A Universal Temperature-Dependence of Mutational Fitness Effects

David Berger[1], Josefine Stångberg[1], Julian Baur[1] & Richard J. Walters[2].*

1. Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University. Norbyvägen 18D, 75236 Uppsala, Sverige.

2. School of Biological Sciences, University of Reading. Whiteknights, Reading, RG6 6BX**,** United Kingdom

* Corresponding author: David Berger

Email: David.berger@ebc.uu.se

Telephone: +46 18 4712662

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INTRODUCTORY PARAGRAPH

Natural environments are constantly changing so organisms must also change 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 discriminates among this variation to favour the survival of the fittest. We apply a biophysical model of protein evolution to demonstrate that rising global temperatures are expected to intensify natural selection systematically throughout the genome by increasing the effects of sequence variation on protein phenotypes. Furthermore, warm and cold adapted genotypes are expected to show similar temperature-dependent increases in selection. We tested these predictions by i) estimating selection on induced mutations in seed beetles adapted to either ancestral or warm temperature, and ii) calculating 100 paired selection estimates on de novo mutations from the literature in a diverse set of unicellular and multicellular ectothermic organisms. We show that environmental stress *per se* generally does not increase the strength of selection on new mutations. However, elevated temperature systematically increased selection on genome-wide polymorphism. Our model and the data suggest that this increase corresponds to a doubling of genome-wide selection for a predicted 2-4°C climate warming scenario in organism living at temperatures close to their thermal optimum. These results have fundamental implications for global patterns of genetic diversity and the rate and repeatability of evolution under climate change.

The strength of natural selection impacts on a range of evolutionary processes, including rates of adaptation[1,2], the maintenance of genetic variation[3,4] and extinction risk[5,6]. However, surprisingly little is known about whether certain types of environments systematically impose stronger selection pressures than others[7–9]. 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 more dynamic landscape, in which the fitness surface itself responds to both environmental and mutational input[9–11]. Mapping of the biochemical basis of developmental constraints and the environment's influence on phenotype is therefore of paramount importance to understanding why certain evolutionary trajectories are favoured over others[12–16], and how evolution can be repeatable despite mutation being considered as an inherently random process[17–20]. Indeed, such information will ultimately be necessary to predict species adaptability and persistence under environmental change.

Environmental change should increase the strength of directional selection on traits underlying local adaptation. However, the fitness consequences associated with maladaptation in such key traits may be relatively small compared to the variance in fitness attributed to segregating polymorphisms across the entire genome[5,21]. This reservoir of genetic variation is expected to have a fundamental impact on species' adaptability and extinction risk[6,22], but how the environment influences the expression and consequences of this genetic variation remains poorly understood[7,23–25]. For example, it is sometimes argued that fitness effects of sequence variation are magnified in new environments due to compromised phenotypic robustness under novel environmental conditions[26–30]. Yet, others

have argued that environmental change is bound to have idiosyncratic effects on the mean strength of selection on genome-wide polymorphism[23,24]. These somewhat conflicting predictions suggest that only by understanding the mechanistic basis for how environments mould the effects of sequence variation will it be 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 fundamental insights about how climate change and regional temperatures 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[31–36], resulting in a universal temperature-dependence of organismal behaviour, life-history and fitness[37–42]. By applying an existing biophysical model of enzyme kinetics we first demonstrate how elevated temperatures cause a drastic increase in the fitness effects of de novo mutation over the biologically relevant temperature range. Second, we show that while increased protein stability is predicted to offer robustness to both temperature and mutational perturbation, warm and cold adapted taxa are expected to show similar temperature-dependent increases in selection when occupying their respective thermal niches in nature. The model thus predicts 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 elevated temperature in replicate experimental evolution lines of the seed beetle, *Callosobruchus maculatus*, adapted to either ancestral or warm temperature. Second, we collate and analyse 100 published paired estimates of selection coefficients

against genome-wide de novo mutations in benign versus 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 on de novo mutations, but provide unequivocal support for the prediction that elevated temperature leads to a universal increase in genome-wide selection and genetic variance in fitness. These results have implications for global patterns of genetic diversity and suggest that evolution will proceed at an ever accelerating rate under continued climate change.

RESULTS

Enzyme kinetics theory predicts temperature-dependence of mutational effects

Fitness of cold blooded organisms shows a well-characterised relationship with temperature that closely mirrors the thermodynamic performance of a rate-limiting enzyme[37,43] (Fig 1a). This close relationship reflects the fact that biological rates are ultimately governed at the biochemical level by the enzymatic reaction rate, r:

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

where r_0 is a rate-specific constant, ΔH is the enthalpy of activation energy of the enzymatic reaction (kcal mol^{-1} K^{-1}), R is the universal gas constant (0.002 kcal mol^{-1}) and T is temperature measured in degrees Kelvin[44]. Equation (1) thus describes an exponential increase in reaction rate kinetics, where a higher value of ΔH results in a lower reaction rate at a given temperature, as observed in warm-adapted species[31].

The decline in biological rate that occurs at temperatures exceeding the organism's thermal optimum (Fig. 1a) is attributed to a reduction in the proportion of functional enzyme available at high temperature due to protein misfolding[31–36]. This temperature-dependence of protein folding is described as a function of the Gibbs free energy, ΔG, which is a measure of protein stability[34]:

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

The Gibbs free energy itself comprises both an enthalpy term (ΔH_G) and a temperature-dependent entropy term (ΔS) and is equal to: $\Delta G = \Delta H_G + T\Delta S$[45]. At benign temperature most natural proteins occur in the native active state and the value of the Gibbs free energy of folding is negative (mean $\Delta G_{T=298} \approx -7$ kcal mol^{-1};[34,46]). From equation (2) it is clear that as temperatures increase the Gibbs free energy becomes less negative, reducing the proportion of active protein. Following Chen and Shakhnovic (2010), the reaction rate kinetics of equation (1) can be combined with the protein folding of equation (2) to derive a fitness function (Fig. 1a) to provide a theoretical framework to investigate the consequences of mutation in a metabolic pathway consisting of Γ rate-determining proteins[47]:

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

Here we use equation (3) as the basis to derive predictions of the effects of temperature on the strength of selection on de novo mutations.

