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# Selection in a fluctuating environment leads to decreased genetic variation and facilitates the evolution of phenotypic plasticity

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## Abstract

Changes in the environment are expected to induce changes in the quantitative genetic variation, which influences the ability of a population to adapt to environmental change. Furthermore, environmental changes are not constant in time, but fluctuate. Here, we investigate the effect of rapid, continuous and/or fluctuating temperature changes in the seed beetle *Callosobruchus maculatus*, using an evolution experiment followed by a split-brood experiment. In line with expectations, individuals responded in a plastic way and had an overall higher potential to respond to selection after a rapid change in the environment. After selection in an environment with increasing temperature, plasticity remained unchanged (or decreased) and environmental variation decreased, especially when fluctuations were added; these results were unexpected. As expected, the genetic variation decreased after fluctuating selection. Our results suggest that fluctuations in the environment have major impact on the response of a population to environmental change; in a highly variable environment with low predictability, a plastic response might not be beneficial and the response is genetically and environmentally canalized resulting in a low potential to respond to selection and low environmental sensitivity. Interestingly, we found greater variation for phenotypic plasticity after selection, suggesting that the potential for plasticity to evolve is facilitated after exposure to environmental fluctuations. Our study highlights that environmental fluctuations should be considered when investigating the response of a population to environmental change.

## Introduction

The global environment is changing, characterized, for instance, by an increase in temperature. The resulting environmental conditions are novel, often suboptimal or even stressful. Populations are exposed to these changes and respond to them. Their response to new conditions can be genetic and/or plastic, or mediated by nongenetic parental effects, and disentangling their roles in response to a changing climate is both important and difficult (Gienapp *et al.*, 2008). A plastic response of a quantitative trait is expected and might be sufficient to cope with a change in the environment in the short term (Przybylo *et al.*, 2000; Chevin & Lande, 2010). However, there are limits to plastic responses (Pigliucci, 1996; DeWitt *et al.*, 1998; De Jong, 2005), and they are unlikely to provide long-term solutions for challenges faced by populations experiencing continued directional environmental change. Thus, adaptive trait evolution is often thought to be a more likely response in the long term (Davis & Shaw, 2001; Réale *et al.*, 2003a,b; Berteaux, 2004; Pulido & Berthold, 2004; Davis *et al.*, 2005; Thomas, 2005). Furthermore, the environment is not constant in time, and thus, a fluctuating environment is the most biologically relevant and realistic way of how we can expect the environment to change (e.g. Boyce *et al.*, 2006; Lundberg *et al.*, 2000; Schreiber, 2010). Thus, in order to understand how a population will cope with environmental change, we need to investigate how it will respond to fluctuating changes in the environment.

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The quantitative genetic variation expressed within populations has been shown to vary with environment (Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999; Husby *et al.*, 2011). Thus, changes in the environment are expected to cause changes in quantitative genetic variation, changes that in turn are expected to influence the adaptability of the population (Fisher, 1930; Endler, 1986), and therefore they are important for the study of evolution. Also plasticity itself can change with changes in the environment, and the occurrence of gene by environment interactions (Via & Lande, 1985) highlights the potential for changes in genetic interactions with altered environmental conditions (Pigliucci, 2001). However, the question of how phenotypic plasticity evolves has been subject of vigorous debate (reviewed in Via *et al.*, 1995; Sarkar, 2004; Windig *et al.*, 2004), and generally, very little is known about the genetic architecture and evolution of phenotypic plasticity (Scheiner, 1993; Promislow, 2005; Czesak *et al.*, 2006).

Many studies have addressed the question of how quantitative traits, their underlying genetic architecture and plasticity behave under constant environmental conditions, as well as in response to changes in the environment (Lynch and Walsh, Hoffmann & Merilä, 1999; Husby *et al.*, 2011; Pigliucci, 2001; Stillwell *et al.*, 2007). However, most models concerning responses of quantitative genetic traits to environmental change deal with responses in the short term, that is, after a rapid shift in environmental conditions (e.g. Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999). Studies investigating the response of quantitative traits over several generations of selection in different kinds of changing, increasing and fluctuating environments are rare (Pélabon *et al.*, 2010). Thus, it is rather unclear what effect long-term directional and/or fluctuating selection has on components of variation as well as on plasticity and its potential for evolution.

In a constant environment, buffering mechanisms that limit deviations from genetically determined patterns of development tend to be favoured by selection. Traditionally, the buffering of genetic and environmental differences among individuals has been referred to as genetic and environmental canalization, respectively (Waddington, 1942; Stearns *et al.*, 1995; Wagner *et al.*, 1997; Flatt, 2005). Under stabilizing selection, the phenotypic variance is predicted to decline over generations (e.g. Bulmer, 1980; Shnol & Kondrashov, 1993). Also genotypic variances (Curnow, 1964; Gavrilets & Hastings, 1994) and environmental variances (Gavrilets & Hastings, 1994; Wagner *et al.*, 1997; Zhang & Hill, 2005) are predicted to be low under stabilizing selection (for a review, see Stillwell *et al.*, 2010). Additionally, one can expect that a substantial amount of hidden genetic variation accumulates. This accumulation can, but does not have to, be due to canalization (Waddington, 1940; Stearns *et al.*, 1995; but see Hermisson & Wagner, 2004). Theoretical work reveals that in a constant environment, plasticity will be lost, genetic assimilation will take place and the degree of canalization will be high (Lande, 2009).

