Elevated temperature increases genome-wide selection on de novo mutations

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ABSTRACT

Adaptation in new environments depends on the amount and type of genetic variation available for evolution, and the efficacy by which natural selection discriminates among this variation to favour the survival of the fittest. However, whether some environments systematically reveal more genetic variation in fitness, or impose stronger selection pressures than others, is typically not known. Here, we apply enzyme kinetic theory to show that rising global temperatures are predicted to intensify natural selection systematically throughout the genome by increasing the effects of DNA sequence variation on protein stability. We tested this prediction by i) estimating temperature-dependent fitness effects of induced random mutations in seed beetles adapted to ancestral or warm temperature, and ii) calculating 100 paired selection estimates on mutations in benign versus stressful environments from a diverse set of unicellular and multicellular organisms. Environmental stress *per se* did not increase the mean strength of selection on de novo mutation, suggesting that the cost of adaptation does not generally increase in new environments to which the organism is maladapted. However, elevated temperature increased the mean strength of selection on genome-wide polymorphism, signified by increases in both mutation load and mutational variance at elevated temperature. The theoretical predictions and empirical data suggest that this increase may correspond to a doubling of genome-wide selection for a predicted 2-4°C climate warming scenario in ectothermic organism living at temperatures close to their thermal optimum. These results have important implications for global patterns of genetic diversity and the rate and repeatability of evolution under climate change.

Impact 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, the biochemical properties of molecules and proteins that underpin the link between genotype and phenotype can exert a major influence over how the physical environment affects the expression of phenotypes and the fitness consequences of DNA sequence polymorphism. Yet, the constraints set by these molecular features are often neglected within eco-evolutionary theory trying to predict evolution in new environments. Here we combine predictions from existing biophysical models of protein folding and enzyme kinetics with experimental data from ectothermic organisms across the tree of life, to show that rising global temperatures are predicted to increase the mean strength of selection on DNA sequence variation in cold-blooded organisms. We also show that environmental stress *per se* generally does not increase the mean strength of selection on new mutations, suggesting that genome-wide natural selection is not stronger in new environments to which an organism is maladapted. Theoretical predictions and data suggest that an expected climate warming scenario of a 2-4˚C temperature raise within the forthcoming century will result in roughly a doubling of genome-wide selection for organisms living close to their thermal optima. However, our results also point to substantial variability in the temperature-dependence of selection on different proteins within and between organisms, suggesting scope for compensatory adaptation to shape this relationship. These results bear witness to and extend the universal temperature dependence of biological rates and have important implications for global

68 patterns of genetic diversity and the rate and repeatability of genome evolution under
69 environmental change.
70

Introduction

The strength of natural selection impacts on the rate and repeatability of evolution (Haldane 1927; Fisher 1930; Kimura 1968; Orr 2005; Svensson and Berger 2019), the maintenance of genetic variation (Lande 1975; Turelli 1984) and extinction risk (Haldane 1937; Bürger and Lynch 1995). However, despite being central to predicting the impacts of climate change, there is little information about whether some environmental factors impose stronger selection pressures than others (Chevin et al. 2010; Kokko et al. 2017). Mapping of the environment's influence on phenotype is therefore of paramount importance to understanding species persistence (Kingsolver et al. 2001; Agrawal and Whitlock 2010; Siepielski et al. 2013, 2017; Caruso et al. 2017). It is widely recognized that environmental change should increase the strength of directional selection on the key traits and genes underlying local adaptation. However, the fitness consequences associated with maladaptation at such genes may be relatively small compared to the variance in fitness attributed to segregating polymorphisms across the entire genome (Haldane 1937; Agrawal and Whitlock 2012). This reservoir of genetic variation is expected to have fundamental impact on adaptability and extinction risk (Haldane 1937; Bürger and Lynch 1995; Agrawal and Whitlock 2012), but how the environment influences the expression and consequences of genome-wide polymorphism remains poorly understood (Martin and Lenormand 2006; Agrawal and Whitlock 2010; Chevin et al. 2010; Caruso et al. 2017). For example, it is sometimes argued that fitness effects of sequence variation are magnified in new environments due to compromised phenotypic robustness and the release of "cryptic genetic variation" (de Visser et al. 2003; Paaby and Rockman 2014; Siegal and Leu 2014). Yet, empirical evidence for this prediction remains ambiguous (Hoffmann and Merilä 1999; Rowiński and Rogell 2017; Noble et al. 2019) and others have argued that environmental

95 change will have idiosyncratic effects on the mean strength of selection (Martin and
96 Lenormand 2006; Agrawal and Whitlock 2010). These conflicting predictions suggest that
97 only by understanding the mechanistic basis for how environments mould phenotypic
98 effects of genetic variation will it be possible to predict adaptation under future climate
99 change.
100
101 Here we demonstrate how considerations of biophysical constraints on protein function can
102 generate insights about how climate change and regional temperatures affect the strength
103 of selection on DNA sequences. Thermodynamics pose a fundamental constraint on protein
104 function, and although there is room for organismal adaptation to circumvent these
105 deterministic effects (Hochachka and Somero 2002; Clarke 2004; Angilletta 2009), there is a
106 strong temperature-dependence of ectotherm behaviour, life-history and fitness (Huey and
107 Kingsolver 1989; Hochachka and Somero 2002; Brown et al. 2004; Angilletta 2009). By
108 adopting a biophysical model of enzyme kinetics and protein stability, we demonstrate that
109 elevated temperatures are predicted to cause a mean increase in the fitness effects of de
110 novo mutation.
111
112 We test these predictions by measuring selection on induced mutations at benign and
113 elevated temperature in experimental evolution lines of the seed beetle, *Callosobruchus*
114 *maculatus*, adapted to ancestral or warm temperature. Second, we analyse 100 published
115 estimates of paired selection coefficients against de novo mutations in benign versus
116 stressful environments from a diverse set of ectothermic organisms, ranging from unicellular
117 bacteria and fungi, to multicellular plants and animals. Our analyses demonstrate that
118 environmental stress *per se* does not affect the mean strength of selection but provide

support for the prediction that elevated temperature leads to an average increase in genome-wide selection and genetic variance in fitness. These results have important implications for extinction risk and global patterns of genetic diversity and suggest that the evolution of DNA sequences could proceed at an ever-accelerating pace under continued climate warming.

