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Temperature effects on life-history trade-offs, germline maintenance and mutation rate under simulated climate warming

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Mutation has a fundamental influence over evolutionary processes, but how evolutionary processes shape mutation rate remains less clear. In asexual unicellular organism, increased mutation rates have been observed in stressful environments and the reigning paradigm ascribes this increase to selection for evolvability. However, this explanation does not apply in sexually reproducing species, where little is known about how the environment affects mutation rate. Here we challenged experimental lines of seed beetle, evolved at ancestral temperature or under simulated climate warming, to repair induced mutations at ancestral and stressful temperature. Results show that temperature stress causes individuals to pass on a greater mutation load to their grand-offspring. This suggests that stress-induced mutation rates, in unicellular and multicellular organisms alike, can result from compromised germline DNA repair in low condition individuals. Moreover, lines adapted to simulated climate warming had evolved increased longevity at the cost of reproduction, and this allocation decision improved germline repair. These results suggest that mutation rates can be modulated by resource allocation trade-offs encompassing life-history traits and the germline and have important implications for rates of adaptation and extinction as well as our understanding of genetic diversity in multicellular organisms.

1. Background

Mutation rates are pivotal in determining fundamental evolutionary and demographic processes, including the evolution of sex and ageing, the maintenance of genetic variation and prevalence of genetic disease, as well as rates of adaptation, speciation and extinction. Although numerous efforts have been devoted to documenting variation in mutation rate both within and across taxa [1–3], the ultimate causes for this variation are still poorly understood [3–7]. Selection for modifiers of mutation rate is generally expected to be weak and proportional to the reduction in the genome wide deleterious mutation rate [8,9]. This suggests that variation in the mutation rate among phyla can to a large extent be explained by differences in population size and resulting limits to the efficacy of selection for a reduced mutation rate [3,10]. However, there is considerable intraspecific variation in the mutation rate [4,6,11–13], suggesting that mutation rates can be contingent upon a range of extrinsic and/or intrinsic factors.

An organism's ability to repair DNA damage seems to be a major determinate of its realized mutation rate, and DNA repair is thought to be associated with sizeable costs related to protein synthesis and replication speed [14]. Thus, the benefits of a decreased mutation rate may be counterbalanced by costs of increased DNA repair fidelity (the *cost-of-fidelity* hypothesis: [11,15]), and if so, both genetic and ecological factors could influence the equilibrium of this trade-off. For example, bacteria [16–18] and unicellular eukaryotes [19–20] exposed to stressful

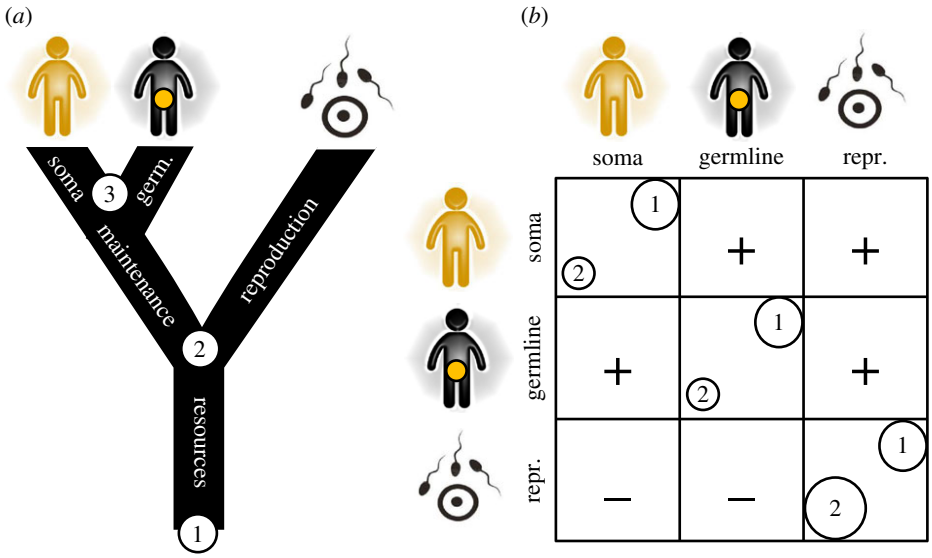

Figure 1. Germline maintenance and life-history trade-offs. (*a*) Energy acquisition and allocation based on the 'Y-model' [23]. The width of each branch gives the amount of resources allocated to a specific function, and individuals are assumed to vary in both the total amount of resources available (1), the proportion of resources allocated to reproduction versus maintenance (2) and how maintenance is allocated between soma and germline (3). Depending on for which trade-off (1–3) there is most individual variation, phenotypic and/or genetic correlations between the three functions can take on different signs and magnitudes. Two cases are illustrated in (*b*). When individuals primarily differ in their overall resource acquisition (1) individuals in high phenotypic condition will outperform low condition individuals across the board, leading to positive correlations between the three traits (above diagonal). When all individuals acquire similar resource levels and instead differ primarily in how they allocate resources between maintenance and reproduction (2), reproductive effort is negatively correlated to both somatic and germline maintenance (below diagonal). On the diagonal, the two cases are denoted with the size of the circle giving the amount of resource invested in each trait by the individuals allocating the highest absolute amount of energy into reproduction and offspring production. Hence, depending on the effect of germline maintenance on mutation rate and the materialization of trade-offs, individuals contributing most offspring to the next generation could either contribute with the most or the least number of mutations. (Online version in colour.)

environments evolve elevated mutation rates. Such observations have been ascribed to mutator alleles hitch-hiking with the beneficial mutations that they happen to create. However, alternative explanations instead invoke trade-offs in the form of direct selection for a maintained replication rate at the cost of reduced DNA repair fidelity under stress, leading to extensive debate about the ultimate causes of stress-induced mutation in unicellular organisms (e.g. [4,11,13,21,22]).