A novel and stressful environment is known to increase phenotypic variation, and this is often thought to be due to an increase in genetic variation (Holloway *et al.*, 1990; Kawecki, 1995; Pigliucci *et al.*, 1995; Guntrip *et al.*, 1997; Imasheva, 1997; Sgrò & Hoffmann, 1998; Bubliy & Loeschcke, 2000; Perez & Garcia, 2002). For example, temperature stressors have generally been found to increase the levels of quantitative genetic variation (Hoffmann & Merilä, 1999; Husby *et al.*, 2011). Exposure to stress or a novel environment might reveal genetic variance that is hidden in more benign conditions (cryptic genetic variance; Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999). This might be due to the expression of genes in the new environment that have been neutral and thus not subject to elimination by natural selection in the usual environment (Mather, 1943; Holloway *et al.*, 1990; Holt & Gaines, 1992; Kawecki, 1995; Pigliucci *et al.*, 1995; Bennington & McGraw, 1996; Guntrip *et al.*, 1997). It can also be due to conditional expression of genes that leads to the accumulation of mutations (Van Dyken & Wade, 2010), activation of stress-induced genes or enhanced frequency of mutations (Debat & David, 2002). It should be noted that even though an increase in genetic variance is thought to be the general trend (Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999), decreases and no changes have been observed. Theoretical work addressing phenotypic plasticity and environmental change reveals that after a sudden major change in the average environment selection produces a rapid transient increase in mean plasticity, followed by a slow genetic assimilation decreasing the mean plasticity (Pal, 1998; Lande, 2009).

Generally, very little is known on how quantitative genetic traits, their underlying genetic architecture as well as their plasticity and its genetic variation change in response to long-term changes in the environment, including fluctuations. The few existing studies investigating the changes in genetic variation use quantitative genetic models. Under directional selection, genetic variance can be shown to increase (Bürger & Lynch, 1995; Hansen, 2006). Kawecki (2000) suggested that under directional selection, phenotypic expression of genetic variation should increase due to a decreased genetic canalization (i.e. 'genetic de-canalization'), whereas under fluctuating selection, the reversal of direction of selection over time should lead to genetic canalization (Kawecki, 2000). Even less well understood is the effect of selection on environmental variance. However, Zhang & Hill (2005; Zhang, 2005) found a tendency for the evolution of environmental de-canalization under fluctuating selection.

In this study, we use an experimental evolution approach and a quantitative genetic breeding design to

investigate how populations respond to changes in the temperature environment in both the short and long term and target whether their response is dependent on the presence of fluctuations in the changing temperature. The aim of our study is three-fold: first, we analyse short-term responses to a rapid change in the environment; second, we analyse the long-term responses to selection in a changing environment and test whether the selective past alters the response to a rapid change in the environment; and third, we analyse the role of fluctuation in the environment by investigating whether the response of a population depends on experienced fluctuations in the environment during selection. In order to target these aims, we investigated the response of four quantitative traits (developmental time, mass at emergence, fecundity and hatching) to different short- and long-term selection regimes in the seed beetle *Callosobruchus maculatus.*

## Material and methods

We conducted an evolution experiment over 18 generations followed by a split-brood experiment in the laboratory using the seed beetle *Callosobruchus maculatus* as a model organism (stocks were provided by Peter Credland, University of London). The seed beetles (Nigerian mixed strain) were adapted to 30 °C for approximately 90 generations in the laboratory before the experiment. Beetles were kept on black-eyed beans as substrate. The generation time is approximately 25 days at 30 °C. During the experiment, we separated generations by transferring about 200 adult individuals to 100 g of fresh beans at peak of emergence.

Individuals were exposed to three different selection regimes including a trend of successive increase in temperature (from 30 to 36 °C; about 0.3 °C every generation) over 18 generations in total. The first treatment involved only a linear trend from 30 to 36 °C, hereafter called 'Trend'. In the other two selection regimes, environmental fluctuations (noise) were incorporated. The trajectories of temperature change of these fluctuation treatments were generated using computer simulations. Two types of environmental fluctuations were simulated around a trend of temperature increase, called red or white noise fluctuations, respectively. The red noise fluctuation is characterized by a high positive temporal autocorrelation between consecutive values of temperature, whereas the white noise fluctuation has no temporal autocorrelation in the time series. In the simulated temperature trajectories, the temperature increased 0.3 °C (as in Trend lines) in each generation, and fluctuation (red or white, respectively) was added upon this increase. Thus, the simulated temperature value of the previous generation served as a starting point for the temperature simulation for the next generation. The treatment with the added red noise fluctuation is called 'Red' treatment, and the treatment with the added white noise fluctuation is called 'White' treatment hereafter. Both Red and White noise treatments are collectively referred to as 'Fluctuation' treatment lines from here on. A control (called Control) was kept at a constant temperature of 30 °C. Trend, Red and White treatment lines were reared in 36 °C in their 18th and last generation of the selection experiment. The Control and the three different treatments contained four replicates each, resulting in 16 lines in total.