Methods

Enzyme kinetics theory predicts temperature-dependence of mutational fitness effects

Ectotherm fitness shows a well-characterised empirical relationship with temperature that closely mirrors the thermodynamic performance of a rate-limiting enzyme (Huey and Kingsolver 1989; Hochachka and Somero 2002; Angilletta 2009). This empirical relationship reflects the fact that biological rates are governed at the biochemical level by the enzymatic catalytic rate, k_{cat}, described by the Eyring equation:

$$k_{cat} = k_0 e^{-\Delta G^a/RT} \qquad \text{(Eq. 1)}$$

Where k_0 is a rate-specific constant, ΔG^a is the Gibbs free energy of activation required for the enzymatic reaction (kcal mol^{-1}), R is the universal gas constant (0.002 kcal mol^{-1}) and T is temperature measured in degrees Kelvin (Schoolfield et al. 1981). Equation (1) thus describes an exponential increase in reaction rate with temperature (Hochachka and Somero 2002).

142 The observed decline in biological rate at temperatures exceeding the organism's thermal
143 optimum is attributed to a reduction in the proportion of functional enzyme due to
144 reversable inactivation via protein unfolding (DePristo et al. 2005; Tokuriki and Tawfik 2009;
145 Sikosek and Chan 2014; Bershtein et al. 2017; Echave and Wilke 2017) (Fig. 1a,b). Protein
146 folding can be described by a Boltzmann probability as a function of the Gibbs free energy of
147 folding, ΔG^f, which is thus a measure of protein stability (DePristo et al. 2005):
148

149 $$\text{Pr(folded)} = 1/\left(1 + e^{\Delta G^f/RT}\right). \qquad \text{(Eq. 2)}$$

150

151 At a benign temperature of 25˚C, most natural proteins occur in functional state and the
152 value of the Gibbs energy is negative (mean ΔG^f ~ -7 kcal mol^{-1}; DePristo et al. 2005; Chen
153 and Shakhnovich 2009). However, both ΔG^a and ΔG^f are comprised of an enthalpy term
154 (ΔH) and a temperature-dependent entropy term (ΔS) that reduces to: $\Delta G(T) = \Delta H + T\Delta S$
155 over the ecologically relevant temperature range of most organisms (Chen and Shakhnovich
156 2010). Based on values from the literature (Chen and Shakhnovich 2010; Dill et al. 2011), ΔS^f
157 ~ 0.25-1 kcal/mol K^{-1} for a protein of typical length. From equation (2) it is therefore clear
158 that warm temperatures increase entropy making ΔG^f less negative, which leads to a rapid
159 and non-linear reduction in the fraction of functional enzyme. This non-linearity suggests
160 that any mutational change in protein stability should be more consequential for folding
161 when temperatures rise (Fig 1a).
162

163 The reaction rate kinetics of equation (1) can be combined with the protein folding of
164 equation (2) to describe the "fitness" of a rate-determining protein, *i*, as a function of

temperature and the Gibbs energies for activation and folding (Chen and Shakhnovich 2010) (Fig. 1b):

$$\omega(\Delta G^a, \Delta G^f, T) \propto k_0 \frac{e^{-\Delta G_i^a(T)/RT}}{1+e^{\Delta G_i^f(T)/RT}} \qquad \text{(Eq. 3)}$$

Here we use equation (3) to explore the effect of temperature on the strength of selection on de novo mutations that impact catalytic rate and/or protein folding stability. We introduce a mutational change in the Gibbs free energy of activation ($\Delta\Delta G^a$) and folding ($\Delta\Delta G^f$), respectively:

$$\omega^*(\Delta\Delta G^a, \Delta\Delta G^f, T) \propto k_0 \frac{e^{-(\Delta G_i^a(T)+\Delta\Delta G_i^a)/RT}}{1+e^{(\Delta G_i^f(T)+\Delta\Delta G_i^f)/RT}}. \qquad \text{(Eq. 4)}$$

We then calculate mean selection against a de novo mutation:

$$s(T) = 1 - \omega_T^*/\omega_T \qquad \text{(Eq. 5)}$$

where $\boldsymbol{\omega}_T{}^*$ and $\boldsymbol{\omega}_T$ is fitness of the mutant and the wildtype at temperature *T*. Assuming fitness effects are multiplicative across proteins, substituting equations (3) and (4) into equation (5) yields the following expression:

$$s_i(T) = 1 - \frac{1+e^{(\Delta G_i^f(T))/RT}}{1+e^{(\Delta G_i^f(T)+\Delta\Delta G_i^f)/RT}}\, e^{-\frac{\Delta\Delta G_i^a}{RT}} \qquad \text{(Eq. 6)}$$

187 Based on equation (6), selection against mutations affecting enzymatic reaction rate ($\Delta\Delta G^a$)
188 is expected to be independent of the original catalytic rate of the wildtype (ΔG^a) and remain
189 largely unaffected by any ecologically relevant (i.e. 283-313˚K) change in temperature (Fig.
190 1c, see further: Supplementary 1.1). Note, however, from equations (2) and (4) and Fig. 1a
191 how mutation and temperature-driven increases in entropy both have non-linear effects on
192 protein folding. To illustrate the consequences of this synergism we applied equation (6) in
193 numerical simulations to calculate the expected mean selection on a mutation in a random
194 protein with its original stability drawn from a truncated gamma distribution ($\Delta G^f \sim -\Gamma(k$
195 $= 5.50$, $\theta = 1.89$) derived from empirical data from bacteria, yeast and nematodes (Ghosh
196 and Dill 2010). We compared the resulting temperature dependence of selection in three
197 enzyme ensembles with different hypothetical distributions of protein stabilities by shifting
198 the empirical gamma distribution so that mean ΔG^f = -6, -9 or -12, respectively (Fig. 1a-d).
199 In the example presented in figure 1, we set ΔS^f = 0.25, but in Supplementary 1 we show
200 results for different values of ΔS^f.
201
202 The majority of mutations with effects on folding ($\Delta\Delta G^f$) are expected to destabilize proteins
203 (DePristo et al. 2005; Drummond and Wilke 2008; Sikosek and Chan 2014; Echave and Wilke
204 2017) and the net impact of a single mutation has been estimated to $\Delta\Delta G^f \sim 0.9$ kcal mol^{-1}
205 (SD = 1.7) (Zeldovich et al. 2007; Tokuriki and Tawfik 2009), a value more or less independent
206 of the original protein stability (ΔG^f) (Chen and Shakhnovich 2009). Hence, each protein was
207 mutated by sampling a single folding mutation from this empirically estimated normal
208 distribution. Empirical estimates of mutational effects on the activation energy of random
209 enzymes are likely very biased (see Supplementary 1.1). In the simulations we therefore
210 chose parameter values of $\Delta\Delta G^a$ that yielded negative selection coefficients of magnitudes

