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 theory. Here, by applying a biophysical model of protein evolution, we 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. Our model further suggests that 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 randomly induced mutations were stronger at elevated temperature for both ancestral and warm-adapted lines. We then calculated 98 estimates from the literature, comparing selection on induced mutations across stressful and benign environments in 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* generally does not increase the strength of selection on new mutations. However, according to prediction, elevated temperature systematically increased the mean strength of selection on genome-wide polymorphism. 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 genome evolution under environmental change.

SIGNIFICANCE STATEMENT

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. Here we integrate theory from the fields of ecology, genetics and biophysics and combine mathematical modelling with 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 genome evolution and biodiversity patterns, and suggests that evolution will proceed at an ever accelerating pace under continued climate warming.

INTRODUCTION

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 the same temperature-dependent increase 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 98 estimates from the literature on 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. 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 1B). This close relationship reflects the fact that biological rates are ultimately governed at the biochemical level by the enzymatic reaction rate, r:

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

where r_0 is a rate-specific constant, $H^{\#}$ 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 the initial exponential increase in biological rates with temperature arising from reaction rate kinetics (Fig. 1A), with a higher value of $H^{\#}$ resulting in a lower rate for a given temperature, as predicted for species adapted to warmer temperatures (31).

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

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

The Gibbs free energy is itself comprised of both an enthalpy term (ΔH) and a temperature-dependent entropy term (ΔS) and is equal to: $\Delta G = \Delta H + T\Delta S$ (45). At benign temperature 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 thus clear that most natural proteins occur in native active state, and that elevated temperatures increasing the Gibbs free energy (i.e. making ΔG less negative), can cause proteins to become misfolded and inactive. Following Chen and Shakhnovic (2010) we combine the reaction rate kinetics of equation 1 with the protein folding of equation 2, to derive a fitness function (Fig. 1B) providing a theoretical framework to investigate the consequences of mutation in a metabolic pathway with Γ rate-determining proteins (47):

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

Here we use equation 3 as the basis to derive predictions of the effects of temperature on the mean selection coefficient against de novo mutation. First let us consider the effect of a

mutation that compromises the catalytic rate of the enzyme (Eq 1), be it directly by increasing the enthalpy of activation energy ($H^{\#}$), or indirectly by limiting substrate concentration within the living cell, following Michaelis-Menten kinetics (45). To understand the consequences for selection we can introduce the term, θ, which is equal to 1 minus the net proportional loss in catalytic rate for the mutant genotype:

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

As a simple scaling factor mutational effects on catalytic rate, introduced via θ, are not dependent on temperature.

Most de novo mutations, however, are expected to decrease fitness by destabilising protein structure on the premise that natural selection leads to inherently stable protein configurations (32–34, 48). The net impact of mutations on the free energy of folding has been estimated empirically to be $\Delta\Delta G \approx +0.9$ kcal mol^{-1} (SD = 1.7: (49, 50)) and to be more or less independent of the original ΔG value (46). Hence, the effects of temperature and mutation act additively to increase ΔG. Indeed, on the basis that $\Delta S \approx -0.25$ kcal mol^{-1}, it can be deduced that the net effect of mutation on protein stability has an equivalent impact to a 3.6°C rise in temperature (47). This additive action causes a disproportionate increase in the fraction of misfolded protein (Fig. 1A).

We can explore the consequences of this synergism between mutation and temperature by estimating the mean selection coefficient ***s*** against de novo mutations across temperature *T*:

$s(T) = 1 - \omega_T^*/\omega_T$, where ω_T^* and ω_T is fitness of a mutant carrying a destabilizing mutation ($\Delta\Delta G = 0.9$) and the wildtype, respectively. Expanding and simplifying Eqs. 3 and 4 while holding the number of rate-determining proteins constant, yields:

$$s(T) = 1 - \omega_T^*/\omega_T = 1 - \theta^{-1}\frac{1+e^{\Delta G(T)/RT}}{1+e^{(\Delta G(T)+\Delta\Delta G)/RT}}. \qquad \text{(Eq. 5)}$$