After the 18th and last generation of selection, we conducted a split-brood experiment. With this approach, we aimed to mirror the response of the different treatment lines to exposed change in temperature, to compare the response among treatments and to test whether the response of each population is due to genetic and/or phenotypic plastic changes. We measured mass at emergence, developmental time, fecundity (fitness) and number of hatched individuals of all lines (including Control).

The split-brood experiment lasted over two generations. We formed mating pairs of virgin beetles and let them mate and lay eggs on 40 g of substrate for 72 h in room temperature (23 °C). Ten pairs per replicate per line were formed, resulting in 160 pairs in total. Beans with attached eggs were split up into two temperature environments: one environment (30 °C) representing the environment of origin that all lines had been adapted to at the beginning of the long-term experimental evolution experiment and the other environment (36 °C) representing the environment that the treatment lines have been selected towards during the experiment. The split-brood design ensured that full-siblings of each family were raised in both environments. Eggs were incubated. Hatching individuals were collected and counted daily during their emergence period, separating sexes (developmental time data). Mass at emergence was measured on days two, four and seven of the emergence period. To accurately measure the mass, individuals were sexed and frozen, then dried in an oven at 50 °C for 48 h and their mass was obtained to the nearest 0.01 mg (using a Sartorius Genius Microbalance model ME235P). In order to assess the fecundity of the individuals hatching in the two environments, up to 10 males and females of each family in each temperature were collected (mainly on day three, but also on days five and six). Females of each family were mated to a random male of a different family (but within the same replicate line, treatment and split temperature) to avoid the fecundity measure of the female being affected by inbreeding. Mating pairs were given 40 g of substrate and allowed to lay eggs until death. Incubation took place in the same temperature the parents had been raised in and offspring were allowed to hatch. After the emergence period (33 days after their parents' mating), offspring of all pairs were frozen and subsequently counted.

In total, 1668 individuals were measured for mass at emergence (i.e. on average 26 observations per treatment, replicate, temperature and sex), 3457 for developmental time (approximately 54 per treatment, replicate, temperature and sex), 581 for hatching (i.e. approximately 9 per treatment, replicate, temperature and sex) and 829 for fecundity (i.e. approximately 25 per treatment, replicate and temperature).

### Expectations – before selection

The Control line was kept at constant temperature, and thus, its environment is stable and predictable and selection is stabilizing. We expect the Control lines to be adapted to 30 °C and therefore to show a high degree of genetic and environmental canalization in this environment (i.e. low genetic and environmental variance). However, a higher-temperature environment is novel to the population, and we do not expect them to be adapted to it. We expect that their short-term response to a rapid shift in the environment is plastic and characterized by an increase in both genetic and environmental variation, accompanied by a reduction in fitness (Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999; Mather, 1943; Holloway *et al.*, 1990; Holt and Gaines, 1992; Kawecki, 1995; Pigliucci *et al.*, 1995; Bennington & McGraw, 1996; Guntrip *et al.*, 1997; Van Dyken & Wade, 2010).

### Expectations – after selection

After selection in a changing environment, we expect populations to exhibit a response that differs from expected responses to short-term change. We expect all the selection lines to potentially be adapted to the higher-temperature environment, due to the exposure to increasing temperature during selection. Although the Trend and Fluctuation lines experience the same mean temperature increase with the same frequency, the variance around the mean of temperature increase is higher in the fluctuation selection regime. Thus, in the fluctuating selection regime, selection within a generation is directional, but the direction and intensity of selection changes between generations and the predictability of the environmental change is low. This is in contrast to the Trend selection regime where selection is directional with direction and intensity of selection remaining the same throughout the experiment, which makes the predictability of the environment high. Thus, because an evolutionary response in a constant or constantly changing environment is expected to be faster than in an environment where changes are irregular, we expect the Trend line to have a higher degree of adaptation compared to the Fluctuation lines.

The Fluctuation lines are expected to show an increased degree of genetic canalization and thus a decreased genetic variance compared to both Control and Trend lines (Kawecki, 2000). We expect the degree of environmental canalization to be decreased in the Fluctuation lines (Zhang & Hill, 2005), manifested in a higher environmental variance compared to the Control lines. Additionally, we expect Fluctuation lines to have increased plasticity (Lande, 2009). This is because the Fluctuation lines are expected to respond according to the Phase I of Lande's (2009) scenario in which plasticity is shown to increase after a shift in the environment; under the fluctuating selection regime, individuals are exposed to sudden and potentially large changes in the environment in a repeated fashion. We expect the Trend lines to show an increase in genetic variation (Bürger & Lynch, 1995; Kawecki, 2000; Hansen, 2006). We expect plasticity to increase as well, but less than the expected increase in the Fluctuation lines (Lande, 2009). This is because traits under strong directional selection are suggested to be highly dependent on the environmental condition and therefore sensitive to a change in the environment to which they respond in a highly plastic way (Bonduriansky, 2007a,b; for review, see Stillwell *et al.*, 2010). However, the magnitude and severity of the environmental change of the Trend selection regime is considered to be less strong than in the Fluctuation selection regime.