While the cost-of-fidelity hypothesis has its origins in research on microorganisms, it remains poorly explored in multicellular species [4,7] and little is known about how diploid mutation rates evolve in changing environments. Importantly, indirect selection for evolvability via genetic hitch-hiking is not a viable explanation for elevated mutation rates in sexually reproducing organisms, because sex and recombination breaks up genetic linkage between mutator alleles and the beneficial mutations they may create [8]. Natural selection seems to have reduced the mutation rate of the diploid germline to relatively low levels, evidenced by its several times lower mutation rate [10] and disproportionately high maintenance costs [7] compared to somatic tissue. Despite this apparent independence, the soma and germline share a majority of the molecular pathways involved in the DNA damage response [14]. Therefore, it seems likely that germline maintenance and resulting mutation rates in multicellular eukaryotes could be affected by allocation decisions between reproductive effort and somatic maintenance, with the optimal solution to these life-history trade-offs set by ecological conditions.

Here we tested the cost-of-fidelity hypothesis within a life-history theory framework (figure 1), to investigate germline DNA repair in a sexually reproducing multicellular eukaryote adapting to simulated climate warming in the laboratory. Within this framework, resource acquisition is assumed to differ between individuals in high and low phenotypic condition, and limited resources need to be allocated between competing physiological demands [23,24]. Consequently, mutation rate is predicted to depend on both the total amount of resources carried by an individual, and the trade-off between allocating those resources to reproduction or maintenance of the soma and germline. Moreover, whether germline repair is primarily determined by variation in overall condition or in resource allocation decisions is predicted to have important implications. If repair is chiefly regulated by variation in condition, then individuals contributing the most offspring to the next generation would contribute with relatively few mutations [25,26]. However, if repair foremost depends on an allocation trade-off between reproduction and maintenance, the situation could be reversed and the mutation rate might increase in subsequent generations (figure 1).

We explored these hypotheses by testing how a stressful temperature regime affected plastic and genetic responses in life-history traits and germline maintenance in replicated long-term experimental evolution lines of the seed beetle *Callosobruchus maculatus*. We present evidence suggesting that germline maintenance is compromised under thermal stress. We further show that compensatory adaptation to increasing temperature has led to the evolution of increased allocation to longevity at the expense of reproduction, and that this allocation decision is associated with increased germline maintenance under thermal stress. These results have bearing on predictions of diploid mutation rates and evolutionary responses under climate warming, and more generally support the hypothesis that the evolution of life-history trade-offs can lead to correlated responses in germline mutation rate.