211 that are typically observed at benign temperatures (s = 0–10^{-2}, T=298˚K, see Supplementary
212 1.1). Mutational effects on the Gibbs energy ($\Delta\Delta G$) can derive from a combination of both
213 enthalpic ($\Delta\Delta H$) and entropic ($\Delta\Delta S$) terms. In the examples in Figure 1 we assumed that all
214 mutational effects are enthalpic in origin. However, qualitative conclusions hold when
215 mutational effects are modelled through only the entropic term or enthalpic and entropic
216 terms combined (Supplementary 1.2). R code available upon request. We centre our
217 discussion in the *Results* on the temperature-dependence of the mean strength of selection,
218 as these predictions were most readily testable with our collated empirical data (see further
219 below). However, the temperature-dependence of selection on individual mutations is
220 predicted to be variable both across genes and organisms, and more quantitative predictions
221 strongly depend on model assumptions, which is further explored in Supplementary 1.
222

223 **Temperature-dependent fitness effects of mutations in seed beetles adapted to**
224 **contrasting thermal regimes**

225 To test model predictions, we measured fitness effects of induced random mutations at
226 30°C and 36°C in lines of the seed beetle *Callosobruchus maculatus*, evolved at benign 30°C
227 (three ancestral lines) or stressful 36°C (three warm-adapted lines) for more than 70
228 generations (see Supplementary 2). Previous studies have revealed significant differentiation
229 in key life history traits between the regimes (Rogell et al. 2014; Berger et al. 2017). Here we
230 further detailed thermal adaptation across the regimes by first analysing differences in
231 offspring production in the lines at the two assay temperatures in the current experiment
232 (see further below). Second, we quantified reaction norms for juvenile survival and
233 development rate across five temperatures (23, 29, 35, 36 & 38°C) following 100 generations
234 of experimental evolution and two generations of common-garden acclimation at 30°C to

235 remove non-genetic parental effects. Newly laid eggs were subsequently randomized to
236 each assay temperature with larval food provided ad libitum. Effects of assay temperature
237 and selection regime on survival and development rate (1/development time) of 2755
238 offspring, evenly split over the five assay temperatures and six replicate lines, were analysed
239 in linear mixed effects models in the lme4 package (Bates et al. 2014) for R (see further:
240 *Extended Supplementary Methods*).
241
242 We compared fitness effects of induced mutations at 30°C and 36°C for each line of the two
243 evolution regimes. A graphical depiction of the design can be found in Supplementary 2. All
244 six lines were maintained at 36°C for two generations of common-garden acclimation. The
245 emerging virgin adult offspring of the second generation were used as the F0 individuals of
246 the experiment. We induced mutations by exposing half of the F0 males to gamma radiation
247 at a dose of 20 Grey (20 min treatment), with the other half kept as controls. All males were
248 mated with virgin females from their own population at benign 30°C. The mated females
249 were immediately placed on beans presented ad libitum and randomized to a climate
250 cabinet set to either 30°C or 36°C (50% RH) and allowed to lay their lifetime storage of F1
251 eggs. To exclude non-genetic effects of the irradiation treatment, we measure mutational
252 effects in the F2 generation. To this end we applied a Middle Class Neighborhood breeding
253 design to nullify selection on all but the unconditionally lethal mutations amongst F1
254 juveniles (Shabalina et al. 1997); from the F1 survivors, we crossed two randomly selected
255 male and female offspring per family with another family from the same treatment and line,
256 so that each family contributed equally to the F2 generation (irrespective of the mutation
257 load it carried). This approach allowed us to quantify the cumulative deleterious fitness
258 effect of all but the unconditionally lethal mutations induced in F0 males (i.e. mutation load)

259 by comparing the production of F2 adults in irradiated lineages, relative to the number of
260 adults descending from F0 controls (Fig. S2). We also used F1 adult counts to derive an
261 estimate of load, acknowledging that it may include non-trivial paternal effects from the
262 irradiation treatment, in addition to pure mutational effects. However, results based on F1
263 and F2 estimates were consistent (Fig 3). To estimate the effects of elevated temperature on
264 mutational fitness effects in the two genetic backgrounds, we analysed the number of
265 offspring produced as a Poisson response, using generalized linear mixed effects models,
266 testing for interactions between radiation treatment, assay temperature and evolution
267 regime. To better illustrate the results graphically, we also ran Bayesian analyses using the
268 MCMCglmm package (Hadfield 2010) with the same model structure, but assuming a
269 normally distributed response. We then calculated the posterior estimates of mutation load
270 in each environment for each evolution regime as: $\boldsymbol{\Delta\omega} = 1\text{-}\ \boldsymbol{\omega}_{\mathrm{IRR}}/\boldsymbol{\omega}_{\mathrm{CTRL}}$ (where $\boldsymbol{\omega}$ is the
271 number of adult offspring) directly from these models (Fig. 3) (see *Extended Supplementary*
272 *Methods*).
273

274 **A meta-analysis of temperature dependent mutational fitness effects**

275 To test the hypothesis that elevated temperature increases the mean strength of genome-
276 wide selection more generally, we retrieved 100 paired estimates comparing selection on de
277 novo mutations across a benign and stressful environment from 28 studies on 11 organisms,
278 spanning viruses and unicellular bacteria and fungi, to multicellular plants and animals. The
279 original studies measured effects of mutations accrued by mutation accumulation,
280 mutagenesis, or targeted insertions/deletions. This was done by estimating fitness in form of
281 Malthusian growth rate, survival, or reproduction in mutants relative to wildtypes
282 (Supplementary 3). Hence, the strength of selection against mutants in each environment

283 (i.e. environment-specific mutation load) could be estimated as: $\Delta\boldsymbol{\omega}_i = 1-\boldsymbol{\omega}_i^*/\boldsymbol{\omega}_i$, where $\boldsymbol{\omega}_i^*$
284 and $\boldsymbol{\omega}_i$ is the fitness in environment *i* of the mutant and wildtype respectively. An estimate
285 controlling for between-study variation was retrieved by taking the log-ratio of mutation
286 load in the stressful relative to benign environment in each study: $\mathrm{Log}_e[\boldsymbol{\Delta\omega}_{\mathrm{stress}}/\boldsymbol{\Delta\omega}_{\mathrm{benign}}]$,
287 with a log-ratio above (below) 0 indicating stronger (weaker) selection against mutations
288 under environmental stress.
289
290 We analysed the log-ratios in meta-analysis using Bayesian mixed effects models to estimate
291 if log-ratios differed from 0 for three levels of environmental stress: cold temperature, warm
292 temperature, and other types of stress pooled, as well as for the total effect of stress
293 averaged across all studies. We also tested if log-ratios differed between the three types of
294 abiotic stress. Each estimated log-ratio's contribution to the final meta analytic result was
295 weighted by its sampling variance by passing the standard error (SE) of each log-ratio to
296 MCMCglmm using the idh(SE):units command. The standard errors were calculated by
297 propagation of measurement errors from the original studies. All models included stress-
298 type, mutation induction protocol and fitness estimate as main effects, although effects of
299 the latter two were never significant. We included study organism and study ID as random
300 effects. Additionally, study organism was crossed with stress type to control for species
301 variation and phylogenetic signal. To further explore large scale signals in the data we
302 performed an analysis including a fixed factor encoding uni- or multicellularity, which was
303 crossed with stress type, allowing us to test for differences in selection between the two
304 groups (see *Supplementary Methods*). We explored any potential publication bias by plotting
305 the precision of each estimate (1/standard error) against its mean. This showed no clear
306 evidence for such bias (Supplementary 3).