In summary, we expect the Trend line to show the most rapid adaptation and to maintain greater genetic variance compared to the Fluctuation lines (Bürger & Lynch, 1995; Hansen, 2006; Kawecki, 2000). The Fluctuation lines are expected to show the greatest plastic response (Lande, 2009), a decrease in genetic variation (Kawecki, 2000) and an increase in environmental variation (Zhang & Hill, 2005). The expected responses for all treatments are summarized in Table 1.

### Statistical analysis

The analysis was conducted using the MCMCglmm package (Hadfield, 2010) in R 2.13.0 (R Development Core Team, 2011). We fitted linear mixed models separately for each trait (developmental time, mass,

**Table 1** Summary of expected changes in quantitative genetic parameters before and after selection. Response of Control across environments (before selection); response of selection lines in relation to Control (after selection)*.

| | Before selection | After selection (response in relation to Control) | |
|---|---|---|---|
| Expected changes | Control | Trend line | Fluctuation lines |
| Phenotypic plasticity | Yes | Yes (+) | Yes ++ |
| $CV_G$ | + | + | - |
| $CV_E$ | + | ? | + |

*Legend: (+) slight increase; + increase; ++ strong increase; - decrease; Yes/No: expected presence of effect, ? no literature/studies on this effect

fecundity, hatching) and each treatment (Control, Trend, Red, White). Temperature (30 °C vs. 36 °C) and sex of the offspring were fitted as fixed-effect predictors with family and replicate identity being included as random effects. As we were interested in the covariances between sexes and between temperature environments, we also fitted the interactions of sex and temperature with the two random effects. Thus, the model takes into account that families differ in their intercept (i.e. in their response to temperature) and in their slopes (i.e. that different families have different slopes of reaction across temperatures). For the family identity and the replicate identity random effects, we estimated unstructured variance–covariance matrices, that is, one variance for each temperature and sex (four variances) and all covariances between sexes and temperatures (six covariances). For the residual variance–covariance matrix, we fixed all covariances to zero, because each individual was measured in only one environment and hence there is no replication in our data to estimate a residual covariance.

We used uninformative proper priors for both fixed and random effects. Fixed-effect priors were normally distributed with expected value (mean) zero and degree of belief (variance) of $10^8$. Random-effects priors (one for each random effect and its interactions with fixed effects) were inverse Wishart distributed. We allowed the Markov chain for a burn-in period of 3000 iterations; after which we ran 15 000 iterations and sampled every 10th iteration from the posterior distribution, resulting in a recommended number of 1500 stored values per chain (Hadfield, 2011; http://stat.ethz.ch/CRAN/web/packages/MCMCglmm/index.html, Vignette MCMCglmm Course Notes). These settings resulted in appropriate convergence of the chain. Convergence was assessed by checking for potential autocorrelations of consecutive values in the chain and via visual inspection of potential trends in the chain as well as of the shape of the posterior density distribution of fixed and random effects, respectively. Autocorrelation between consecutive values was low (<0.04), and there were no trends in the chain as well as the posterior distributions were not skewed. We present the posterior mode and 95% credible intervals unless explicitly stated otherwise. Within each treatment line, significance of estimates of the fixed effects, phenotype and environment can be assessed by their posterior distribution not overlapping zero. We tested for significance of variance components using model comparison based on the deviance information criterion (DIC) (Wilson *et al.*, 2010).

In order to evaluate the differences in variance and covariances between selection line treatments, we had to compare estimates between models. This was carried out by testing whether the distribution of difference (= delta distribution) differed significantly from zero. Delta distributions were generated from posterior distributions of fixed and random effects, respectively. Thus, we calculated the difference between the posterior distribution of each parameter in one treatment line and the posterior distribution of corresponding parameter in the other treatment. Mean and standard deviation of the resulting delta distribution were calculated, and the estimate was considered significant if the mean (±2 SD) did not include zero.

### Parameter estimates

Our models estimate all relevant phenotypic and genotypic variance–covariance matrices for each trait. We converted variances to standardized coefficients of variation by dividing variance by the phenotypic mean trait value (Houle, 1992). The coefficient of genetic variation ($CV_G$) measures the variation among families in each environment (covariance of full-sibs; Falconer & Mackay, 1996) and thus the amount of genetic variation in a treatment line in a certain environment. Typically, it includes dominance and maternal effects. Thus, we can regard the estimated $CV_G$ as an upper bound to $V_A$ (Falconer & Mackay, 1996; Conner & Hartl, 2004). The coefficient of environmental variation ($CV_E$) measures the variation among individuals within each family and environment and thus represents environmental variability/environmental canalization (Stearns *et al.*, 1995). Typically, $CV_E$ contains all the variance due to nongenetic origin including variation due to external environmental conditions (e.g. climatic factors) and internal conditions (e.g. developmental noise). It also may include the effects of environmental heterogeneity, individual genotype, phenotypic plasticity, G × E and maternal effects (Falconer & Mackay, 1996). In our study, we were able to control for environmental heterogeneity (half of the individuals of a family are raised in a common environment) and assume effects related to individual genotype to be small (see discussion). Thus, we interpret changes in $CV_E$ to be due to external environmental (experimental temperature treatment) and/or internal conditions. For the trait hatching, we estimated heritability ($h^2$) and not $CV_G$ and $CV_E$. This is because $CV_E$ cannot be assessed due to the data structure, that is, the response variable was the number of hatched individuals per family and thus an estimate of within-family variation and thereby $CV_E$ is missing. $CV_G$ and $CV_E$ are measures of genetic and environmental canalization, respectively (Stearns *et al.*, 1995). We use two measurements of G × E. By testing for differences in among-family variation across environments, we obtain an estimate of variance G × E. We also estimate the genetic correlation across environments $r_G$, which is a measurement of both variance and crossing G × E (Falconer & Mackay, 1996; Via & Lande, 1985). Any difference of $r_G$ from ±1 is caused by a difference in among-family variance across environments (variance G × E) and/or a crossing of family reaction norms across environment (crossing G × E) (Via & Lande, 1985). Any G × E (variance and/or crossing) indicates the variation