Firstly let us consider the effect of a possible mutation that impacts the catalytic rate of the enzyme by introducing a term to denote a mutational change in the enthalpy of activation energy ($\Delta\Delta H$) in equations 1 and 3:

$$\omega^*(\Delta\Delta H, T) \propto r_0 \frac{e^{-(\Delta H+\Delta\Delta H)/RT}}{\prod_{i=1}^{\Gamma}(1+e^{\Delta G(T)_i/RT})}. \qquad \text{(Eq. 4)}$$

Little is known about the size of such mutational effects, but inspection of equation 4 reveals that the mean selection coefficient against such de novo mutations is expected to remain largely unaffected by a change in temperature[45] (Fig. 1c).

The introduction of a mutational change in Gibbs free energy, $\Delta\Delta G$, into equations (4), is in contrast expected to disproportionately impact protein fitness at higher temperatures:

$$\omega^*(\Delta\Delta H, \Delta\Delta G, T) \propto r_0 \frac{e^{-(\Delta H+\Delta\Delta H)/RT}}{\prod_{i=1}^{\Gamma}(1+e^{(\Delta G(T)_i+\Delta\Delta G_i)/RT})}. \qquad \text{(Eq. 5)}$$

The majority of de novo mutations are expected to decrease fitness by destabilising protein structure since natural selection has led to inherently stable protein configurations[32–34,48]. The net impact of a single mutation on the free energy of folding has been estimated to $\Delta\Delta G \approx +0.9$ kcal mol^{-1} (SD = 1.7)[35,49,50], a value found to be more or less independent of the stability of the targeted protein (i.e. the original ΔG value)[46]. Note from equation (2) and (5) how mutation and temperature have synergistic effects on biological rate given their additive effects on ΔG. Indeed, on the basis that $\Delta S \approx -0.25$ kcal mol^{-1} [47], the net impact of a mean mutational effect of +0.9 $\Delta\Delta G$ on protein stability is equivalent to a 3.6^{o}C rise in

157 temperature. To examine the consequences of this synergism for the temperature
158 dependence of mutational fitness effects, we calculated the mean selection coefficient
159 against a de novo mutation across temperature as:
160
161 $$s(T) = 1 - \omega_T^*/\omega_T \qquad \text{(Eq. 6)}$$
162
163 where $\boldsymbol{\omega}_T$* and $\boldsymbol{\omega}_T$ is fitness of the mutant and the wildtype at temperature T. If we assume
164 that fitness is multiplicative, when equations (3) and (5) are substituted into equation (6) we
165 can yield the following simple expression for selection against a single mutation ($\Delta\Delta G$) in a
166 protein with a given stability (ΔG):
167
168 $$s(T) = 1 - \omega_T^*/\omega_T = 1 - \theta\,\frac{1+e^{\Delta G(T)/RT}}{1+e^{(\Delta G(T)+\Delta\Delta G)/RT}}\,. \qquad \text{(Eq. 7)}$$
169
170 Where θ is the relative catalytic performance of the mutant (i.e.
171 $r_0\, e^{-(\Delta H+\Delta\Delta H)/RT} / (r_0\, e^{-\Delta H/RT})$), which remains largely unchanged over the ecologically
172 relevant temperature range (Fig. 1c).
173
174 We applied equation (7) in numerical simulations to calculate the expected mean selection
175 coefficient on a mutation in a metabolic pathway by averaging across all possible rate-
176 determining proteins with stabilities randomly drawn from a truncated gamma
177 distribution ($\Delta G \sim -\Gamma(k = 5.50, \theta = 1.89)$, for $\Delta G < -5$) based on empirical data from
178 bacteria, yeast and nematodes[51]. Each protein was mutated by sampling a single folding
179 mutation from the empirically estimated normal distribution $\Delta\Delta G \sim N(\mu = 0.9, \sigma =$
180 $1.7)$[35,49,50]. Because little is known about the distribution of mutational effect sizes on

catalytic rate, we chose parameter values of $\Delta\Delta H$ that yielded reasonable negative selection coefficients at ecologically relevant temperatures (T: 0-50°C, s = 10^{-2} – 10^{-4}). Finally, we compared the resulting temperature dependence of selection in three genotypes with different hypothetical distributions of protein stabilities thought to reflect differences in thermal adaptation[31], by shifting the empirical gamma distribution so that mean ΔG = -6, -9 and -12, respectively (Fig 1).

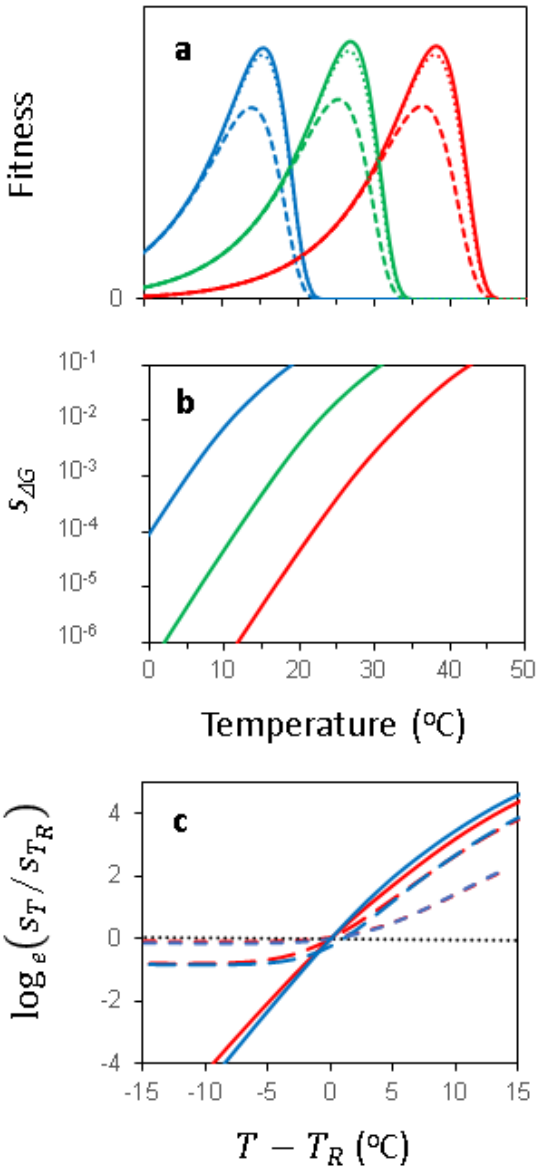
Equation (7) yields three predictions: First, the strength of selection increases with temperature (Fig. 1b) as a predictable consequence of the effect of de novo mutations on protein folding ($\Delta\Delta G$). Second, while the evolution of increased protein thermostability in response to hot climates (increasingly negative values of ΔG) produces proteins that are also more robust to mutational perturbation (Fig. 1b), we predict that cold- and warm-adapted genotypes will experience the same strength of selection on de novo mutations in their respective thermal environments, all else being equal (Fig. 1c), though thermal specialists will show a stronger temperature dependence (Fig S1.2, see also[52]). Third, while mutational effects on catalytic rate (ΔΔH) are largely unaffected by temperature (Eq. 4; Fig 1c), they can weaken the temperature-dependence of genome wide mutational fitness effects. The extent to which they do depends on their effect size and frequency relative to mutational effects on folding ($\Delta\Delta G$) (Eq. 5, Fig. 1c).