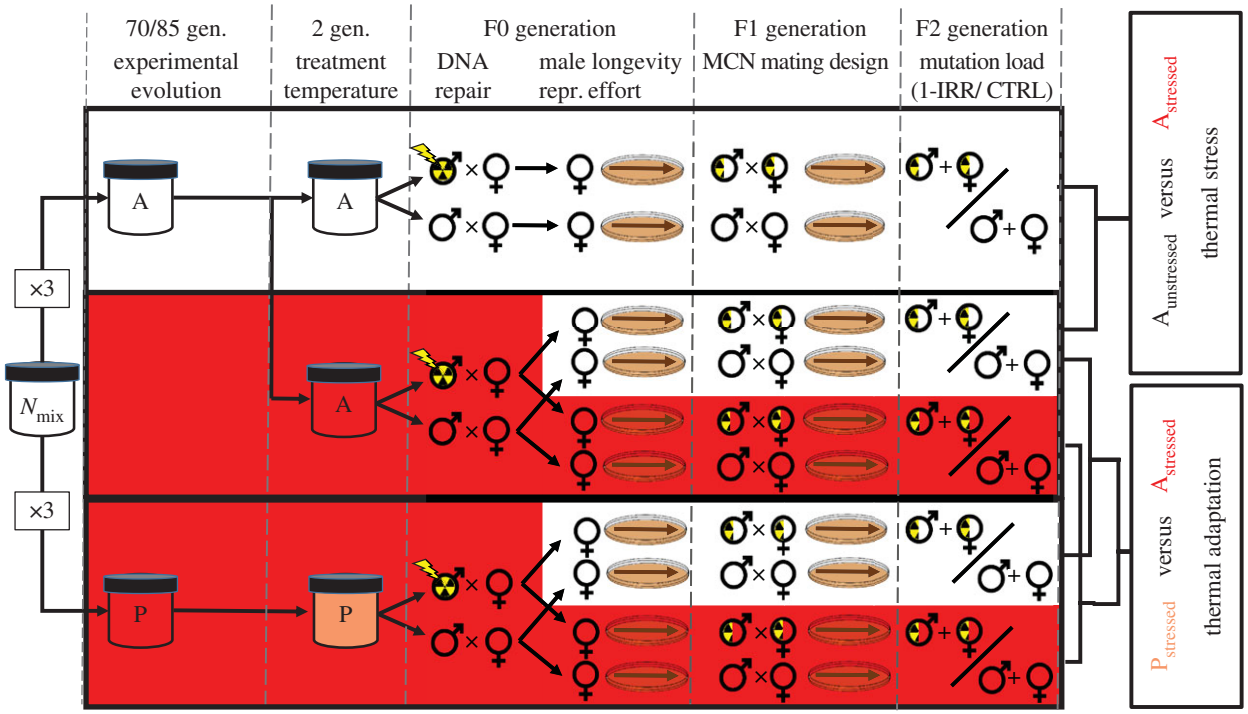

Figure 2. A schematic overview of the experimental design. An outbred laboratory population (N_{mix}) was used to create the three replicate ancestral (A) and preadapted (P) populations, adapting for 70 (2015 experiment) and 85 (2016 experiment) generations to either ancestral 30°C (white background) or stressful 36°C (red/dark background). Each population's investment into longevity and reproductive effort was assayed in the F0 generation. Radiation symbols indicate induction of mutations in F0 males via gamma radiation. F1 descendants are expected to receive half of this load from fathers, given no mutational filtering in the F0 generation. Petri dishes indicate the transferring of individuals onto black eyed beans for egg laying. Mutation load was quantified by first applying a middle class neighbourhood (MCN) breeding design to F1 juveniles, relaxing selection on all but the unconditionally lethal mutations, allowing these to pass onto the adult stage. The F1 adult descendants were mated in a round robin design and assayed for the number of adult F2 offspring produced, relative to the number of F2 offspring produced by mating couples descending from F0 control males of the same population and rearing temperature. The effect of thermal stress on germline maintenance was assessed by comparing mutation load in unstressed and stressed ancestral populations, and the effect of compensatory thermal adaptation was assessed by comparing mutation load of stressed ancestral and preadapted populations averaged across the two assay temperature. For details see main text. (Online version in colour.)

2. Methods

(a) Study populations

Callosobruchus maculatus is a capital breeding beetle that has colonized most of the tropical and subtropical regions of the world. Adults do not require food or water to reproduce at high rates and both sexes start reproducing on the day of adult eclosion [27]. The juvenile phase is completed in approximately 22–25 days, and egg to adult survival rate is above 90% at 30°C, which is a benign temperature for this species [28–31]. The experimental populations were derived from an outbred population created by mixing beetles collected at three nearby sites in Nigeria [32]. This population was reared at 30°C on black eyed beans (*Vigna unguiculata*), and maintained at large population size (minimizing inbreeding) for more than 90 generations (reducing linkage disequilibrium) prior to experimental evolution. Four replicate populations were kept at 30°C (ancestral populations). Four other populations were exposed to gradually increasing temperatures from 30 to stressful 36°C for 18 generations (i.e. 0.3°C/generation) and then kept at 36°C (preadapted populations). While this experimental evolution protocol lacks some of the realism associated with ongoing climate change in terms of temperature variability and associated correlated responses in other abiotic factors, it efficiently isolates the effect of increasing temperature means and represents a rate of warming predicted for many semivoltine ectotherms under projected scenarios of climate warming. Population size was kept at 200 individuals for the first 18 generations and then increased to 500 individuals. One of the ancestral populations was lost due to mishandling in generation 65. Therefore, we decided to randomly discard one preadapted population, comparing three populations of each regime. At the onset of our 2015 and 2016 experiments, the populations had been maintained for 70 and 85 generations, respectively. Previous studies have revealed significant differentiation in key life-history traits between the regimes [31,33].

(b) Experimental design

Our aims were to first explore the effect of thermal stress on life-history traits and germline maintenance, and then attempt to disentangle if putative effects on germline maintenance were related to variation in phenotypic condition and life history. A graphical depiction of the design can be found in figure 2. We employed a common garden design including the two experimental evolution temperatures (30 and 36°C). We created two replicates each of the three ancestral populations. One replicate remained at 30°C (unstressed) and the other was moved to 36°C (stressed). The three preadapted populations were maintained at 36°C. Following a full generation of acclimation, emerging adults were allowed to lay eggs on fresh beans for 48 h, after which these beans were isolated individually and allowed to develop at the respective temperature. The emerging virgin adults were used as the focal F0 individuals for whom we assessed germline maintenance and life-history variation. This design allowed us to test if thermal stress impaired germline maintenance by comparing stressed (36°C) and unstressed (30°C) ancestral populations, with the expectation to find reduced germline maintenance in the stressed populations. Comparison of ancestral and preadapted populations when reared at 36°C allowed us to test the effect of adaptation to increasing temperature on germline maintenance,

with the expectation that adaptation generally should improve germline maintenance. To further detail the effect of temperature on phenotypic condition and life-history traits, we measured longevity and reproductive effort in all populations (see further below). Adult longevity without access to water and nutrition is strongly genetically correlated in the sexes, and a very good measure of body condition in this species [34,35].

(i) Inducing mutations

To test the efficiency of germline repair, we induced DNA damage by exposing F0 males to gamma radiation at a dose of 20 Gy (20 min treatment). Gamma radiation causes double and single stranded breaks in the DNA, which in turn induces DNA repair mechanisms [14,36]. Such breaks occur naturally during recombination, and in yeast to humans alike, point mutations arise due to errors during their repair [14,37]. Importantly, variation in the mutation rate is almost entirely attributable to the density and type of repair molecules, and not to the number of DNA lesions induced by a given dose of gamma radiation [36,37], which is surprisingly constant per DNA base pair from bacteria to humans [38]. The use of gamma radiation is thus an ideal method to evaluate the efficacy of the DNA repair system [39,40]. Importantly, as the majority of new mutations are neutral or deleterious [41,42], the number of mutations transferred from parents to offspring can be approximated by the decline in relative fitness of lineages that in previous generations were challenged to repair induced mutations.