307

308 ## Results

309 **Enzyme kinetics theory predicts temperature-dependence of mutational fitness effects**

310 Enzyme kinetic theory parameterized with empirical estimates of protein stabilities (Eq. 6)
311 predicts that the mean strength of selection against random de novo mutations should
312 increase with temperature (Fig. 1c). This effect is further attributed to the synergistic effect
313 of temperature and de novo mutation to destabilize proteins (Fig. 1a). In Supplementary 1.2
314 and 1.3 we show that this is also predicted to lead to a substantial increase in evolutionary
315 potential in form of mutational variance in fitness and a larger fraction of both highly
316 deleterious and beneficial mutations. Second, the evolution of increased protein
317 thermostability is predicted to produce enzymes that are more robust to mutational
318 perturbation (Fig. 1a, c), and hence, mutations in proteins with low stability are predicted to
319 contribute disproportionally to temperature dependent mutational fitness effects
320 (Supplementary 1.3, see also: Agozzino and Dill 2018). Third, if differences in thermostability
321 of proteins evolve in response to environmental temperatures, cold- and warm-adapted
322 ecotypes are predicted to experience the same strength of selection on de novo mutations
323 at an environmental temperature standardized relative to the ecotypes' respective thermal
324 optima (Fig. 1d). We note here, however, that while individual proteins isolated from
325 mesophilic and thermophilic organisms do show evolved differences in stabilities, this seem
326 not to hold for the organismal-wide repertoires of proteins measured in vitro (Leuenberger
327 et al. 2017). Fourth, variation in the temperature dependence of mutational effects might be
328 generated by differences in ΔS^f, governing the temperature sensitivity of protein stability
329 (i.e. $\Delta G^f(T)$) (Supplementary 1.4, see also Sawle and Ghosh 2011). Hence, this could account
330 for variability in the temperature-dependence of selection across genes within an organism,

331 or general variability in the temperature-dependence of selection between organisms. Fifth,
332 while the mean mutational effect on catalytic rate ($\Delta\Delta G^a$) is predicted to be largely
333 independent of temperature (Supplementary 1.1), $\Delta\Delta G^a$ mutations are expected to weaken
334 the overall temperature-dependence of mutational fitness effects. The extent to which they
335 do depends on their effect size relative to that of folding mutations ($\Delta\Delta G^f$) (Fig. 1d).
336
337 [FIGURE 1]
338
339 These predictions arise from two well-established principles: i) enzymes show
340 disproportionate reversible inactivation at high temperatures driven by increases in the
341 entropy of folding (Hochachka and Somero 2002), and ii) the majority of de novo mutations
342 act to destabilize proteins (DePristo et al. 2005; Drummond and Wilke 2008; Tokuriki and
343 Tawfik 2009; Sikosek and Chan 2014; Bershtein et al. 2017; Echave and Wilke 2017). The
344 qualitative results are therefore robust to the particular mathematical formulation of the
345 enzyme-kinetic model. In Supplementary 1.5 we also show that a model of metabolic flux
346 theory incorporating protein toxicity (Serohijos and Shakhnovich 2014; Echave and Wilke
347 2017) makes the same qualitative predictions as outlined above. This model makes the
348 additional prediction that the temperature dependence should, all else equal, be stronger
349 for more abundant proteins. This is in line with documented greater fitness costs of folding
350 mutations in more expressed genes, which has been postulated as a reason for why such
351 genes evolve more slowly (Drummond et al. 2005; Serohijos et al. 2012).
352
353 **Temperature-dependent fitness effects of mutations in seed beetles adapted to**
354 **contrasting thermal regimes**

355 As expected, there was considerable signs of thermal adaptation across the compared
356 evolution regimes of seed beetle; offspring production assayed in the non-irradiated
357 lineages kept at the two temperatures (30°C vs 36°C) demonstrated local adaptation to
358 temperature (Fig. 2a). Thermal reaction norms for juvenile development rate and survival
359 showed that warm-adapted lines develop slightly slower but survive slightly better across
360 the temperature range (Fig. 2b-c), a sign of counter-gradient adaptation (Hochachka and
361 Somero 2002; Angilletta 2009) (i.e. counteracting the general effect of elevated temperature
362 to increase development rate and decrease survival).

363

364 [FIGURE 2]

365

366 Despite the observed thermal adaptation, there was no obvious difference in the
367 temperature-dependence of mutational fitness effects seen in control and warm-adapted
368 lines (interaction: P_{F1} = 0.43, P_{F2} = 0.90; Fig. 3); Elevated temperature caused a general
369 increase in mutation load in both the F1 (X^2 = 13.0, df = 1, P < 0.001, total n = 713, Fig. 3a)
370 and F2 generation (X^2 = 7.44, df = 1, P = 0.006, total n = 1449, Fig 3b), consistent with model
371 predictions (compare Fig. 1c and Fig. 3). The fact that mutational fitness effects in control
372 and warm-adapted genotypes, locally adapted to the alternative assay temperatures,
373 showed qualitatively similar thermal dependence suggests that high temperature, rather
374 than stress *per se*, caused the increase in selection. We note here that, while the greater
375 overall mutational effects seen in control relative to warm adapted lineages also are
376 consistent with model predictions (compare Fig. 1c and Fig 3), this difference is not easily
377 interpretable and may have several alternative explanations (see *Extended Supplementary*
378 *Methods*).