In Supplementary 1 we show that also mutational variance in fitness (i.e. the distribution of fitness effects of de novo mutations) also conforms to these general predictions. Elevated temperature leads to a substantial increase in mutational variance and the release of cryptic genetic variation in fitness, as well as a larger fraction of both highly deleterious and

beneficial mutations (Fig. S1.1). Moreover, the strongest selection in any single organism is predicted to act on mutations in genes encoding proteins with low stabilities (see also[51]), and these genes thus contribute disproportionally to temperature dependent effects (Fig. S1.1).

Our predictions arise from two fundamental and well-established principles: i) enzymes show reversible inactivation at high temperatures[31], and ii) the majority of de novo mutations act to destabilize protein structure[32–36,48]. Our qualitative results are therefore robust to the particular mathematical formulation of the enzyme-kinetic model, an assertion we confirmed by extending this analysis to various alternative equations recently reviewed by[53] (results available upon request). We also note that while we here have focused on the very essential features of protein fitness in terms of the fraction of active enzyme and its catalytic rate, the model can be expanded to, and is consistent with, a broader scope of temperature-dependent reductions in fitness, including effects from protein toxicity and aggregation arising from misfolded proteins in the cell [34,48] and RNA (mis)folding[54].

Figure 1: An enzyme-kinetic model of temperature dependent mutational fitness effects



Predicted consequences of mutation ($\Delta\Delta G$ = +0.9 ± 1.7 SD) on temperature dependent selection. In A) fitness for three genotypes with sets of proteins with different mean stabilities (ΔG) (blue, green and red lines reflect mean ΔG values of -6, -9 and -12). Solid lines = wildtype, short-dashed lines = mutant carrying a single folding mutation, long-dashed lines = mutant carrying 10 folding mutations with multiplicative effects on fitness. Reaction norms are based on an example using the respective ΔH values = 19.25, 20.00 and 20.76 and Γ = 500. In B) the expected mean selection coefficient against a single folding mutation occurring at a random gene for each of the three genotypes. In C, 'warm' and 'cold' adapted genotypes experience equivalent strengths of selection when fitness effects are assessed at a temperature standardised relative to each genotype's thermal optimum ($T_R = T_{OPT} - 10$ °C, for clarity only reaction norms for ΔG = -6 and -12 are shown). Mutational fitness effects on catalytic rate ($\Delta\Delta H$) show no discernible temperature dependence (black dotted line; here $\Delta\Delta H$ lowers fitness at T_{opt} by $s_H = 10^{-2}$). However, if a mutation has pleiotropic effects on both $\Delta\Delta H$ and $\Delta\Delta G$, the temperature dependence of selection against the mutant brought about by its effects on stability can be masked (long-dash and short-dash lines equate to a $s_H = 10^{-3}$ and 10^{-2} at T_{opt}, respectively).

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

250 To test these predictions, we measured fitness effects of induced mutations at 30°C and
251 36°C in replicate lines of the seed beetle *Callosobruchus maculatus*, evolved at benign 30°C
252 (3 ancestral lines) or stressful 36°C (3 warm-adapted lines) for more than 70 generations
253 (overview in SI Fig. 2.1). Previous studies have shown that the warm-adapted lines have
254 evolved considerably increased longevity[55,56]. Moreover, while lifetime offspring production
255 is decreased at 36°C relative to 30°C (X^2 = 62.5, df = 1, P < 0.001, n = 698), this decrease is
256 less pronounced in warm-adapted lines (interaction: X^2 = 7.35, df = 1, P = 0.007; Fig. 2a). To
257 characterize thermal adaptation further and relate it to the biophysical model, we quantified
258 thermal performance curves for juvenile development rate and survival; two traits that
259 presumably reflect variation in biochemical reaction rates (Eq. 1) and protein stability (Eq. 2),
260 respectively [31]. In line with expectations based on the thermodynamics of enzyme function
261 [43], elevated temperature generally decreased juvenile survival (X^2 = 76.0, df= 3, P < 0.001, n =
262 2755) and increased development rate (X^2 = 1723, df= 3, P < 0.001, n = 2755). Divergence
263 between ancestral and warm-adapted lines in the temperature-dependence of these two
264 traits was weak (interaction for survival: X^2 = 5.43, df= 3, P = 0.14, Fig. 2b; interaction for
265 development: X^2 = 6.71, df= 3, P = 0.082, Fig. 2c). Instead, ancestral lines showed consistently
266 faster development (X^2 = 27.2, df= 1, P < 0.001, Fig 2b) and marginally lower survival in
267 general (X^2 = 3.74, df= 1, P = 0.053, Fig. 2c). These results thus demonstrate considerable
268 divergence between the selection regimes and are qualitatively consistent with the
269 biophysical model of protein kinetics.