Newly emerged (0–24 h old) virgin males from all populations were isolated into 0.3 ml ventilated Eppendorf tubes and acclimated at room temperature (22°C) for 2 h prior to and during the application of irradiation. Males were randomly assigned to a treatment category and either placed inside a Gamma Cell-40 radiation source (irradiated), or on top of the machine for the endurance of the treatment (controls). After an additional 2 h at room temperature post-irradiation, all males were emptied of ejaculate and mature sperm by mating with females (that later were discarded) on heating plates set to 30°C. The males were subsequently put back at their respective acclimation temperature and given 26 h to regenerate new ejaculate and repair DNA damage in their germline cells. This comprehensive procedure was performed to (i) maximize the opportunity for variation in male germline repair to contribute to variation in the number of mutations in transferred sperm (as males typically do not repair fully matured sperm, see: [43]) and (ii) discard the first ejaculate, which might have contained seminal fluid proteins that had been damaged by the irradiation treatment [38] and could therefore have caused unwanted paternal effects in offspring. Simultaneously, the procedure also challenged males to mature a new ejaculate to reveal variation in allocation to reproduction versus longevity among populations. After the 26 h, all males were mated with virgin females from their own population on heating plates set to 30°C and then individually put back in Eppendorf tubes and censused daily to retrieve an estimate of each population's F0 longevity. Previous experiments suggest that there is no effect of irradiation on male mating or sperm competition ability [29,30]. Indeed, irradiation did not have a mean effect on male longevity in this experiment ($n = 1049$, $\chi^2 = 0.30$, $p = 0.58$, electronic supplementary material, figure S1), nor did it affect the relative ranking in male longevity among the studied populations (correlation between irradiated and control population mean longevity across years and temperatures: $r = 0.94$, $n = 30$, $p < 0.001$). This suggests that paternal effects (other than the mutations carried in the sperm) owing to the irradiation treatment were negligible. The mated females were placed on beans presented ad libitum and allowed to lay eggs that would produce the F1 adults used to assess mutation load (see below). All adult F1 offspring produced by control mating pairs were counted to retrieve an estimate of each population's F0 reproductive effort. As the female sex is known to repair mutations in male sperm in fruit flies [40] and various vertebrates [43], it can be assumed that we measured the combined effect of germline maintenance across both sexes.

(ii) Estimating germline maintenance

We applied a middle class neighbourhood (MCN) breeding design to nullify selection on all but the unconditionally lethal mutations among F1 juveniles. To achieve this we randomly selected 2–4 adult male and female F1 descendants from each F0 family (see [44]). To exclude inbreeding, F1 virgin adults were assigned a mating partner descending from another F0 family within the same population and treatment. This approach allowed us to measure the heterozygous effects of the mutations induced in F0 males on the production of F2 adult offspring, and this was important for three reasons. First, it made sure that we could compare load in individuals that had been reared their entire life at the same temperature, irrespective of differences in the F0 rearing temperature, excluding potentially confounding effects stemming from differential expression of mutational effects at different temperatures (see below). Second, it minimized the risk that paternal effects from the irradiation treatment (other than the mutations themselves) affected estimates of load. Third, it allowed us to retrieve statistically independent measures of each population's reproductive effort and longevity (both assayed in F0 controls) and mutation load (assayed by F2 adult offspring counts) that we later used to infer biologically meaningful links between life-history variation and germline maintenance (see Results and Discussion).

Thus, to assess the number of deleterious mutations passed on from F0 mating pairs we assayed the mutation load as the lifetime number of F2 offspring produced by F1 mating pairs descending from irradiated F0 males, relative to the number of F2 offspring produced by F1 mating pairs descending from respective F0 control males: $\Delta\omega = 1 - \omega_{IRR}/\omega_{CTRL}$ (figure 2). Mutational effects, used here to approximate the amount of DNA damage left unchecked in the germline, can be dependent on genotype × environment interactions [2,45]. When assessing the effect of compensatory thermal adaptation on germline maintenance we therefore quantified differences in mutation load between ancestral and preadapted populations as the difference in marginal mean load assayed across the two experimental evolution temperatures. Therefore, F0 females from the populations reared at 36°C were randomly assigned to lay their eggs at either 30 or 36°C immediately following mating. Stressed and unstressed ancestral populations were compared by estimating load at the ancestral (30°C) temperature (figure 2).

(c) Statistics

(i) Thermal stress, compensatory adaptation and life history

We employed maximum-likelihood (ML) estimation using linear mixed effects models available in the lme4 package [46] for R [47]. These and all subsequent analyses modelled normally distributed response variables. To estimate the effect of temperature stress on condition and life history, we first compared F0 male longevity and mating pair offspring production (i.e. reproductive effort) of control individuals in stressed and unstressed ancestral populations. We only included the offspring counts of females from the stressed populations that had been moved back to 30°C for egg laying, as their offspring were the ones used to assess the effect of thermal stress on germline maintenance. To assess if compensatory adaptation had led to improved condition under stress we employed models comparing ancestral and preadapted populations reared at stressful 36°C. These models were similar to those described above with the addition of including female egg laying temperature (see above and figure 2) and its interaction with evolution regime as fixed effects when analysing reproductive effort. All these, as well as the subsequent models described below,