This expression yields three novel predictions: First, the strength of selection increases with temperature (Fig. 1C) as a predictable consequence of the effect of de novo mutations on protein folding ($\Delta\Delta G$), rather than on reductions in catalytic rate (θ). Second, while the evolution of increased protein thermostability in response to hot climates (a more negative ΔG) produces proteins that are also more robust to mutational perturbation (Fig. 1C), cold- and warm-adapted genotypes are expected to experience the same mean strength of selection on de novo mutations, and the same increase in selection with rising temperature, when occupying their respective thermal niches (Fig. 1D). Third, while mutations affecting catalytic rate themselves have unconditional fitness effects with respect to temperature (Eq. 4), they can weaken the temperature dependence of genome wide mutational fitness effects, and the extent to which this happens depends on their effect and frequency relative to mutations with effects on protein folding (Fig. 1D).

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 (51) (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 (52).

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

To test these predictions, we measured 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). Previous studies have shown that the warm-adapted lines have evolved a considerably increased longevity (53, 54). Moreover, while offspring production is decreased at 36□C relative to 30□C (X^2 = 62.5, df = 1, P < 0.001), this decrease is much less pronounced 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 biophysical model (Fig. 1A), we quantified thermal performance curves for juvenile development rate and survival; two traits that presumably reflect variation in the rate of catalysis (Eq. 1) and protein stability (Eq. 2), respectively (31). In line with expectations based on the thermodynamics of enzyme function (43), elevated temperature generally decreased juvenile survival (X^2 = 76.0, df= 3, P < 0.001) and increased 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 showed consistently faster development (X^2 = 27.2, df= 1, P < 0.001, Fig 2B) and marginally lower survival in general (X^2 = 3.74, df= 1, P = 0.053, Fig. 2C). These results demonstrate considerable local adaptation among the selection regimes, qualitatively consistent with the biophysical model of protein kinetics (compare: Fig. 1A & B with Fig. 2B & C).

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: $\boldsymbol{\Delta\omega} = 1 - \boldsymbol{\omega}_{IRR}/\boldsymbol{\omega}_{CTRL}$, and compare it across the two assay temperatures in ancestral and warm-adapted lines. Elevated 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). 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 kinetics (compare Fig. 1C 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.

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 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 2.1). Hence, selection against accumulated mutations could be estimated as mutation load: $\Delta\omega_i = 1-\omega_i^*/\omega_i$, where ω_i^* and ω_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: $\text{Log}_e[\Delta\omega_{stress}/\Delta\omega_{benign}]$, 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 crossed with the form of environmental stress (see further below) as random effects. This analysis 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 = 0.21, 95% CI: -0.04-0.48; $P_{MCMC} = 0.094$, Fig 4).

A universal temperature dependence of mutational fitness effects

Next 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 ≤ 0; $P_{MCMC} < 0.004$, n = 20), whereas there was no increase in selection at low temperature

stress (log-ratio ≤ 0; P_{MCMC} = 0.94, n = 18) or for the other forms of stress pooled (log-ratio ≤ 0; P_{MCMC} = 0.29, n = 60). Moreover, elevated temperature led to a significantly larger increase in selection relative to both cold stress (P_{MCMC} = 0.012; Fig. 4) and the other stressors pooled (P_{MCMC} = 0.008; Fig. 4). We found no evidence suggesting that multicellular and unicellular species differed in these patterns; mutational fitness effects were greater at elevated temperature in 8/10 and 9/10 cases in multicellular and unicellular species, respectively (combined binomial test: P = 0.0026). These results are robust to analysis method and do not change when using maximum likelihood estimation (SI Table 2.2). 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 follows the same pattern as the mutation load (Fig 4 and SI Table 2.3).