270

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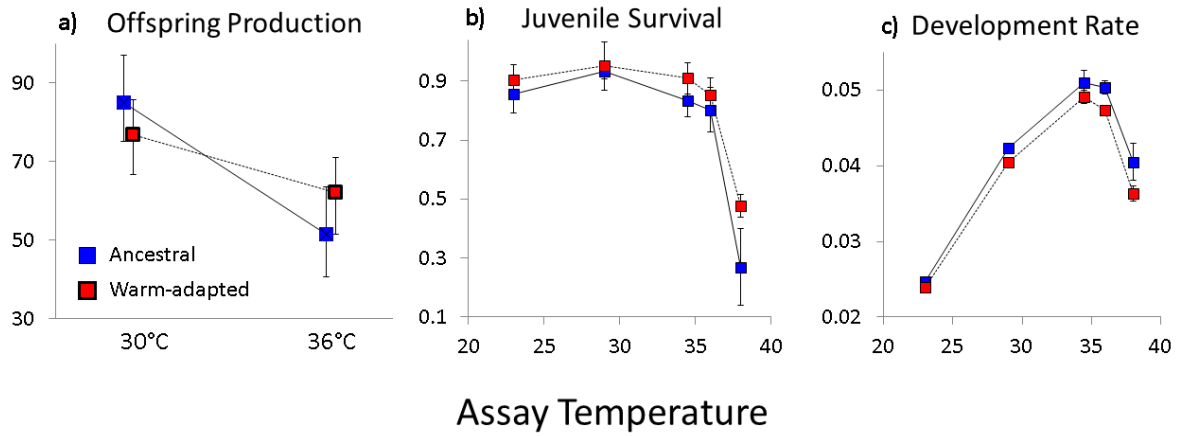
Figure 2: Thermal adaptation during experimental evolution

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 a genotype by environment interaction for offspring production (P = 0.007), reaction norms for survival and development rate show no clear differences in temperature dependence between ancestral and warm-adapted lines. Instead, ancestral lines show generally faster development (P < 0.001) but lower survival (P = 0.053) across temperatures.



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. 2.2), we could quantify the cumulative fitness effect of the mutations (i.e. mutation load): $\boldsymbol{\Delta\omega = 1\text{-}\ \omega_{IRR}/\omega_{CTRL}}$, and compare it across the two assay temperatures in ancestral

and warm-adapted lines (Fig. 3). Elevated temperature increased **Δω**, assayed in both the F1 (X^2 = 13.0, df = 1, P < 0.001, n = 713, Fig. 3a) and F2 generation (X^2 = 7.44, df = 1, P = 0.006, n = 1449, Fig 3b). These temperature effects were consistent across ancestral and warm-adapted lines (interaction: P_{F1} = 0.43, P_{F2} = 0.90; Fig. 3), lending support to the model predictions of temperature-dependent mutational fitness effects based on protein biophysics (compare Fig. 1b and Fig. 3). Indeed, the fact that ancestral and warm-adapted genotypes showed similar responses supports the tenet that high temperature, rather than thermal stress *per se*, caused the increase in selection against the induced mutations.

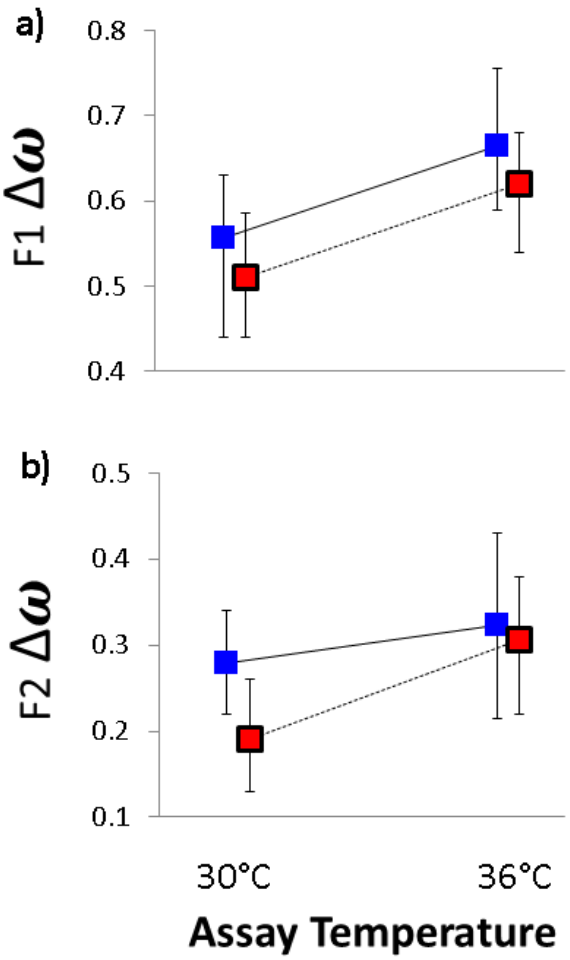


Figure 3: The evolution of temperature dependent mutational fitness effects

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).

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

To test model predictions further, we retrieved 100 paired estimates comparing the strength of selection on de novo mutations across benign and stressful abiotic environments from 28 studies on 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 3.1). Hence, selection against accumulated mutations could be estimated as the mutation load: $\Delta\boldsymbol{\omega}_i = 1-\boldsymbol{\omega}_i^*/\boldsymbol{\omega}_i$, 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 mutation load 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 in meta-analysis using Bayesian mixed effects models incorporating study ID and organism crossed with the form of environmental stress (see further below) as random effects. In addition, the contribution of each measure to the final model was weighted by the approximated standard error of the estimated log-ratio (see *Methods*). We further explored any potential publication bias in the collated data by plotting the precision of each estimate of the log-ratio (1/standard error) against its mean in a funnel plot (Fig. SI 3.5). This showed no clear evidence for such bias.

A universal temperature dependence of mutational fitness effects

Analysing all collated log-ratios together confirmed predictions from fitness landscape theory[23,24] suggesting that selection against de novo mutation does not generally seem to be

greater under stressful abiotic conditions (log-ratio = 0.19, 95% CI: -0.07-0.45; P_{MCMC} = 0.13, Fig 4). Next we analysed the 40 estimates derived at high and low temperature stress separately from the 60 estimates derived from various other stressful environments (of which increased salinity, other chemical stressors, and food stress, were most common: SI Table 3.1). This revealed that selection on de novo mutation increases at high temperature stress (log-ratio ≤ 0; P_{MCMC} < 0.001, n = 21, studies = 10), whereas there was no increase in selection at low temperature stress (log-ratio ≤ 0; P_{MCMC} = 0.67, n = 19, studies = 11) or for the other forms of stress pooled (log-ratio ≤ 0; P_{MCMC} = 0.48, n = 54, removing 6 estimates for s in each environment ~ 0, studies = 22). Moreover, elevated temperature led to a significantly larger increase in selection relative to both cold stress (P_{MCMC} = 0.004) and the other stressors pooled (P_{MCMC} = 0.002) (Fig 4 & SI Table 3.2).