for phenotypic plasticity of the trait under observation and thereby the potential for phenotypic plasticity to evolve. Additionally, $r_G$ indicates the extent to which a trait is correlated among environments, and thus how independent trait evolution is across environments (Scheiner, 1993; Via, 1994). A genetic correlation across environments of +1 suggests complete genetic dependence of character expression across environments, whereas a correlation of −1 suggests complete reciprocity or trade-off between values of character states across environments. We present $r_G$ for developmental time (sex specific) and for mass at emergence (pooled for sex due to low sample size). We do not present genetic correlations for fecundity, because it was hard to detect a significant genetic variance in some treatment–temperature combinations.

## Results

### Before selection (= Control)

Body mass at emergence, fecundity and hatching success were significantly lower at 36 °C than at 30 °C, whereas developmental time was unchanged (Table 2, Fig. 1a–d). $CV_G$ increased from 30 to 36 °C for body mass (females only) and developmental time, but decreased for fecundity (Table 2, Figs 2a, 3a and 4a). $CV_E$ increased for both mass at emergence and developmental time for females but not males (Table 2, Figs 2b and 3b), and G × E was significant for developmental time in males (Table 2, Table S1A). The genetic correlation across environments was low (around 0.3), and the credible interval included zero for body mass and developmental time (females only; Table S3).

### After selection, no fluctuations (= Trend)

Body mass and fecundity decreased significantly at 36 °C, whereas developmental time and hatching success did not change significantly across temperatures (Table 2, Fig. 1a–c). $CV_G$ increased significantly across environments for developmental time, but for body mass differences were not significant; we could not detect significant $CV_G$ for fecundity (Table 2, Figs 2a, 3a and 4a). $CV_E$ increased significantly for fecundity, developmental time and female mass at emergence, and there was a significant G × E for developmental time (Table 2, Figs 2b, 3b and 4b). The genetic correlation between the two temperature environments was positive for body mass and developmental time (Table S3).

Comparing the response of populations before (Control) and after selection (Trend), the Trend lines had a significantly higher body mass than the Control lines (Table 2, Fig. 1a). The change in fecundity across temperatures was larger in the Trend lines than in the Control lines (Fig. 1c, Table S2, significant environment × treatment interaction). Trend females showed a significantly greater increase in genetic variation between temperatures for developmental time (Fig. 3a). There was a significant difference in the G × E between Trend and Control lines (Table S2). The trait that differed most in response before and after selection was hatching success. Compared to the Control, the Trend lines had a significantly decreased mean hatching success, a significantly lower degree of plasticity and a significant family variation (Fig. 1d, Table S2).

### After selection, fluctuations (= Red and White)

In the Fluctuation lines, fecundity decreased significantly from 30 to 36 °C (Fig. 1c, Table S1A), but body mass, developmental time and hatching success did not. $CV_G$ did not change significantly across temperatures for all traits measured, apart from females in the Red treatment line, which showed a significant increase in $CV_G$ for developmental time (Fig. 3a).

Both Fluctuation lines showed a significant increase in $CV_E$ across temperatures for developmental time

**Table 2** Observed changes in quantitative genetic parameters for fecundity, mass at emergence and developmental time (dev. time). Response of Control across environments (before selection) and response of selection lines in relation to Control, that is, difference to Control (after selection). Results that are in line with expectation are indicated in boldface*

| Observed changes | Before selection | | | After selection (difference to control) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Trend line | | | Fluctuation lines | | |
| Trait | Fecundity | Mass | Dev. time | Fecundity | Mass | Dev. time | Fecundity | Mass | Dev. time |
| Phenotypic plasticity | **Yes** | **Yes** (females only) | No | Yes, 0 | Yes, 0 (females only) | No, 0 | Yes, 0 | - | No, 0 |
| $CV_G$ | - | **+** | **+** | ? | 0 | 0 | **?** | (-) | **-** |
| $CV_E$ | 0 | **+** | **+** (females only) | + | 0 | - | **+** (Red only) | - | - |
| G × E (Variation for plasticity) | No | No | Yes (males only) | ? | No | Yes, (change in variance G × E) | ? | No, (but White; change in sign of $r_G$ in Red) | Yes, (reduced variance G × E, also reduced $r_G$) |

* Legend: (+) slight increase; + increase; ++ strong increase; - decrease; 0 no change; ? nonsignificant family variance; Yes/No presence of effect.