Using the 38 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} > 0.3). However, a shift towards warmer assay temperature *per se* caused a substantial increase in mutation load (b = 0.063, CI: 0.025-0.10, P_{MCMC} = 0.002, Fig 5B). There was also a moderate non-linear effect of temperature (b = 0.10, CI: 0.004-0.19, P_{MCMC} = 0.032, Fig 5A), similar to that predicted to result from unconditional mutational effects (compare Fig. 1D and Fig. 5). These results thus confirm that selection against de novo mutations generally increases with temperature in ectotherms. Interestingly, there was a tendency for the temperature dependence to be stronger for the unicellular compared to

multicellular species studied (Fig. 5B), however, we could not find any statistical support for this difference (change in slope: 0.039, CI: -0.02-0.11, $P_{MCMC} = 0.22$).

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 (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, 55). 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 empirically demonstrated. Here we show that elevated temperature increases selection genome-wide, an observation that is consistent with the applied biophysical model of enzyme kinetics, which ascribes this increase to magnified allelic effects on protein folding at elevated temperature (Fig. 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 the same for cold- and

warm adapted taxa occupying their respective thermal niches in nature. In contrast, environmental stress *per se* did not have a significant effect on the strength of selection on de novo mutations in any of our analyses, implying that mutational robustness is not generally greater in benign relative to stressful environments (23, 24).

Our analyses have been limited to purifying selection as a consequence of the fact that the very majority of de novo mutations are deleterious. However, increased conditional genetic variation in protein phenotypes at elevated temperature are in rare cases predicted to confer fitness benefits (25, 30, 50). Clearly, the increase in mutational effects at warm temperature is predicted to influence regional patterns of standing genetic variation and future evolutionary potentials under climate change. Previous studies have highlighted a range of possible consequences of temperature on evolutionary potential in tropical versus temperature regions, including faster generation times (38), higher maximal growth rates (56) higher mutation rates (40, 54) and more frequent recombination (57, 58) 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 greater levels of biodiversity in this area (59, 60). However, implications for species persistence under climate change will crucially depend on demographic parameters such as reproductive rates and effective population size (6, 9, 61) and greater selection in tropical areas may even result in increased extinction rates if evolutionary potential is limited (37, 56, 62, 63). 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 (64). Moreover, protein stability has itself been suggested to increase

evolvability and innovation by allowing slightly destabilizing mutations with conditionally beneficial effects on other aspects of protein fitness to be positively selected (65–67). Hence, the destabilizing effect of rising global temperatures on protein folding may, by reducing this buffering capacity, limit the potential for evolutionary innovation.

The observed temperature dependence of mutational effects also builds a scenario in which contemporary climate warming may lead to molecular signatures of increased purifying selection and 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 (68). 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 (69). Following the same reasoning, increased mutational effects in warm climates could select for increased mutational robustness (70–72). As mutation pressure on single genes is weak, the evolution of such increased genome integrity would likely involve mechanisms regulating mutation rate and/or robustness globally (73), such as the upregulation of chaperone proteins, known to assist both protein folding (31, 74) and DNA repair (75). It remains an open question, however, whether the increase in selection is strong enough to result in improved genome integrity in species with medium to small effective population sizes where genetic drift may dominate the evolution of genome architecture (76, 77). Alternatively, mutational

robustness may be more likely to result indirectly from selection for genome features leading to increased environmental robustness (26–29, 34), in line with predictions from the presented biophysical model of enzyme kinetics, suggesting that increased protein thermostability confers increased robustness to de novo mutation (Fig. 1C).