Next we explored whether there were differences in the effects of environmental stress on selection between unicellular and multicellular species in our dataset by incorporating cellularity as a two-level factor in the analysis. There was a tendency for cold stress to decrease selection in unicellular species and increase it in multicellular species, but this effect was marginally non-significant (interaction: P_{MCMC} = 0.066). Moreover, 5 of the 6 estimated log-ratios at cold stress for multicellular species derive from *D. melanogaster* and drive this trend (Fig. 5b). We found no evidence for differences in the effect of elevated temperature on selection between the four multicellular and three unicellular species (P_{MCMC} = 0.45). Indeed, mutational fitness effects were greater at elevated temperature in 8/10 and 10/11 cases in multicellular and unicellular species, respectively (combined binomial test, 18/21 cases: P_{binom} = 0.0015). Notably, the 12 log-ratios that were significantly different from 0 (>1.96SE) at high temperature stress were all positive, signifying increased selection (P_{binom}

= 0.0005, Fig S3.5). These results are robust to analysis method and do not change when using maximum likelihood estimation (SI Table 3.2). Additionally, by analysing a reduced number of studies for which we could extract 64 paired estimates of mutational variance, we show that this alternative measure of mutational effects also increases with temperature and follows the same general patterns as the mutation load (Fig 4 and SI Table 3.3).

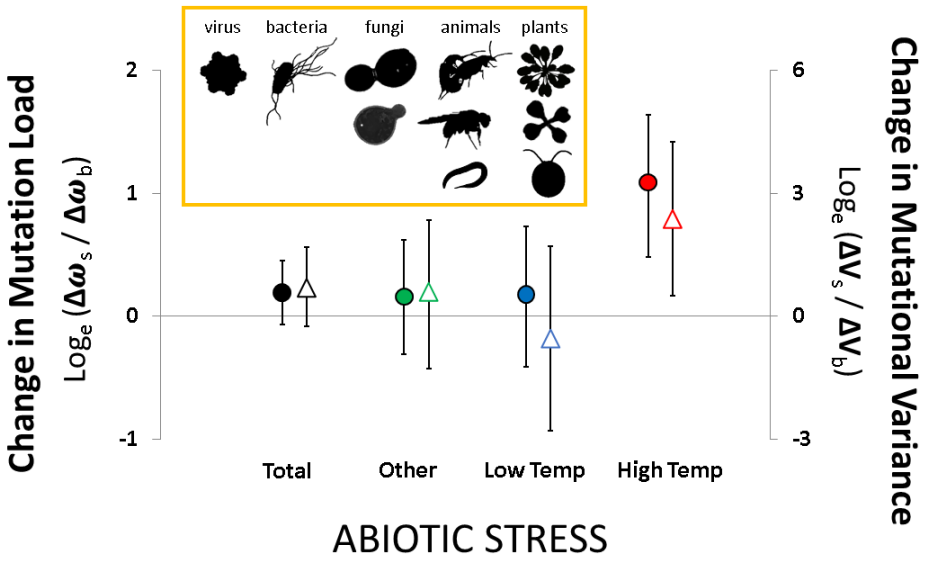
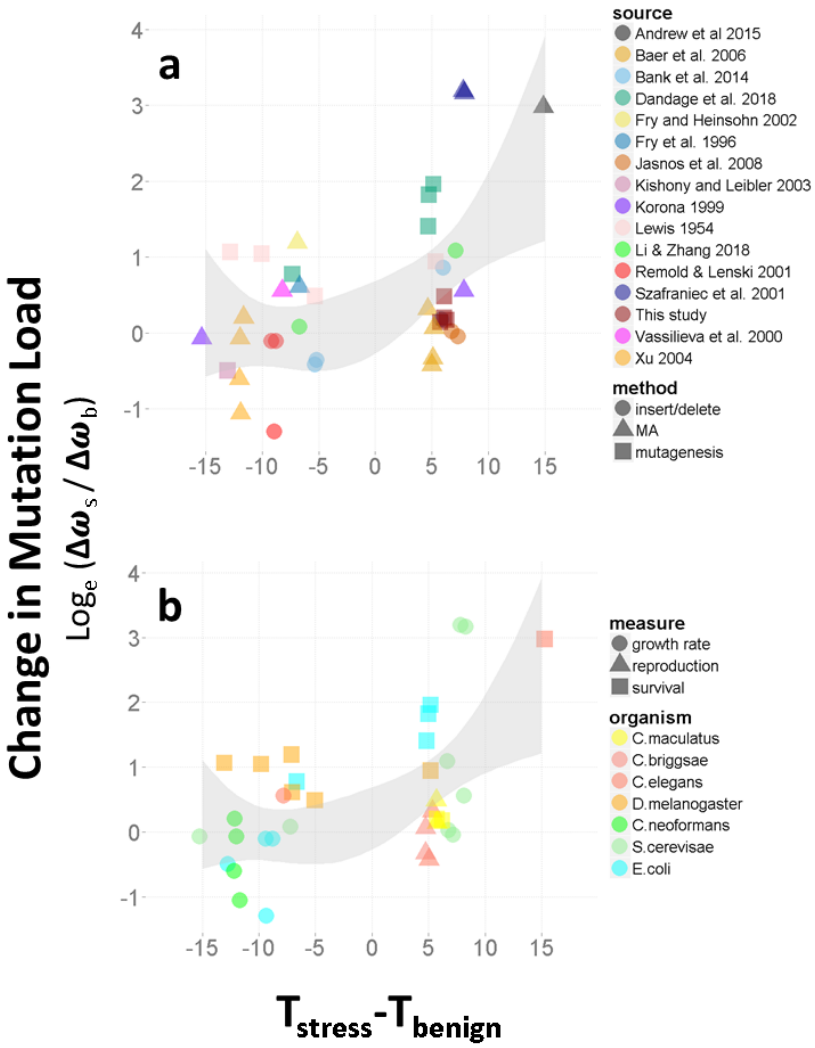


Figure 4: Meta-analysis of mutational fitness effects in stressful environments

Meta-analysis of the effect of abiotic stress on the mean strength of selection against de novo mutations (filled points) and mutational variance (open triangles) analysed by log-ratios (Bayesian posterior modes ± 95% credible intervals): $\boldsymbol{\Delta\omega}_{stress}/\boldsymbol{\Delta\omega}_{benign}$ and $\mathbf{\Delta V}_{stress}/\mathbf{\Delta V}_{benign}$ > 0 correspond to greater mutational fitness effects under environmental stress. The 94 paired estimates of $\boldsymbol{\Delta\omega}$ (filled circles) show that selection is not greater in stressful environments overall (P = 0.13) and highly variable across the 25 studies analyzed. However, estimates of $\boldsymbol{\Delta\omega}$ at high temperature are greater than their paired estimates at benign temperature (P < 0.001). These results were qualitatively the same when analysing the fewer available estimates of mutational variance ($\mathbf{\Delta V}$: open triangles, P = 0.02). The box shows the eleven species included in the analysis (of which two were roundworms), covering four major groups of the tree of life. See main text and Supplementary 3 for further details.