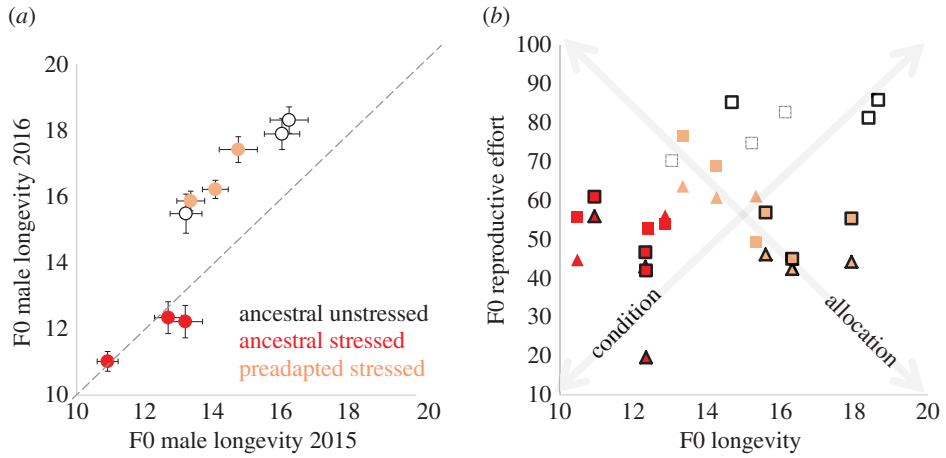

Figure 3. Effects of thermal stress and compensatory adaptation on life history. White and red symbols indicate ancestral populations reared at ancestral (30°C) and stressful (36°C) temperature respectively, and orange symbols preadapted populations reared at stressful temperature. In (*a*) the population mean (±1 s.e.) longevity in the two experiments. The hatched line denotes a 1 : 1 relationship. In (*b*) the population mean reproductive effort plotted against the corresponding longevity. A positive correlation between reproductive effort and longevity represents variation in overall condition among populations, and a negative correlation represents an allocation trade-off between devoting resources to reproduction or longevity. Squares and triangles denote reproductive effort assayed at ancestral and stressful temperature, respectively. Symbols with lacking/thin outline designate the 2015 experiment, and symbols with thick outline the 2016 experiment. (Online version in colour.)

included interactions with year in the fixed effects to ensure that results were qualitatively similar in the two experiments, and population identity and its interactions with relevant treatments to ensure that these were consistent across replicate populations. *p*-Values were calculated by log-likelihood ratio-tests using type-III sums of squares.

(ii) Thermal stress, compensatory adaptation and germline maintenance

To test the effects of thermal stress and compensatory adaptation on germline maintenance, we used three complementary approaches. First, we applied ML linear mixed effects models to test for interactions between radiation treatment and rearing temperature or experimental evolution regime. As mutation load is quantified as the F2 adult offspring production in irradiated lineages *relative* to the F2 offspring number produced by their corresponding controls, offspring counts were log-transformed before this analysis. Second, we employed Bayesian mixed effects models using Markov chain Monte Carlo simulations in the MCMCglmm package [48] for R. This allowed us to directly calculate posterior estimates and 95% credible intervals of mutation load ($\Delta\omega = 1 - \omega_{IRR}/\omega_{CTRL}$) for all groups compared, and these posterior distributions were used to calculate Bayesian *p*-values. Third, we estimated environment- and population-specific variance components with credible intervals via MCMC resampling. This allowed us to assess if there was excess (mutational) variance associated with the irradiation treatment, and if this variance was greater in stressed populations. For each population and environment we retrieved a posterior distribution of its coefficient of variation (CV = s.d./mean), for control and irradiated treatments separately. An additional inference about the number of passed on mutations could then be given by calculating each population's posterior distribution of the ratio: $\log_e[CV_{IRR}/CV_{CTRL}]$, giving the relative inflation of variance in the irradiated treatment relative to its control [49]. The MCMC resampling ran for 1 000 000 iterations, preceded by 500 000 burn-in iterations that were discarded. Every 1000th iteration was stored, resulting in 1000 independent posterior estimates from each model. We used weak and unbiased priors for the random effects ($V = 1$, $nu = 10^{-6}$ for all variance components).

Bayesian and ML models were identical in their build. When comparing stressed and unstressed ancestral populations we included radiation treatment and rearing temperature, as well as their interaction, as fixed effects. When estimating effects of compensatory adaptation, we built equivalent models with the addition of including assay temperature and its interaction with experimental evolution regime as main effects. In this model, the main hypothesis of how germline maintenance was affected by compensatory adaptation was estimated by comparing marginal mean load of the ancestral and preadapted populations averaged across the two assay temperatures (figure 4*b*,*d*).

(iii) Life-history trade-offs and germline maintenance

To explore more directly if phenotypic condition and allocation into longevity versus reproduction were associated with variation in germline maintenance, we employed two complementary multiple regression analyses. First we extracted two orthogonal (i.e. uncorrelated) principal components (PCs) based on each population's F0 longevity and reproductive effort, where population scores along PC1 described variation in overall condition (loading positively on both original variables), and PC2 described variation in allocation (figure 3*b*). Estimates of mutation load were then regressed on the PC scores. In the alternative analysis, mutation load was regressed directly onto longevity and reproductive effort. These analyses were fit using ML linear mixed effects models that, apart from the three focal variables, also included assay temperature and year as main effects. Population identity was included as a random effect, accounting for the fact that there were only six populations but 30 averages for the three traits in total across years, rearing and assay temperatures. Again we complemented the ML analysis with an identical Bayesian analysis which also allowed us to test if the variance standardized partial regression coefficients for longevity and reproductive effort differed from each other.