379

380 [FIGURE 3]

381

382 **A meta-analysis of temperature dependent mutational fitness effects**

383 Analysing all collated log-ratios together showed that selection against de novo mutation
384 was not greater under stressful abiotic conditions on average (log-ratio = 0.19, 95% CI: -0.07-
385 0.45; P_{MCMC} = 0.13, Fig 4). We continued by analysing the 40 estimates derived at high and
386 low temperature stress separately from the 60 estimates derived from various other
387 stressful environments (summarized in Supplementary 3). This revealed that selection on de
388 novo mutation increases at elevated temperature (log-ratio ≤ 0; P_{MCMC} < 0.001, n = 21,
389 studies = 10), whereas there was no increase at low temperature (log-ratio ≤ 0; P_{MCMC} = 0.67,
390 n = 19, studies = 11) or for the other forms of stress pooled (log-ratio ≤ 0; P_{MCMC} = 0.48, n =
391 54, removing 6 estimates that were not possible to analyse due to *s* in each environment ~
392 0, studies = 22). Elevated temperature led to a significantly larger increase in selection
393 relative to both cold stress (P_{MCMC} = 0.004) and the other stressors pooled (P_{MCMC} = 0.002)
394 (Fig 4). There was a tendency for cold stress to decrease selection in unicellular species and
395 increase it in multicellular species, but this effect was marginally non-significant (interaction:
396 P_{MCMC} = 0.066). Moreover, 5 of the 6 estimates at cold stress for multicellular species derive
397 from survival data on *D. melanogaster* and drive this trend (Fig. 5b). We found no evidence
398 for differences in the effect of elevated temperature on selection between the four
399 multicellular and three unicellular species in the data (P_{MCMC} = 0.45); mutational effects were
400 greater at elevated temperature in 8/10 and 10/11 cases in multicellular and unicellular
401 species, respectively (combined two-sided binomial test, P = 0.0015). Notably, the 12 log-
402 ratios that were significantly different from 0 (>1.96SE) at elevated temperature were all

403 positive, signifying increased selection (two-sided binomial test, P = 0.0005). An analysis of
404 64 paired estimates of mutational variance followed the same general pattern as mutation
405 load (Fig. 4 and Supplementary 3), as predicted by the biophysical model (Supplementary 1.2
406 and 1.3).
407
408 [FIGURE 4]
409
410 Using the 40 paired estimates of mutation load at contrasting temperatures we partitioned
411 effects on the strength of selection from i) stress *per se*; quantified as the reduction in mean
412 fitness at the stressful temperature relative to the benign temperature ($1 - \bar{\omega}_{stress}/\bar{\omega}_{benign}$),
413 and ii) that of the temperature shift itself; quantified as the magnitude and direction of the
414 temperature shift: $T_{stress} - T_{benign}$. The strength of selection was not related to stress (P_{MCMC} >
415 0.8). However, a shift towards warmer assay temperature *per se* caused a substantial
416 increase in mutation load (P_{MCMC} < 0.001, Fig 5). There was also a non-linear effect of
417 temperature (P_{MCMC} = 0.01, Fig 5). Again, the effect of cold temperature on the strength of
418 selection seemed to differ between the unicellular and multicellular species studied
419 (interaction: P_{MCMC} = 0.018, Fig. 5b). However, given that this pattern is driven almost solely
420 by the 5 estimates from *D. melanogaster*, more data is needed to say anything concrete
421 about this potential effect of cellularity at cold temperature.
422
423 [FIGURE 5]
424
425 The long term consequences of the revealed relationship will depend on if the predicted
426 effects of temperature on protein stability will change the relative abundance of nearly

427 neutral to strongly deleterious alleles (Kimura 1983; Bershtein et al. 2006; Agrawal and
428 Whitlock 2012; Siegal and Leu 2014). In Supplementary 3 we show that increases in both the
429 number of (conditionally) expressed mutations as well as increases in their average fitness
430 effect are likely to underlie the observed increases in $\Delta\boldsymbol{\omega}$ at elevated temperature.
431
432

433 **DISCUSSION**

434 Here we have presented evidence suggesting that elevated temperature increases the mean
435 strength of genome-wide selection and mutational variance in fitness, an observation
436 qualitatively consistent with the applied biophysical model of enzyme kinetics, which
437 ascribes these increases to magnified allelic effects on protein folding at elevated
438 temperature. In contrast, environmental stress *per se* did not have an effect on the strength
439 of selection in any analysis, implying that mutational robustness is not compromised in
440 stressful environments (Martin and Lenormand 2006; Agrawal and Whitlock 2010). The
441 empirical data suggest that, without adaptation, the depicted scenario of 2-4°C of warming
442 by the end of this century (Fifth Assessment Report - Climate Change 2013) could result in a
443 doubling of genome-wide selection on average. However, the existing data at hand also hint
444 at there being variation between organismal groups in the effect (Fig. 5), a possibility that
445 needs further empirical exploration. Indeed, the exact strength of the temperature
446 dependence of selection is predicted to vary between genes and mutations and depends on
447 model assumptions (Fig. 1, Supplementary 1). Moreover, even though there is a strong
448 qualitative agreement between empirical data and the theoretical predictions, further in-
449 depth studies are urgently needed to prove causality and evaluate model assumptions in
450 more detail.

451

452 An increase in mutational effects at warm temperature is predicted to influence regional
453 patterns of evolutionary potential. Previous studies have highlighted a range of possible
454 consequences of temperature on evolutionary potential in tropical versus temperature
455 regions (Allen et al. 2002, 2006; Mayhew et al. 2008, 2012; Tittensor et al. 2010; Puurtinen
456 et al. 2016; Yasuhara and Danovaro 2016), including faster generation times (Gillooly et al.
457 2002), higher maximal growth rates (Frazier et al. 2006; Walters et al. 2012), higher
458 mutation rates (Berger et al. 2017) and more frequent recombination (Lloyd et al. 2018) in
459 the former. Our results imply that also the efficacy of selection on DNA sequences may be
460 greater in the tropics, which together with the aforementioned factors could result in more
461 rapid evolution and diversification, in line with the greater levels of biodiversity in this area
462 (Jablonski et al. 2006; Tittensor et al. 2010). However, implications for species persistence
463 under climate change will crucially depend on demographic parameters (Bürger and Lynch
464 1995; Kokko et al. 2017), and given that most mutations are deleterious and act to
465 destabilize proteins, greater purifying selection in tropical areas may result in increased
466 genetic loads and extinction rates if evolutionary potential is limited (Kellermann et al. 2009;
467 Hoffmann and Sgrò 2011; Walters et al. 2012). Moreover, protein stability has itself been
468 suggested to increase evolvability by allowing destabilizing mutations with conditionally
469 beneficial effects on other aspects of protein fitness to be positively selected (Bloom et al.
470 2006; Wagner 2008, 2017; Soskine and Tawfik 2010). Hence, the destabilizing effect of rising
471 global temperatures on protein folding could by reducing this buffering capacity also limit
472 the potential for evolutionary innovation.