Using the 40 paired estimates of mutation load at contrasting temperatures we partitioned effects on the strength of selection from i) stress *per se*; quantified as the reduction in mean fitness at the stressful temperature relative to the benign temperature ($1 - \bar{\omega}_{stress}/\bar{\omega}_{benign}$), and ii) that of the temperature shift itself; quantified as the magnitude and direction of the temperature shift: T_{stress} - T_{benign}. The strength of selection was not significantly related to stress ($P_{MCMC} > 0.8$). However, a shift towards warmer assay temperature *per se* caused a substantial increase in mutation load (slope coefficient = 0.070, CI: 0.044-0.10, $P_{MCMC} < 0.001$, Fig 5). There was also a non-linear effect of temperature (non-linear coefficient = 0.007, CI: 0.003-0.012, $P_{MCMC} = 0.002$, Fig 5), equivalent to that predicted to result from combined unconditional (*ΔΔH)* and temperature dependent (*ΔΔG)* mutational effects (compare Fig. 1C and Fig. 5). These results thus further support that selection against de novo mutations generally increases at high temperature in ectotherms. Again the effect of cold temperature on the strength of selection seemed to differ between the unicellular and multicellular species studied (difference in slope: 0.055, CI: 0.008-0.099, $P_{MCMC} = 0.024$, Fig. 5b). However, given that this pattern is driven almost solely by the 5 estimates from *D. melanogaster*, more data is needed to say anything concrete about effects of cellularity on mutational fitness effects at cold temperature.



384

385 **Figure 5: Meta-analysis of temperature-dependent mutational fitness effects**

386 Temperature-dependent mutational fitness effects. The strength of selection on de novo mutations
387 as a function of the direction and magnitude of the temperature shift between the benign and
388 stressful temperature. In (A) the 16 studies analysed and the method used to induce mutations, is
389 depicted. In (B) the seven species analysed, and the fitness measure taken, is depicted. Selection
390 generally increases with temperature ($P_{MCMC} < 0.001$) whereas stress per se (quantified as the mean
391 reduction in relative fitness between the benign and stressful temperature) did not affect the
392 strength of selection ($P_{MCMC} > 0.8$). The grey shaded area represents the 95% CI from a second degree
393 polynomial fit of the log-ratios on temperature, weighted by the statistical significance of each
394 estimate (absolute log-ratio/standard error). Points are jittered for illustrative purposes.

The fitness load at mutation selection balance is predicted to equal the genomic deleterious mutation rate, but to be unrelated to the mean deleterious effect of mutation [5,21]. 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 [29,57]. In SI 3.4 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 suggesting 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 empirically demonstrated. Here we show that elevated temperature increases genome-wide selection and genetic variation in fitness, an observation that is consistent with the applied biophysical model of enzyme kinetics, which ascribes these increases to magnified allelic effects on protein folding at elevated temperature (Fig. 1, Fig. S1.1). The model and data further suggest that, while the evolution of protein thermostability in response to hot climates can indirectly confer mutational robustness, the temperature-mediated increase in the strength of selection will be similar for cold- and warm adapted taxa occupying their respective thermal niches in nature. The data and model predicts that, without adaptation, the depicted scenario of 2-4°C of warming by the end of this century[58] will result in a doubling of

419 genome-wide selection on average, although the effect may vary between organisms (Fig. 5,
420 Fig. S1.2) and depends on model assumptions regarding unconditional mutational effects
421 (Fig. 1c). Nevertheless, the effect may be underestimated, given the non-linear relationship
422 between selection strength and temperature and the predicted increase in occurrence of
423 heat waves[58]. In contrast, environmental stress *per se* did not have a significant effect on the
424 strength of selection on de novo mutations in any of our analyses, implying that mutational
425 robustness is not generally greater in benign relative to stressful environments[23,24].
426
427 Our analyses have been limited to purifying selection as a consequence of the fact that the
428 very majority of de novo mutations are deleterious. However, increased conditional genetic
429 variation in protein phenotypes at elevated temperature are in rare cases predicted to
430 confer fitness benefits[25,30,50], as seen in our model predictions on the distribution of fitness
431 effects of mutations at different temperatures (Fig. S1.1). Thus, the increase in mutational
432 effects at warm temperature is predicted to influence regional patterns of standing genetic
433 variation and future evolutionary potentials under climate change. Previous studies have
434 highlighted a range of possible consequences of temperature on evolutionary potential in
435 tropical versus temperature regions, including faster generation times[38], higher maximal
436 growth rates[59], higher mutation rates[40,56] and more frequent recombination[60,61] in the
437 former. Our results imply that also the efficacy of selection may be greater in the warmer
438 tropical regions, which together with the aforementioned factors predict more rapid
439 evolution and diversification, in line with the greater levels of biodiversity in this area[62,63].
440 However, implications for species persistence under climate change will crucially depend on
441 demographic parameters such as reproductive rates and effective population size[6,9,64], and
442 greater selection in tropical areas may even result in increased extinction rates if