3. Results

(a) Thermal stress and life history

We first assessed the direct effects of rearing temperature (30 versus 36°C) on condition and life-history traits in the F0 generation, when populations were challenged to repair

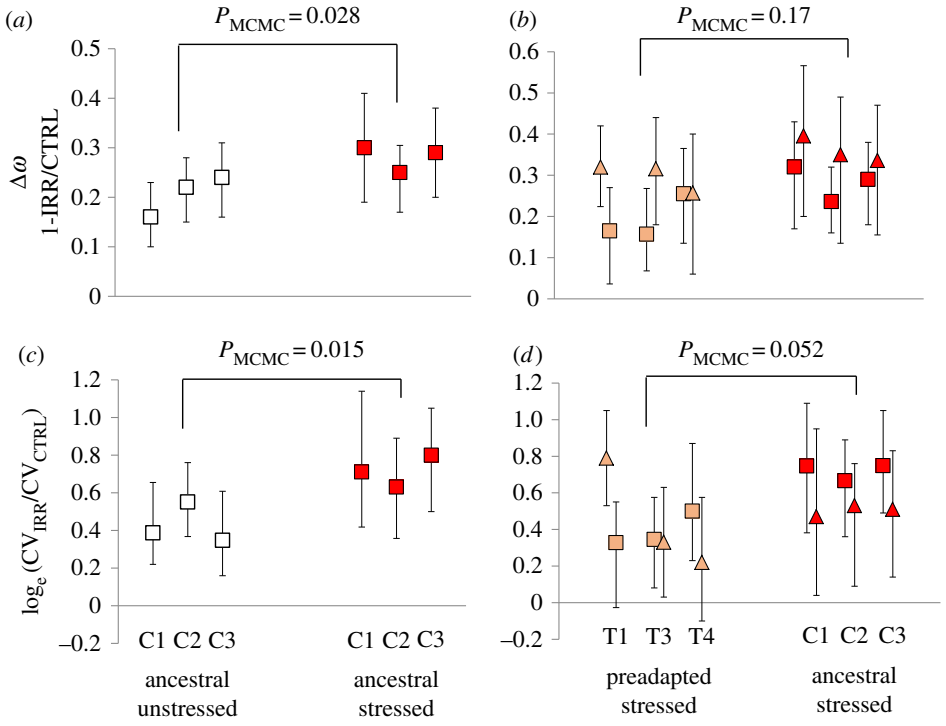

Figure 4. Effects of thermal stress and compensatory adaptation on germline maintenance. In (*a*) and (*c*) comparisons between ancestral populations reared at ancestral 30°C (unstressed) and 36°C (stressed) in the F0 generation when the irradiation treatment was applied. There was a significantly greater mutation load ($\Delta\omega$; *a*) and mutational variance ($\log_e[CV_{IRR}/CV_{CTRL}]$; *c*) in stressed populations when assayed at the ancestral temperature. In (*b*) and (*d*) comparisons between ancestral and preadapted populations reared at 36°C in the F0 generation, and assayed both at 30°C (squares) and 36°C (triangles) in the F2. Preadapted populations showed no statistically significant decrease in $\Delta\omega$ relative to ancestral populations (*b*), but marginally reduced mutational variance (*d*). Bayesian posterior modes $\pm$ 95% credible intervals are given for each replicate population. (Online version in colour.)

the artificially induced mutations. There was a clear direct effect of temperature stress on reproduction ($\chi^2 = 21.4$, d.f. = 1, $p < 0.001$) and longevity ($\chi^2 = 31.0$, d.f. = 1, $p < 0.001$) in the ancestral populations, with the reduction in longevity being stronger in the 2016 experiment (interaction; $\chi^2 = 11.2$, d.f. = 1, $p < 0.001$) (figure 3). Evolution under simulated climate warming had led to compensatory adaptation and increased condition of the preadapted populations relative to the ancestral populations when reared under thermal stress (longevity: $\chi^2 = 11.6$, d.f. = 1, $p < 0.001$; reproduction: $\chi^2 = 3.85$, d.f. = 1, $p = 0.050$). Again, the difference in longevity was stronger in the 2016 experiment (interaction: $\chi^2 = 7.51$, d.f. = 1, $p = 0.006$) (figure 3).

(b) Thermal stress and germline maintenance

Mutation load ($\Delta\omega$ in the F2 generation was greater when the F0 generation had been raised at the stressful temperature compared to when raised at the ancestral temperature ($\chi^2 = 5.23$, d.f. = 1, $p = 0.022$). These results were confirmed by the Bayesian MCMC resampling ($P_{MCMC} = 0.028$, figure 4*a*). Moreover, the greater load was accompanied by an inflation of mutational variance ($P_{MCMC} = 0.015$, figure 4*c*). This suggests that more mutations with deleterious fitness effects were passed on in stressed relative to unstressed ancestral populations.

(c) Compensatory thermal adaptation and germline maintenance

There was a tendency for compensatory thermal adaptation to reduce mutation load under temperature stress, but we did not detect a significant two-way interaction between evolution regime and radiation treatment using either maximum likelihood ($\chi^2 = 2.04$, d.f. = 1, $p = 0.15$) or Bayesian estimation ($P_{MCMC} = 0.17$, figure 4*b*). The Bayesian analysis did, however, show a marginal decrease in the mutational variance in preadapted relative to ancestral populations ($P_{MCMC} = 0.052$) (figure 4*d*), consistent with more mutations with fitness effects being passed on in ancestral relative to preadapted populations when faced with elevated temperature.