473

474 The observed temperature dependence builds a scenario in which climate warming may lead
475 to molecular signatures of increased purifying selection and genome-wide convergence in
476 taxa experiencing climate warming. In support of this claim, Sabath et al. (2013) showed that
477 growth temperatures across thermophilic bacteria tend to be negatively correlated to the
478 non-synonymous to synonymous (dN/dS) nucleotide substitution-rate. Effects could possibly
479 extend to other aspects of genome architecture. For example, Drake (2009) showed that two
480 thermophilic microbes have lower mutation rates than their seven mesophilic relatives and
481 suggested that increased fitness consequences of mutation at hot temperature selects for
482 decreased mutation rate. Increased selection in warm climates could also select for
483 increased mutational robustness (Wagner 2005; Rajon and Masel 2011) via additional
484 mechanisms not explicitly considered by our model, for example via upregulation of
485 chaperones such as heat-shock proteins, known to assist both protein folding and DNA
486 repair (Hochachka and Somero 2002). Indeed, some of the variation in the revealed
487 temperature dependence of mutational fitness effects may be rooted in the use and extent
488 of such compensatory mechanisms (Clarke 2004; Sikosek and Chan 2014; Fields et al. 2015).
489
490 Environmental tolerance has classically been conceptualized by a Gaussian function mapping
491 organismal fitness to an environmental gradient (e.g: Levins 1968; Huey and Kingsolver
492 1989; Bürger and Lynch 1995). In this framework stress is not expected to increase mean
493 selection against de novo mutation (Martin and Lenormand 2006), a prediction supported by
494 our estimates of selection under forms of environmental stress other than temperature (Fig.
495 4). This framework assumes that phenotypic effects of mutations remain constant. The
496 applied biophysical model differs from this assumption in that mutation and temperature
497 are assumed to have synergistic effects on protein stability, causing mutational effects on

498 protein folding to increase with temperature. While supported by a number of targeted
499 studies on mutant proteins (DePristo et al. 2005; Tokuriki and Tawfik 2009; Sikosek and Chan
500 2014; Bershtein et al. 2017; Echave and Wilke 2017; Dandage et al. 2018), it remains less
501 clear how these effects map to the level of physiological, morphological and life history
502 phenotypes (Hoffmann and Merilä 1999; Clarke 2004; Berger David et al. 2011; Husby et al.
503 2011; Berger et al. 2013; Paaby and Rockman 2014; Rowiński and Rogell 2017).
504
505 Another open question is how the unveiled temperature-dependence interacts with other
506 features expected to influence the distribution of fitness effects of segregating genetic
507 variants, such as niche width (Chevin et al. 2010; Walters et al. 2012), genome size (Lynch
508 2007), phenotypic complexity (Orr 2000) and population size (Kokko et al. 2017). Unicellular
509 and multicellular organisms differ in these aspects, and interestingly, our data hint at a
510 difference in mutational fitness effects between the two groups at cold temperature. The
511 model also predicts that the temperature dependence should be weakened by an increased
512 fraction of unconditionally deleterious mutations. Differences between unicellular and
513 multicellular organism could therefore also arise if the link between fitness and rate-
514 dependent processes at the level of enzymes is more direct in unicellular compared to
515 multicellular organisms, resulting in a higher fraction of unconditional mutations and weaker
516 temperature dependence in the latter. Questions such as these will be crucial to answer to
517 understand how the unveiled temperature dependence of mutational fitness effects will
518 affect regional and taxonomic patterns of genetic diversity and evolutionary potentials
519 under climate change.
520
521

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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.

Data accessibility:

http://dx.doi.org/10.5061/dryad.6dd04

Competing Interests

The authors have no competing interests to report

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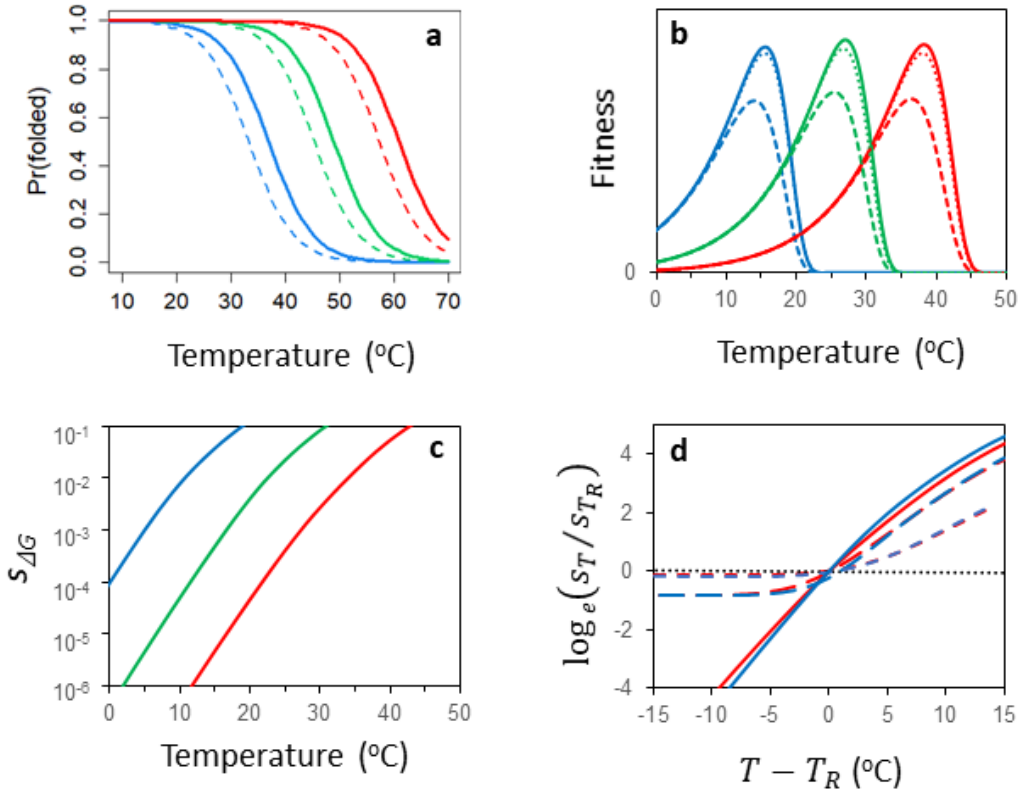
Figure legends