Environmental tolerance has classically been conceptualized and modelled by a Gaussian function mapping organismal fitness to an environmental gradient (e.g. (6, 78)). 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 protein studies (reviewed in: (32–36)), 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 selection and phenotypic effects under environmental stress (79–85). 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 genome size, phenotypic complexity (86, 87) and effective population size (9, 61, 77, 88). 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 (89). The juvenile phase is completed in approximately three weeks, and egg to adult survival is above 90% at benign 30°C (90). The lines were derived from an outbred population created by mixing beetles collected at three nearby sites in Nigeria (91). 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 each line. 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 (53, 54). 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. Two generations prior to the assaying all six lines were moved to 30°C, which is a beneficial temperature to both sets of lines (Fig. 2, (54)), 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 (92) for R. Temperature and selection regime as well as their interaction were included as fixed effects, and line identity crossed by assay 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 (75). Such breaks occur naturally during recombination, and in yeast to humans alike, point mutations arise due to errors during their repair (75). 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 (93), 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 (54), 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 (94). 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) by comparing the production of F2 adults in irradiated lineages, relative to the number of adults descending from F0 controls (SI 1). We also used F1 adult counts to derive this estimate, acknowledging that it may include non-trivial paternal effects from the irradiation treatment. However, results based on F1 and F2 estimates were consistent (Fig 3). Thus, to estimate the effects of elevated 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* (95); 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: $LOG_e[\Delta\omega_{stress}/\Delta\omega_{benign}]$, where $\Delta\omega = 1 - \omega^{mutant}/\omega^{CTRL}$. 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 (96) 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 pooled (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. Additionally, study organism was crossed with stress type to control for species variation and phylogenetic signal. To further explore large scale signals in the data we performed an analysis including a fixed factor encoding uni- or multicellularity, which was crossed with stress type, allowing us to test for differences in selection between the two groups. 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 reduction in mean fitness at the stressful temperature relative to the benign temperature (see SI 2), 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. Study organisms were also allowed to have random slopes for the temperature effect to control for between-species variation in the temperature dependence. Again we added a fixed effect encoding uni- or multicellularity crossed by the temperature covariate to test if the two groups differed in the temperature dependence of mutational fitness effects.

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Figure Legends:

Fig. 1. Predicted consequences of mutation ($\Delta\Delta G$ = +0.9, dashed lines) on A) enzyme kinetics and B) fitness for a cold-adapted (blue; T_{OPT} = 20 °C, ΔG = -4, $H^{\#}$ = 19.02) vs. a hot-adapted genotype (red; T_{OPT} = 36 °C, ΔG = -8, $H^{\#}$ = 20.00). For illustrative purposes the example depicts a wildtype (solid lines) compared to a mutant (dashed lines) carrying mutations in 20 out of a total of 200 rate determining proteins, with mutational effects on enzyme catalys, θ, = 10^{-4}. The strength of selection on a single mutation is depicted in panels C and D. Warm and cold adapted genotypes are predicted to experience the same strength of selection at their respective thermal optima (panel C), and the same increase in selection relative to a standardised benign reference temperature, here defined as $T_r = T_{OPT} - 10$ °C (panel D). The extent to which the strength of selection increases with temperature is reduced by unconditional mutational effects on catalytic rate (panel D: solid to dotted lines: θ = 0, 10^{-4}, 10^{-3} and 10^{-2}, respectively). The entropy term (ΔS) for the Gibbs free energy was held at a value of 0.25 at the reference temperature of 20°C (47).

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 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.

Fig. 3: Mutation load ($\boldsymbol{\Delta\omega}$) (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 $\boldsymbol{\Delta\omega}$ 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 (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 $\boldsymbol{\Delta V}_{stress}/\boldsymbol{\Delta V}_{benign} > 0$ correspond to greater mutational fitness effects under environmental stress. The 98 paired estimates of $\boldsymbol{\Delta\omega}$ (filled circles) 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). These results were qualitatively the same when analysing the fewer available estimates of mutational variance ($\boldsymbol{\Delta V}$: open triangles). 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 2 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 mean reduction in relative fitness between the benign and stressful temperature) did not affect the strength of selection ($P_{MCMC} > 0.3$).