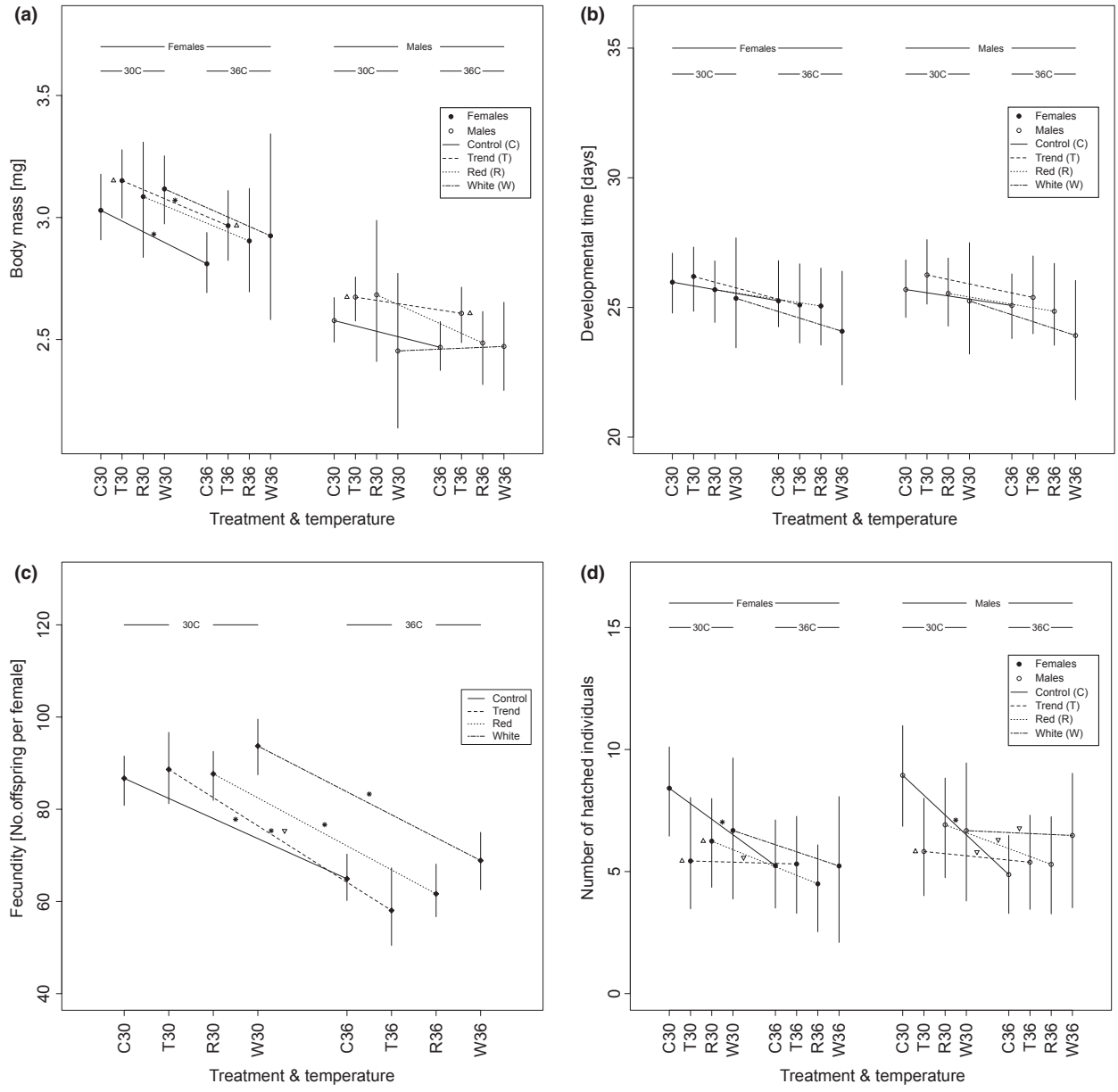


**Fig. 1** Mean phenotype for each treatment line and temperature (with credible intervals based on variation among 40 families) for (a) mass at emergence, (b) developmental time, (c) fecundity and (d) hatching. Control (C), Trend (T), Red (R) and White (W) in 30 and 36 °C are indicated and are abbreviated by C30, C36, T30, T36, etc. Significant effect of environment within each line is indicated by an asterisk (*). Significant differences between treatment lines and Control (i.e. effect of selection) are indicated by triangles; significant difference to the Control within each temperature is indicated by a triangle pointing up (Δ), and significant difference to Control across temperatures (i.e. treatment by environment interaction) is indicated by a triangle pointing down (∇). Estimates of significance are shown in Tables S1 and S2.

(Fig. 3b). $CV_E$ increased significantly in the Red lines, but not in the White lines for fecundity (Fig. 4b). For body mass, the Fluctuation lines tended to show a decrease in $CV_E$ across temperatures (Fig. 2b). There was a significant G × E for developmental time in the Red lines, but not in the White lines (Table S1A). The genetic correlation between 30 and 36 °C for body mass was negative in the Red lines and positive in the White lines. For developmental time, the correlation was low (around 0.3) in both the Red and White lines, and the credible interval included zero (Table S3).