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

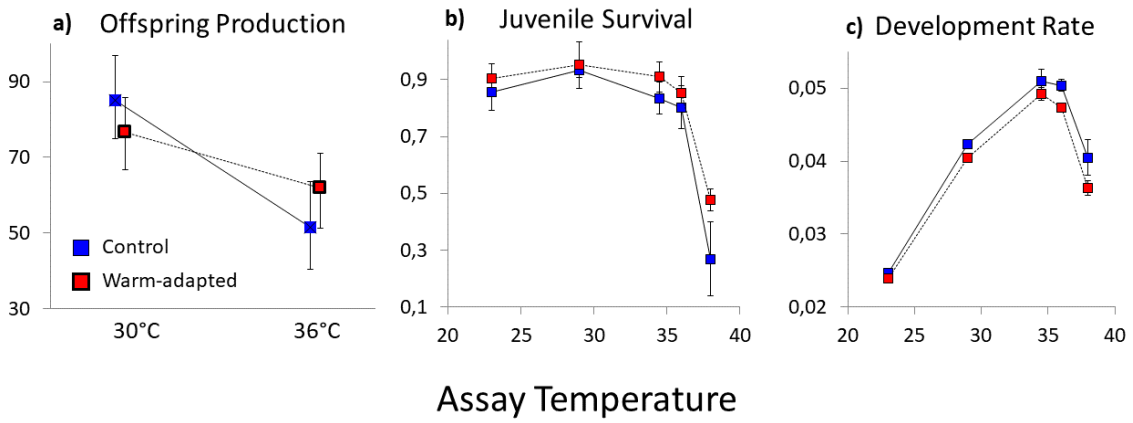


In a) the fraction of folded protein for a wildtype (full lines) and mutant protein with a single stability mutation ($\Delta\Delta G^f$ = 0.9, dashed lines). Illustrated for an unstable (blue) intermediate (green) and stable (red) protein ($\Delta G^f_{T=298}$= -3, 6 and -9 kcal/mol, respectively). In b) expected fitness for three genotypes with an ensemble of 500 multiplicatively acting proteins with different mean stabilities ($\Delta\bar{G}^f$) (blue, green and red lines reflect $\Delta\bar{G}^f$ values of -6, -9 and -12 kcal/mol and ΔG^a values = 19.25, 20.00 and 20.76 kcal/mol). Solid lines = wildtype, short-dashed lines = mutant carrying a single folding mutation, long-dashed lines = mutant carrying 10 folding mutations. Mutations were drawn from the empirical normal distribution ($\Delta\Delta G^f$ = 0.9 ± 1.7 SD). In c) the expected mean selection coefficient against a single folding mutation occurring at a random gene for each of the three genotypes. In d, '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 $\Delta\bar{G}^f$ = -6 and -12 are shown). Mutational fitness effects on catalytic rate ($\Delta\Delta G^a$) show no discernible temperature dependence (black dotted line; here $\Delta\Delta G^a$ lowers fitness at T_{opt} by s = 10^{-2}). However, if a mutation has pleiotropic effects on both $\Delta\Delta G^f$ and $\Delta\Delta G^a$, the temperature dependence of selection against the mutant brought about by its effects on stability can be masked (full, long-dash and short-dash lines equate to a s($\Delta\Delta G^a$) = 0, 10^{-3} and 10^{-2} at T_{opt}). All examples used a value of ΔS^f = 0.25kcal/mol K^{-1} and

753 assumed that mutations affected stability via changes in enthalpy ($\Delta\Delta H^f$). See main text and
754 Supplementary 1 for further details.

755 **Figure 2: Thermal adaptation during experimental evolution**

756



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758

759 The level of adaptation to simulated climate warming measured as (A) adult offspring production at
760 30 and 36°C, and thermal reaction norms for (B) juvenile survival and (C) development rate (means ±
761 95% confidence limits). Blue and red symbols denote control and warm-adapted lines, respectively.
762 While offspring production was generally decreased at 36°C relative to 30°C (X^2 = 62.5, df = 1, P <
763 0.001, total n = 698), this decrease was less pronounced in warm-adapted lines (interaction: X^2 =
764 7.35, df = 1, P = 0.007; Fig. 2a) signifying significant local adaptation to respective thermal regime.
765 Warm-adapted lines showed consistently slower development (X^2 = 27.2, df= 1, P < 0.001, total n =
766 2755, Fig 2b) and marginally higher survival (X^2 = 3.74, df= 1, P = 0.053, total n = 2755, Fig. 2c) across
767 temperatures, in line with counter-gradient adaptation in response to the effect of elevated
768 temperature to increase juvenile development rate but decrease juvenile survival.

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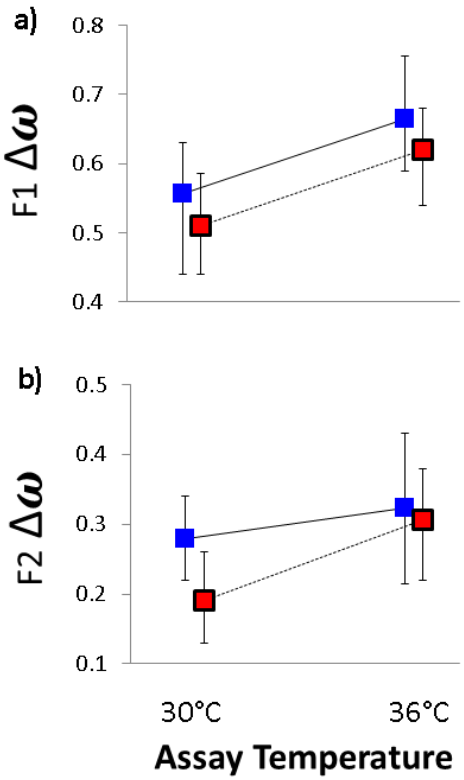
770

771 **Figure 3: The evolution of temperature dependent mutational fitness effects**



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774 Mutation load (**Δω**) (mean ± 95% confidence limits) measured for (A) F1 juvenile survival and (B) F2

775 adult offspring production, at the two assay temperatures. There was an overall strong and

776 significant increase in **Δω** at hot temperature. This effect was similar across the three control (blue)

777 and three warm-adapted (red) lines, in both the F1 and F2 generation.