Figure 1: An enzyme-kinetic model of mutational effects

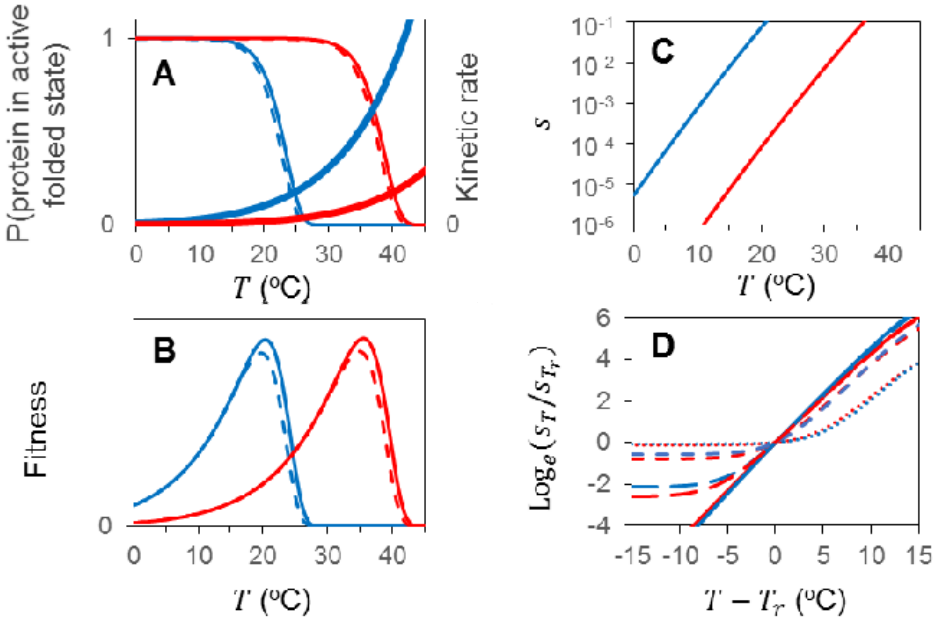

Figure 2: Thermal adaptation during experimental evolution

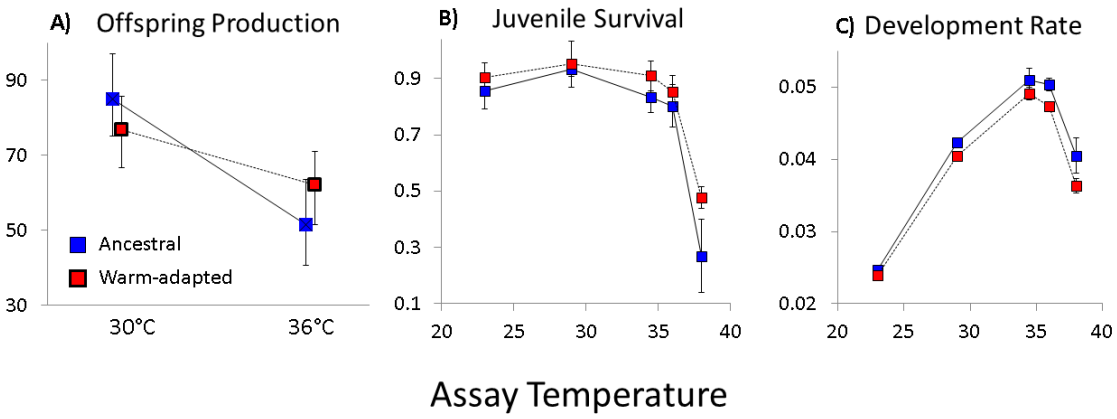

Figure 3: Temperature dependent mutational fitness effects