(d) Life-history trade-offs and germline maintenance

We looked for associations between variation in germline maintenance and life history by regressing each population's mean F2 mutation load on estimates of its F0 reproductive effort and longevity for each experimental year. Populations that showed high phenotypic condition overall and allocation to longevity at a cost of reduced reproductive effort passed on a smaller mutation load (allocation: $\chi^2 = 4.58$, d.f. = 1, $p = 0.032$; overall condition: $\chi^2 = 2.93$, d.f. = 1, $p = 0.087$, figure 5*a*,*b*), suggesting that life-history allocation decisions affected germline maintenance. An alternative analysis using longevity and reproductive effort as explanatory variables revealed that mutation load was negatively correlated to F0 longevity ($b = -0.056 \pm 0.019$, $\chi^2 = 9.08$, d.f. = 1, $p = 0.003$, figure 5*c*, electronic supplementary material, figure S3), whereas F0 reproductive effort showed a non-significant positive relationship with load ($b = 0.018 \pm 0.020$, $\chi^2 = 0.80$, d.f. = 1, $p = 0.37$, figure 5*d*). Resampling of the variance standardized partial regression coefficients confirmed these results and also showed that longevity was more negatively related to mutation load than reproductive effort in 970 out of 1000 MCMC simulations (one-sided $p = 0.030$).

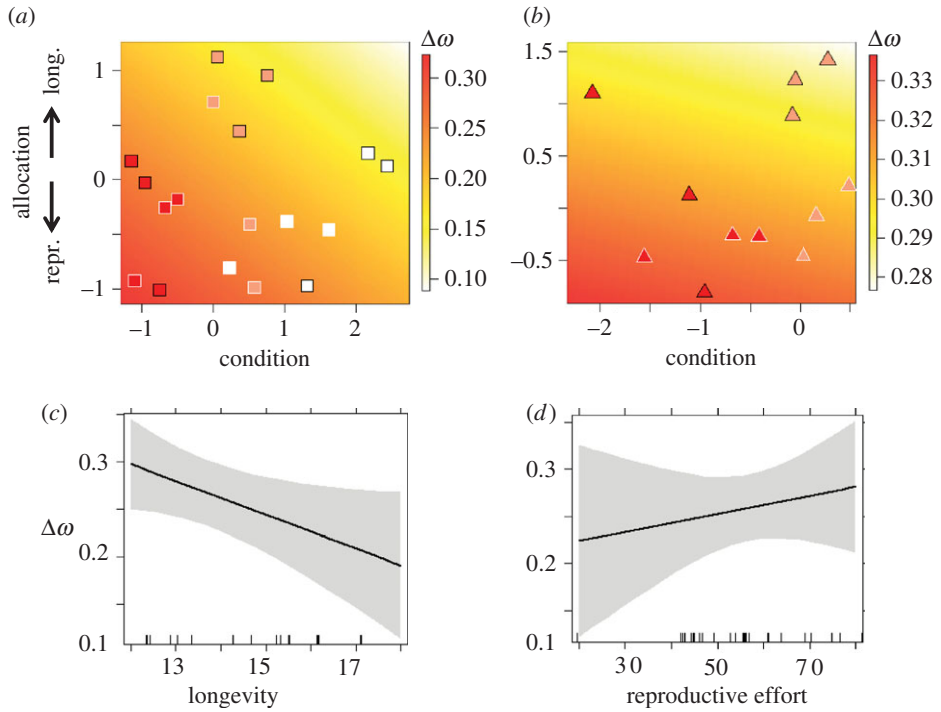

Figure 5. Life-history trade-offs and germline maintenance. Mutation load ($\Delta\omega$) predicted from variation in overall phenotypic condition and resource allocation between reproduction and longevity in the F0 generation. $\Delta\omega$ was lower in populations that, at the time of irradiation, had high condition and allocated resources to longevity. The relationship was qualitatively similar when $\Delta\omega$ was assayed at ancestral (30°C; *a*) and stressful temperature (36°C; *b*). In (*a*) and (*b*), symbols designate; open symbols: unstressed ancestral populations; red: stressed ancestral populations; orange: preadapted populations. Principal component scores for condition and allocation from the 2015 and 2016 experiment are outlined with white and black borders respectively. In (*c*) and (*d*), maximum-likelihood predictions (shaded area depicts 95% CI) of the relationship between $\Delta\omega$ and F0 longevity (*c*) and reproductive effort (*d*). Tick marks on the *x*-axes show the distribution of population longevities (male lifespan in days) and reproductive efforts (mean number of offspring produced per couple). (Online version in colour.)

4. Discussion

We have shown that thermal stress can have a negative impact on phenotypic condition and concomitant germline maintenance. This suggests that mutation rates can be condition-dependent and may increase under continued climate warming if populations are unable to track rising global temperatures. Given that most mutations are deleterious, this scenario is predicted to result in mutational meltdown [50] as stressed populations pass on more mutations to their offspring, decreasing population health and increasing mutation rate further in future generations (but see [25,26]). Our findings mirror previous results showing that thermal stress tends to be associated with overall higher mutation rates [51–53], and recent studies in *Drosophila* reporting correlations between estimates of DNA repair and phenotypic/genetic condition [40,54]. At a mechanistic level, this may be explained by individuals of low condition reducing investment in DNA repair molecules overall, and/or disproportionately using low-fidelity DNA repair pathways that entail reduced energetic costs ([55,56], but see [57]).

