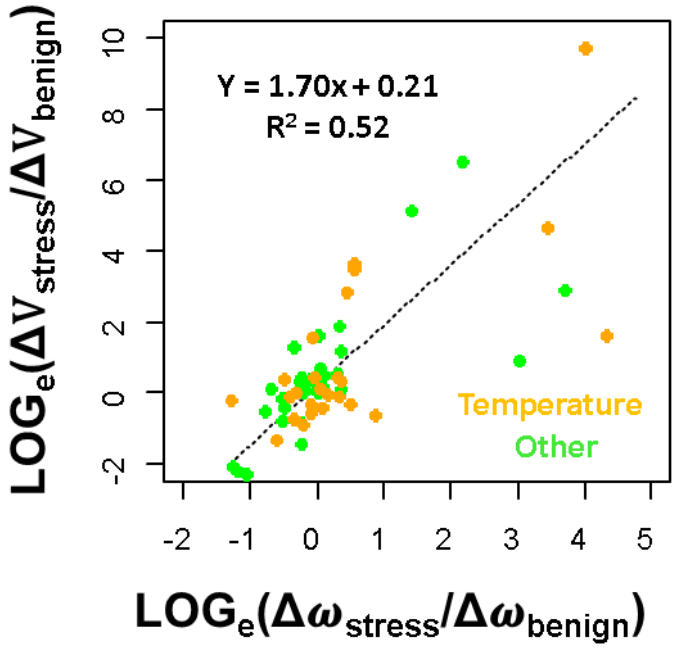


Fig SI 2.4: Relationship between the log-ratio of mutational variance ($\Delta V_{stress}/\Delta V_{benign}$) and mutation load ($\Delta\boldsymbol{\omega}_{stress}/\Delta\boldsymbol{\omega}_{benign}$) in the stressful and benign environment for the full dataset (temperature stress: orange points, other forms of stress: black points). The hatched line gives the Standardized Major Axis regression slope.

The conclusions above rely on that variation in effects of single mutations (CV_m) do not change on average across environments (reviewed in Halligan & Keightley 2009). If not, an increase in CV_m could contribute to ΔV unequally across environments, and therefore complicate interpretations of the relationship between ΔV and $\Delta\boldsymbol{\omega}$. Martin & Lenormand (2006) have suggested a method to account for this potential bias by regressing ratios of the Bateman-Mukai estimator of the average mutational effect across stressful and benign environments (s_{stress}/s_{benign}) on ratios of corresponding variances ($\Delta V_{stress}/\Delta V_{benign}$) (see Martin & Lenormand 2006 for further details). However, since the Bateman-Mukai estimate of $s = \Delta V/\Delta\boldsymbol{\omega}$, this regression violates the assumption of independent measurement error in x and y. There is great uncertainty in estimates of ΔV and s, irrespective of the method used to obtain them (Halligan & Keightley 2009), and regression analysis on this kind of data without accounting for measurement error can lead to very biased estimates and erroneous conclusions (Berger & Postma 2014). Hence, since estimates of measurement error was hard to come by for much of the data, and therefore even harder to correct for, we simply note that there is a potential bias incurred by assuming constant variance in effects of single mutations across environments.

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