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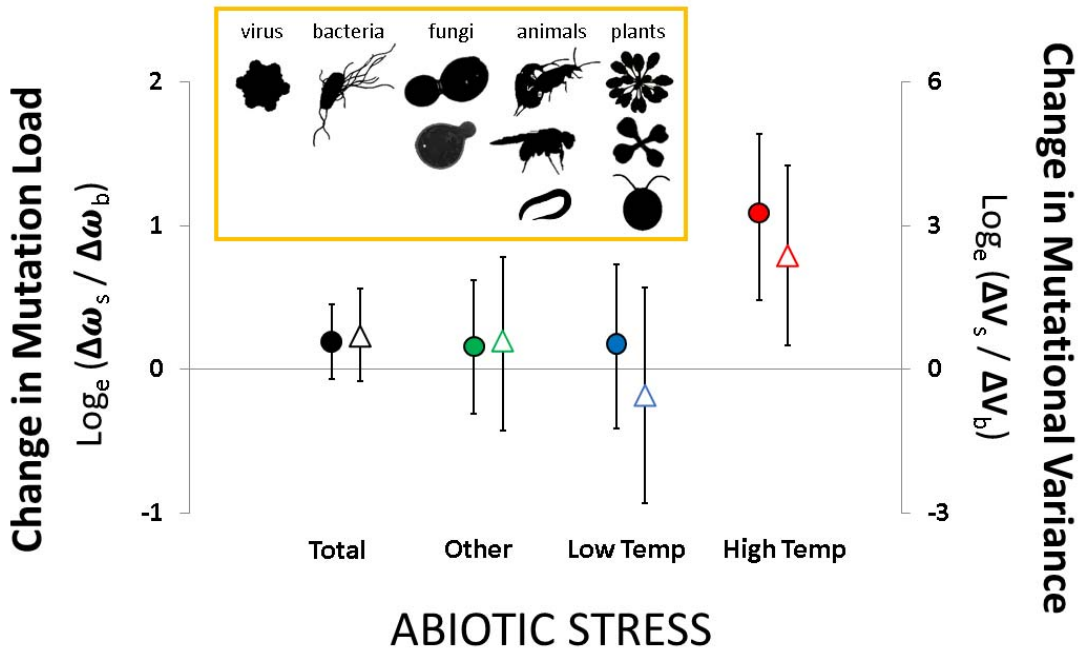
780 **Figure 4: Meta-analysis of mutational fitness effects in stressful environments**



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782

783 Meta-analysis of the effect of abiotic stress on the mean strength of selection against de novo
784 mutations (filled points) and mutational variance (open triangles) analysed by log-ratios (Bayesian
785 posterior modes ± 95% credible intervals): $\Delta_{stress}/_{benign}$ and $\Delta V_{stress}/_{benign}$ > 0 correspond to
786 greater mutational fitness effects under environmental stress. The 94 paired estimates of **Δ** (filled
787 circles) show that selection is not greater in stressful environments overall (P_{MCMC} = 0.13) and highly
788 variable across the 25 studies analysed. However, estimates of **Δ** at high temperature are greater
789 than their paired estimates at benign temperature (P_{MCMC} < 0.001). Results were similar when
790 analysing the fewer available estimates of mutational variance (**ΔV:** open triangles, P_{MCMC} = 0.02). The
791 box shows the eleven species included in the analysis (of which two were roundworms), covering
792 four major groups of the tree of life. See main text and Supplementary 3 for further details.

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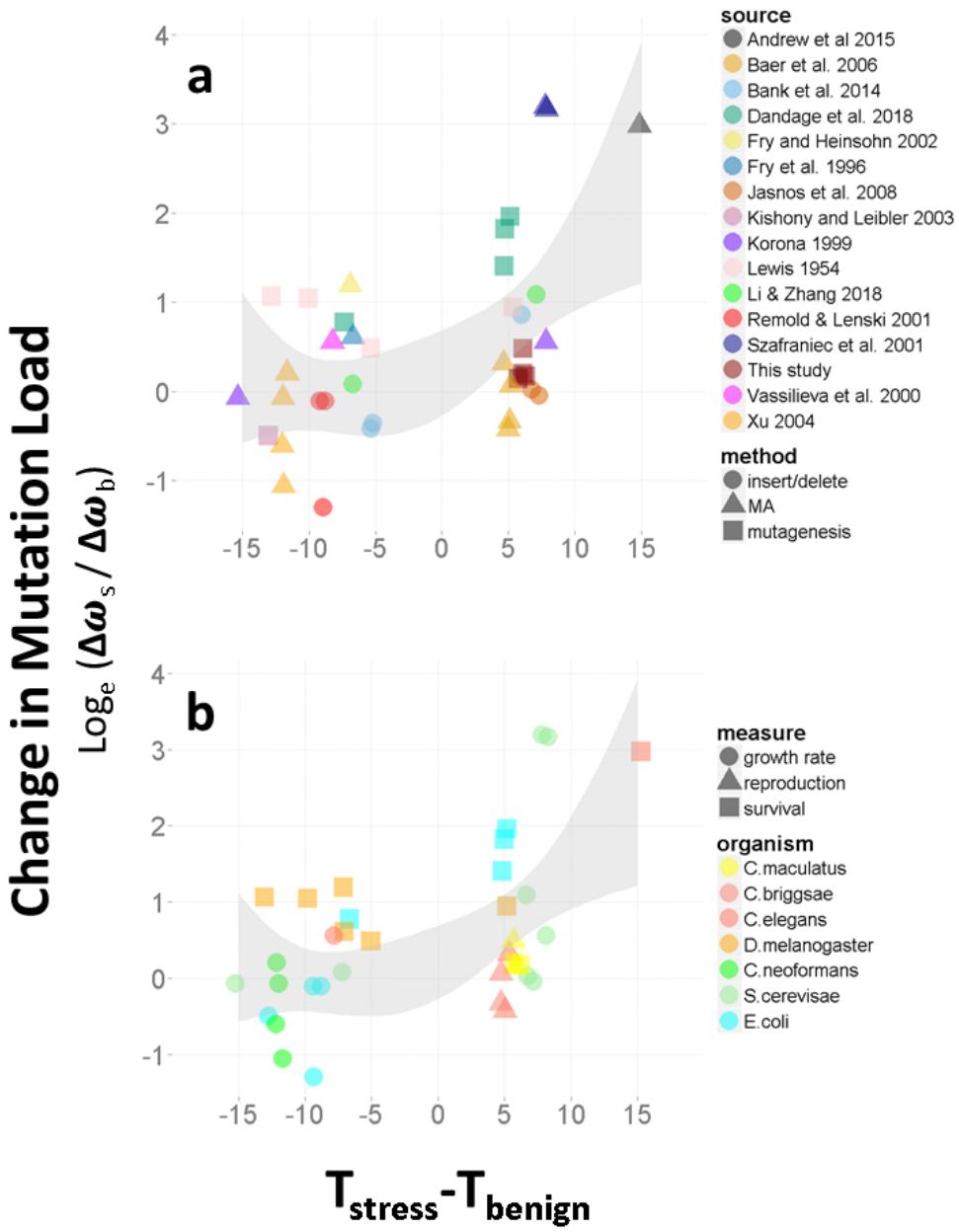
Figure 5: Meta-analysis of temperature-dependent mutational fitness effects



Temperature-dependent mutational fitness effects. 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. In (A) the 16 studies analysed and the method used to induce mutations, is depicted. In (B) the seven species analysed, and the fitness measure taken, is depicted. 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 ($P_{MCMC} > 0.8$). The grey shaded area represents the 95% CI from a second degree polynomial fit of the log-ratios on temperature, weighted by the statistical significance of each estimate (absolute log-ratio/standard error). Points are jittered for illustrative purposes.