Theory suggests that the relationship between mutation rate and phenotypic condition has fundamental consequences for evolutionary demography of sexually reproducing species [25,26]. In support of condition-dependent mutation rates, we found that stressed parents passed on a greater mutation load to their offspring and that compensatory thermal adaptation tended to improve germline maintenance at stressful temperature. Moreover, our findings suggest that allocation into different life-history components (i.e. longevity versus reproduction) can affect mutation rate. Longevity evolved under simulated climate warming and was significantly negatively correlated with mutation load, while reproductive effort was instead unrelated to or even positively correlated with load. These results do not necessarily imply that investment into somatic and germline maintenance will generally be positively correlated or that there is no allocation trade-off between the two [7]. It does, however, suggest that there may be more individual variation in overall condition and allocation between maintenance versus reproduction, than for allocation between somatic versus germline maintenance. More generally, our study provides support for the hypothesis that mutation rates are affected by how environments modulate life-history traits [7], and that the evolution of environmental robustness can lead to lowered germline mutation rates (see also [58]).

While stress-induced mutation rates can have a fundamental influence on evolutionary demography, it remains less clear whether they are adaptive. Mutator genotypes have convincingly been linked to increased rates of adaptive evolution in bacterial cultures exposed to stress [18,59–61]. However, mutator alleles are not predicted to reach high frequencies in organisms with sexual recombination [8,11]. Nevertheless, together with recent studies in *Drosophila*, our findings imply that stress-induced mutation may readily occur also in sexually reproducing organisms. These findings mirror those found for stress-induced recombination rates, which have been demonstrated in a variety of sexually reproducing organisms [62–64] despite that the conditions for evolution of plastic recombination in diploids seem far more restrictive than for haploids [65]. Interestingly, DNA repair often involves recombination, which in turn relies on efficient DNA repair, and these processes are highly conserved across bacteria and higher

eukaryotes [14]. Moreover, cellular responses to DNA damage in the germline and somatic tissue are hugely overlapping with physiological responses to a variety of environmental stressors [14,60,66–68]. This suggests that stress-induced mutation may to some extent reflect a general and conserved stress-response involving allocation trade-offs [58,67,69]. This notion is congruent with the cost-of-fidelity hypothesis [7,11,22] and in line with the stress-mediated correlation between longevity and germline maintenance observed here.

Inferring mutation rates from estimates of mutation load can be problematic because mutational effects can be dependent on genotype and environment [2]. Importantly, our experimental design precluded that differential expression of mutational fitness effects across temperatures or genotypes could have affected our results, because (i) F1 and F2 offspring carrying the induced mutations were always reared and compared for load at the same temperature, and (ii) population longevity at the moment of germline repair, not at the time of offspring production assays, predicted load, and (iii) longevity rather than genotype identity predicted load (ancestral populations exhibited both the lowest and highest loads, depending on parental rearing temperature). Our results thus imply that the number of deleterious mutations that passes through the germline to the next generation can be dependent on the phenotypic condition and life-history decisions of parents.

In addition to condition-dependent DNA repair, there are a couple of other mechanistic explanations for this result that are not mutually exclusive. First, while we have argued here that temperature inflicts stress that lowers condition and DNA repair, another effect of warm temperature could be to increase the rate of mitotic divisions and therefore lower the *relative* efficacy of DNA repair. However, this explanation seems unlikely given that development rates (as a proxy for mitotic rate) show very small differences between 30°C and 36°C and between ancestral and preadapted populations [31]. Moreover, this explanation assumes that the rate of DNA repair does not increase in parallel with mitotic division as temperature rises. Second, it is possible that cryptic female choice inside the reproductive tract acts to discriminate against mutated sperm. If this process was to contribute to our results female choice would need to be condition dependent so that high-condition females are better at weeding out bad sperm. Moreover, females would need to be able to select mutation-free genotypes based on their sperm phenotype (hence a form of haploid selection). Along a similar vein, haploid selection within the male ejaculate could be condition-dependent so that mutated sperm are weeded out more efficiently in high-condition males prior to insemination. While there are numerous examples of female choice in insects [70], haploid selection has received very sparse empirical support (but see [71]) and is expected to be weak on theoretical grounds [72]. Moreover, convincing evidence that either of these two related processes are condition dependent are, to our knowledge, lacking. More importantly, our previous experiments on *C. maculatus* suggest that males irradiated at a dose five-times higher than that used in this experiment still achieve approximately 50% fertilization success in competition with control males [29,30]. These observations thus seem to preclude that haploid selection could have contributed to our results.

The condition-dependent transfer of deleterious mutations reported here has important demographic consequences and implications for our understanding of evolution and patterns of molecular diversity. We have presented a rare empirical demonstration of how variation in life-history and environmental robustness can be linked to germline maintenance in a sexually reproducing organism. However, we only have a budding understanding of how these relationships and their underlying causality mediate variation in mutation rate in multicellular eukaryotes [3–7,10]. Our results thus provide motivation for further empirical efforts toward this end, especially since many organisms are faced with contemporary environmental change.

Ethics. No permits were needed to conduct this study and actions were taken to limit the number of used beetles.

Data accessibility. Data are available at the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.6dd04) [73].

Authors' contributions. D.B., K.G. and J.S. conceived and planned the study. D.B., J.S., I.M.-A. and K.G. collected the data. D.B. analysed the data and wrote the manuscript with input from G.A. All authors commented on the final version.

Competing interests. The authors have no competing interests to declare.

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