When comparing the response of population across temperatures before (Control) and after selection in a

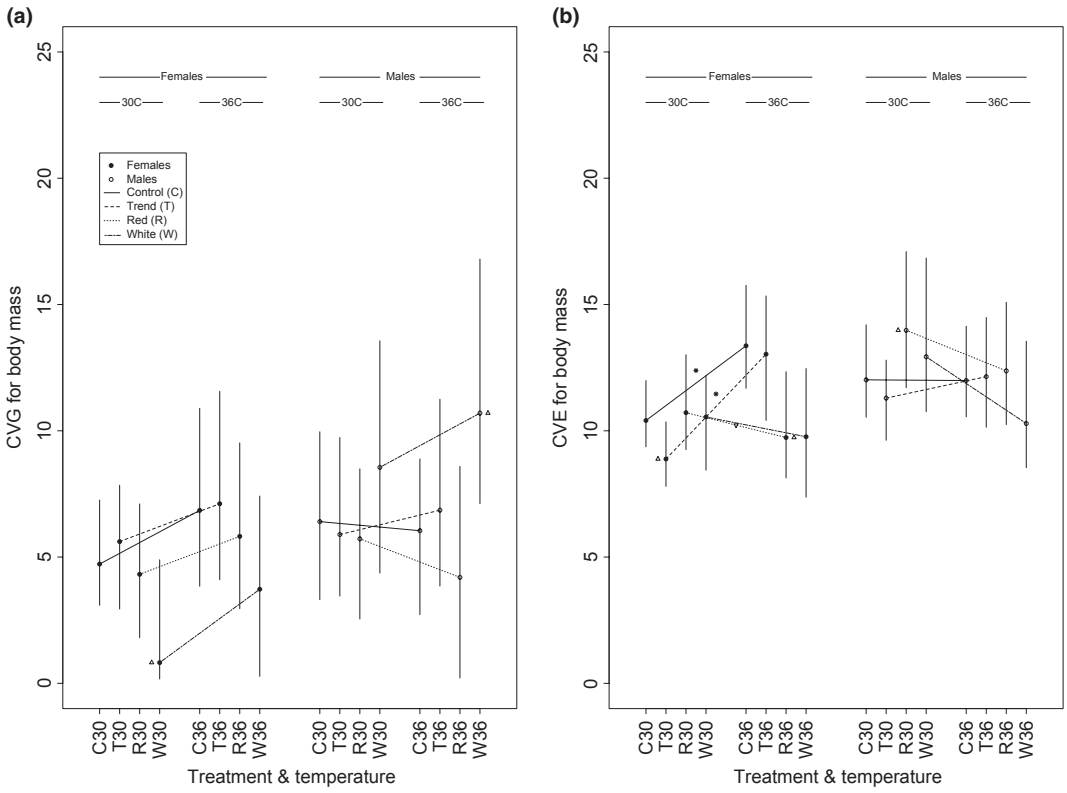


**Fig. 2** (a) Genetic coefficient of variation ($CV_G$) (b) Environmental coefficient of variation ($CV_E$) for mass at emergence (separate sexes). Abbreviation as in Fig. 1; *Significant effect of environment within each treatment line. Triangles indicated significant differences of each treatment line to Control line; difference within temperature (Δ) and across temperatures (∇). Estimates of significance are shown in Appendix S1.

fluctuating environment (Fluctuation lines), we found that the Fluctuation lines (particularly males) differed from the Control lines in their response across environments (plasticity) for hatching success – plasticity was reduced after fluctuating selection (Fig. 1d). There were significant differences in $CV_G$, $G \times E$ and $CV_E$ after selection in a fluctuating environment for body mass, developmental time and hatching success. $CV_G$ was significantly decreased in the Fluctuation lines for developmental time and body mass (White lines, females) (Figs 2a, 3a, Table S2). Heritability ($h^2$) of hatching success increased after selection for the Red line (Fig. 5, Table S2). Both Fluctuation lines (males only) showed an increase in $G \times E$ for developmental time (Table S2); the genetic correlation across environments for developmental time was small (credible interval included zero) in the Fluctuation lines, but not in the Trend and the Control (except females) lines (Table S3). In the Fluctuation lines, the $CV_E$ was significantly reduced for developmental time and mass at emergence (especially the Red treatment line) (Figs 2b and 3b). For mass at emergence, changes in $CV_E$ between temperatures were similar for Control and Trend vs. the Fluctuation lines (Fig. 2b); whereas Control and Trend lines showed an increase in $CV_E$, the fluctuation lines showed the tendency to decrease (Fig. 2b).

## Discussion

### Population response – before selection

There was a strong response to the new environment (36 °C) manifested as an approximately 6% lower mass at emergence, 25% lower fecundity and 42% lower hatching success. Developmental time was largely unaffected (3% change, not significant). This strongly suggests that the new environment was stressful and not optimal for the beetles. The plastic response in body mass, hatching success and fecundity matches expectations, because a rapid shift in the environment is thought to lead to an increase in phenotypic plasticity (Lande, 2009).