443 evolutionary potential is limited[37,59,65,66]. Such a scenario could be envisioned if temperature-
444 mediated selection has led to a greater erosion of genetic variation in ecologically relevant
445 traits, such as reported for thermal tolerance limits in tropical *Drosophila* species[67].
446 Moreover, protein stability has itself been suggested to increase evolvability and innovation
447 by allowing slightly destabilizing mutations with conditionally beneficial effects on other
448 aspects of protein fitness to be positively selected[68–70]. Hence, the destabilizing effect of
449 rising global temperatures on protein folding may, by reducing this buffering capacity, limit
450 the potential for evolutionary innovation.
451
452 The observed temperature dependence of mutational effects builds a scenario in which
453 contemporary climate warming may lead to molecular signatures of increased purifying
454 selection and genome-wide convergence in taxa inhabiting similar thermal environments. In
455 support of this claim, Sabath et al. (2013) showed that growth temperature across
456 thermophilic bacteria tend to be negatively correlated to the non-synonymous to
457 synonymous nucleotide substitution-rate (dN/dS-ratio), suggesting stronger purifying
458 selection in the most pronounced thermophiles[71]. Effects could possibly extend beyond
459 nucleotide diversity to other aspects of genome architecture. For example, Drake (2009)
460 showed that two thermophilic microbes have substantially lower mutation rates than their
461 seven mesophilic relatives, implying that increased fitness consequences of mutation at hot
462 temperature can select for decreased genome-wide mutation rate[72]. Following the same
463 reasoning, increased mutational effects in warm climates could select for increased
464 mutational robustness[73–75]. As mutation pressure on single genes is weak, the evolution of
465 such increased genome integrity would, at least in organisms with small population size[76,77],
466 likely involve mechanisms regulating mutation rate and/or robustness globally[78] such as the

upregulation of chaperone proteins, known to assist both protein folding[31,79] and DNA repair[80]. Additionally, mutational robustness may also result indirectly from selection for increased environmental robustness[26–29,34], in line with predictions from the presented biophysical model suggesting that increased protein thermostability confers increased robustness to de novo mutation (for a given temperature: Fig. 1b).

Environmental tolerance has classically been conceptualized and modelled by a Gaussian function mapping organismal fitness to an environmental gradient (e.g.[6,81]). In this framework stress is not generally expected to increase the mean strength of purifying selection against de novo mutation[23], a prediction supported by our estimates of selection under forms of environmental stress other than elevated 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 terms of protein folding states, are assumed to increase exponentially with temperature. While supported by a number of targeted studies on proteins[32–36,82], it remains less clear how the effects on protein and RNA folding map to the level of morphological and life history traits, which have previously been used with varying outcome to study selection and phenotypic effects under environmental stress[83–89].

Another open question is how the unveiled temperature-dependence interacts with other features expected to influence the distribution of fitness effects of segregating genetic variants, such as thermal niche width (Fig. S1.2), genome size, phenotypic complexity [90,91] and effective population size [9,64,76,92]. Unicellular and multicellular organisms differ greatly in

these aspects, and interestingly, our data hint at a difference in the temperature dependence of mutational fitness effects between these two groups at cold temperature (Fig 5b). Our model shows that the temperature dependence is weakened by an increased fraction of unconditionally (i.e. temperature-independent) deleterious mutations (Fig. 1c). Differences between unicellular and multicellular organism could therefore arise if the link between fitness and rate-dependent processes at the level of enzymes is more direct in unicellular compared to multicellular organisms, resulting in a higher fraction of unconditional mutations and weaker temperature dependence in the latter. Questions such as these 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[93]. The juvenile phase is completed in approximately three weeks, and egg to adult survival is above 90% at benign 30°C[94]. The lines were derived from an outbred population created by mixing beetles collected at three nearby sites in Nigeria[95]. 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 20 generations (i.e. 0.3°C/generation) and then kept at 36°C (warm-adapted lines). Population

514 size was kept at 200 individuals for the first 20 generations and then increased to 500
515 individuals in each line. In this study we compared three replicate lines of each regime.
516
517 *Thermal reaction norms for juvenile survival and development rate*
518 Previous studies have revealed significant differentiation in key life history traits between
519 the regimes[55,56]. Here we quantified reaction norms for juvenile survival and development
520 rate across five temperatures (23, 29, 35, 36 & 38°C) following 100 generations of
521 experimental evolution. Two generations prior to the assaying all six lines were moved to
522 30°C, which is a beneficial temperature to both sets of lines (Fig. 2)[56], to ascertain that
523 differences between evolution regimes were due to genetic effects. Newly emerged second
524 generation adults were allowed to mate and lay eggs for 24h on new *V. unguiculata* seeds
525 that were subsequently randomized to each assay temperature in 90mm diameter petri-
526 dishes with ca. 100 seeds per dish with each carrying no more than 4 eggs to make sure
527 larval food was provided ad libitum. Two dishes were set up per temperature for each line.
528 In total we scored egg-to-adult survival and development time for 2755 offspring evenly split
529 over the five assay temperatures and six replicate lines. Survival was analysed using
530 dead/alive as the binomial response, and development rate (1/development time) as a
531 normally distributed response using generalized and general linear mixed effects models,
532 respectively, in the lme4 package[96] for R. Temperature and selection regime as well as their
533 interaction were included as fixed effects, and line identity crossed by assay temperature
534 was added as random effect.
535
536

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 Supplementary 2. 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 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[80]. Such breaks occur naturally during recombination, and in yeast to humans alike, point mutations arise due to errors during their repair[80]. 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 kept at 30°C. The males were subsequently moved back to the climate cabinet to mature a new ejaculate. This procedure discarded the first ejaculate that will have contained damaged seminal fluid proteins in the irradiated males[97], 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[56], 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

561 virgin females from their own population. The mated females were immediately placed on
562 beans presented ad libitum and randomized to a climate cabinet set to either 30°C or 36°C
563 (50% RH) and allowed to lay their lifetime storage of F1 eggs. We set up 19-38 F0 males (and
564 mating couples) per treatment, assay temperature and line, and 713 males in total.
565
566 To measure mutational effects in the F2 generation, we applied a Middle Class
567 Neighborhood breeding design to nullify selection on all but the unconditionally lethal
568 mutations amongst F1 juveniles[98]; from the F1 survivors, we crossed a randomly selected
569 male and female offspring per family with another family from the same treatment and line.
570 From a few treatment:line combinations with a low number of F0 families set up, we did this
571 procedure twice to get a more balanced sample size. This approach allowed us to quantify
572 the cumulative deleterious fitness effect of all but the unconditionally lethal mutations
573 induced in F0 males (i.e. mutation load) by comparing the production of F2 adults in
574 irradiated lineages, relative to the number of adults descending from F0 controls (Fig. S2).
575 We also used F1 adult counts to derive this estimate, acknowledging that it may include non-
576 trivial paternal effects from the irradiation treatment, in addition to pure mutational effects.
577 However, results based on F1 and F2 estimates were consistent (Fig 3). Thus, to estimate the
578 effects of elevated temperature on mutational fitness effects in the two genetic
579 backgrounds, we analysed the number of offspring produced as a Poisson response, using
580 generalized linear mixed effects models, testing for interactions between radiation
581 treatment, assay temperature and evolution regime. We included each individual
582 observation as a random effect to account for over-dispersion in the data. Mutation load is
583 formally quantified as offspring production in irradiated lineages *relative* to corresponding
584 controls. To better illustrate the results we therefore also ran Bayesian analyses using the