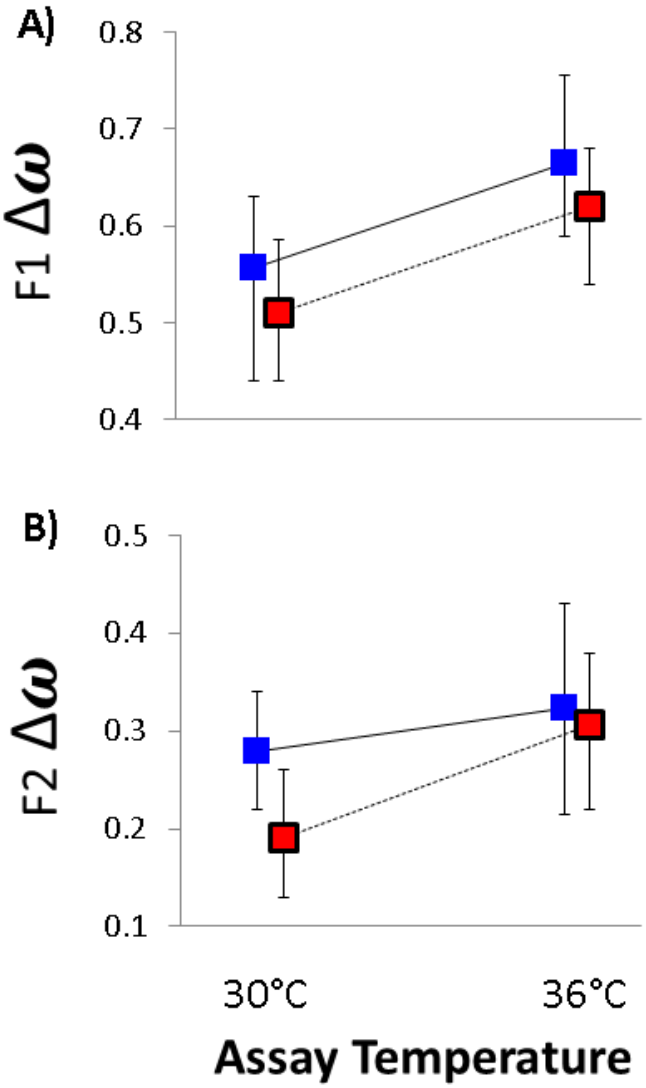

Figure 4: Meta-analysis of mutational fitness effects in stressful environments

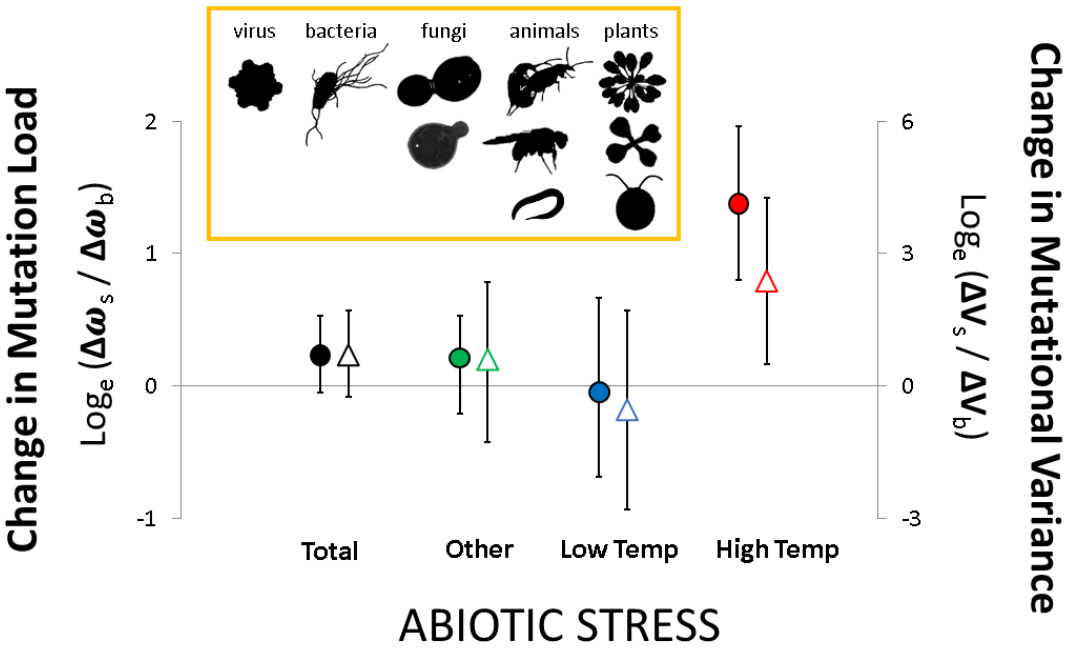

Figure 5: Meta-analysis of temperature-dependent mutational fitness effects