MCMCglmm package[96] with the same model structure, but assuming a normally distributed response, and calculated the posterior estimates of mutation load ($\Delta\omega = 1 - \omega_{IRR}/\omega_{CTRL}$) directly from these models (Fig. 3). The MCMC resampling ran for 1.000.000 iterations, preceded by 500.000 burn-in iterations that were discarded. Every 1000th iteration was stored, resulting in 1000 independent posterior estimates from each model. We used weak priors for the random effects as recommend in[96].

Meta-analysis of selection on de novo mutation in benign and stressful environments

We looked for 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. We started by extracting data from studies reported in two earlier reviews on mutational fitness effects[23,24]. We then used Google Scholar to search the literature citing these papers. In addition we also made own searches including the search terms "mutation", "selection"/"fitness" and "environment"/"stress/"temperature"". We collated selection coefficients along with their standard errors from raw data, tables or figures from the original publications. 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*[99]; 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

609 the protocol used to accrue mutations (mutation accumulation at population sizes >2) may
610 have failed to remove selection, biasing subsequent comparisons of mutational fitness
611 effects across environments. In total we retrieved 100 paired estimates of selection from 28
612 studies and 11 organisms, spanning unicellular viruses and bacteria to multicellular plants
613 and animals (summary in Supplementary 3). Ultimately, three of these studies (and six
614 paired estimates of selection) were discarded since selection coefficients in both the benign
615 and stressful environment were ≈ 0 and could not be analyzed further.
616
617 An estimate controlling for between-study variation was calculated by taking the log-ratio of
618 the cumulative fitness effect of the induced mutations at stressful relative to corresponding
619 benign conditions in each study: $LOG_e[\Delta\omega_{stress}/\Delta\omega_{benign}]$, where $\Delta\omega = 1 - \omega^{mutant}/\omega^{CTRL}$.
620 Hence, a ratio above (below) 0 indicates stronger (weaker) selection against mutations
621 under stress. We used both REML and Bayesian linear mixed effects models (available in the
622 MCMCglmm package[98] for R) to estimate if log-ratios differed from 0 for three levels of
623 environmental stress: cold temperature, warm temperature, and other types of stress
624 pooled (Table SI 3.1), as well as for the total effect of stress averaged across all studies. We
625 also tested if log-ratios differed between the three types of abiotic stress. All models
626 included stress-type, mutation induction protocol and fitness estimate as main effects,
627 although effects of the latter two were never significant. We included study organism and
628 study ID as random effects. Additionally, study organism was crossed with stress type to
629 control for species variation and phylogenetic signal. To further explore large scale signals in
630 the data we performed an analysis including a fixed factor encoding uni- or multicellularity,
631 which was crossed with stress type, allowing us to test for differences in selection between
632 the two groups.

633

634 Using the 40 estimates that compared the strength of selection across temperatures, we
635 partitioned the effect of i) temperature stress; quantified as the reduction in mean fitness at
636 the stressful temperature relative to the benign temperature (Table S3.1), and ii) that of
637 temperature itself; quantified as the linear (1^{st} polynomial coefficient) and non-linear (2^{nd}
638 polynomial coefficient) effect of the magnitude and direction of the temperature shift: T_{stress}
639 - T_{benign}. We included stress and temperature as the two fixed effect covariates, and study
640 organism and study ID as random effects. Study organisms were also allowed to have
641 random slopes for the temperature effect to control for between-species variation in the
642 temperature dependence. Again we added a fixed effect encoding uni- or multicellularity
643 crossed by the temperature covariate to test if the two groups differed in the temperature
644 dependence of mutational fitness effects.

645

646 To weight each estimate's contribution to the final meta analytic results by its sampling
647 variance, we passed the standard error (SE) of each log-ratio to MCMCglmm using the
648 idh(SE):us command. The standard errors were approximated using laws of error
649 propagation for ratios, but since this technique is known to heavily inflate standard errors
650 when the denominator approaches zero[100], we simulated unidirectional standard errors for
651 the 10 log-ratios for which $\boldsymbol{\Delta\omega}_{benign}$ (i.e. the denominator) was smaller than 1.96 SE. This was
652 done by drawing 10.000 samples of $\boldsymbol{\Delta\omega}_{stress}$ and $\boldsymbol{\Delta\omega}_{benign}$ from a normal distribution defined
653 by their reported mean and standard error and then discarding the 50% of the simulations in
654 which values of $\boldsymbol{\Delta\omega}_{benign}$ were below its mean. We then approximated the unidirectional
655 (downwards) error of the log-ratio based on the remaining simulations by calculating the
656 average deviation from the mean log-ratio. Note here that this unidirectional error

657 corresponds directly to whether the log-ratio was significantly different from zero or not (i.e.
658 giving the uncertainty downwards for positive ratios). We present a funnel plot depicting the
659 precision (1/SE) and mean log-ratio in Supplementary 3 (Fig. S3.5).
660
661 In all models, the MCMC resampling ran for 1.000.000 iterations, preceded by 500.000 burn-
662 in iterations that were discarded. Every 1000th iteration was stored, resulting in 1000
663 independent posterior estimates from each model. We used standard priors for the fixed
664 effects and weak priors for the random effects. Variance for the random effect incorporating
665 the within-study standard errors was fixed to 1.
666

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Author Contributions

DB performed the experiments on seed beetles together with JS. RJW and DB performed the modelling and DB and JB performed the meta-analysis. DB wrote the manuscript with considerable input from RJW. All authors commented on manuscript drafts.

Competing Interests

The authors have no competing interests to report