Genetic variance, estimated as $CV_G$, was higher in 36 °C for both mass at emergence (females only) and developmental time. A spontaneous response to exposure to a new environment manifested as an increase in $CV_G$ is in line with expectations (Holloway *et al.*, 1990; Guntrip *et al.*, 1997; Sgrò & Hoffmann, 1998) and could, for example, be due to hidden genetic variation

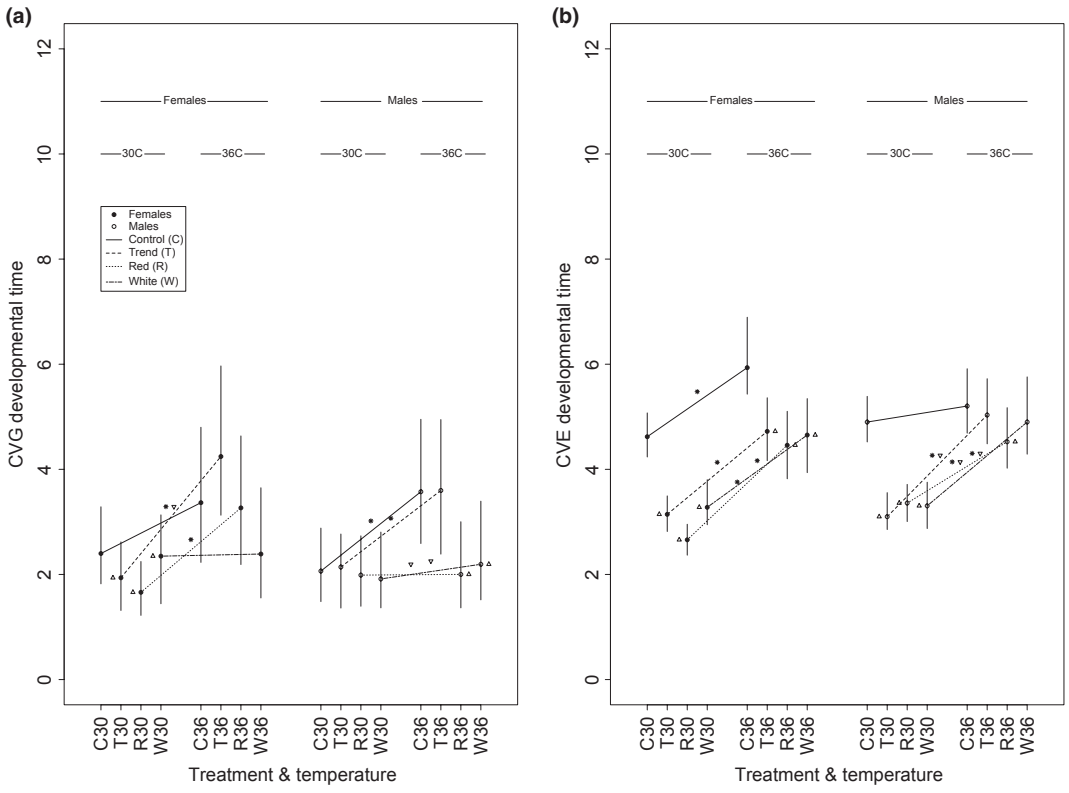


**Fig. 3** (a) $GV_G$ and (b) $CV_E$ for developmental time (separate sexes). Abbreviation as in Fig. 1; *Significant effect of environment within each treatment line. Triangles indicated significant differences of each treatment line to Control line; difference within temperature (Δ) and across temperatures (∇). Estimates of significance are shown in Appendix S1.

being expressed (Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999), or due to the failure of compensatory mechanisms involved in canalization (Pal, 1998). Alternatively, a novel environment may present new environmental disturbances for which no canalization mechanisms exist.

In contrast to body mass and developmental time, $CV_G$ for fecundity could not be detected in the higher temperature. This might be explained by an increased genetic canalization for this trait. Fecundity is a trait that is important for fitness, and is therefore thought to be under strong stabilizing selection. Our result suggests that genetic canalization for this fitness trait is strong, even if the change in the environment is considered to be stressful. Alternatively, the increased environmental variation in the higher temperature could mask the genetic variation, making it hard to detect.

We detected significant G × E for developmental time, but not body size or fecundity. This result is in line with previous studies on *C. maculatus* (Guntrip & Sibly, 1998). Even though males and females differ in their type of G × E (males show variance G × E, whereas females show crossing G × E and low $r_G$; Table S1A), they both exhibit variation for phenotypic plasticity, and thus evolution of phenotypic plasticity for developmental time is possible (Via & Lande, 1985).

## Population response – after selection

We exposed all treatment lines to a successive change in temperature environment over 18 generations; hence, selection acted on these lines to adapt to the changing conditions. We therefore expect the treatment lines to differ from the Control lines, which were kept at constant temperature. We were interested in which ways the selection treatment in general and the details of the selective past in particular affect a population's response. Thus, we compared a population's response to change in temperature after selection (treatment lines) to the response of a population before selection (Control).

Phenotypic responses due to selection could mainly be detected in the Trend lines, whereas lines that had been exposed to a fluctuating increase in temperature responded mainly in $CV_G$, $CV_E$ and G × E. We expected an increase in phenotypic plasticity for the Trend line compared to the Control. Both fecundity and mass at emergence did indeed show significant amounts of plasticity, but plasticity did not increase due to selection.

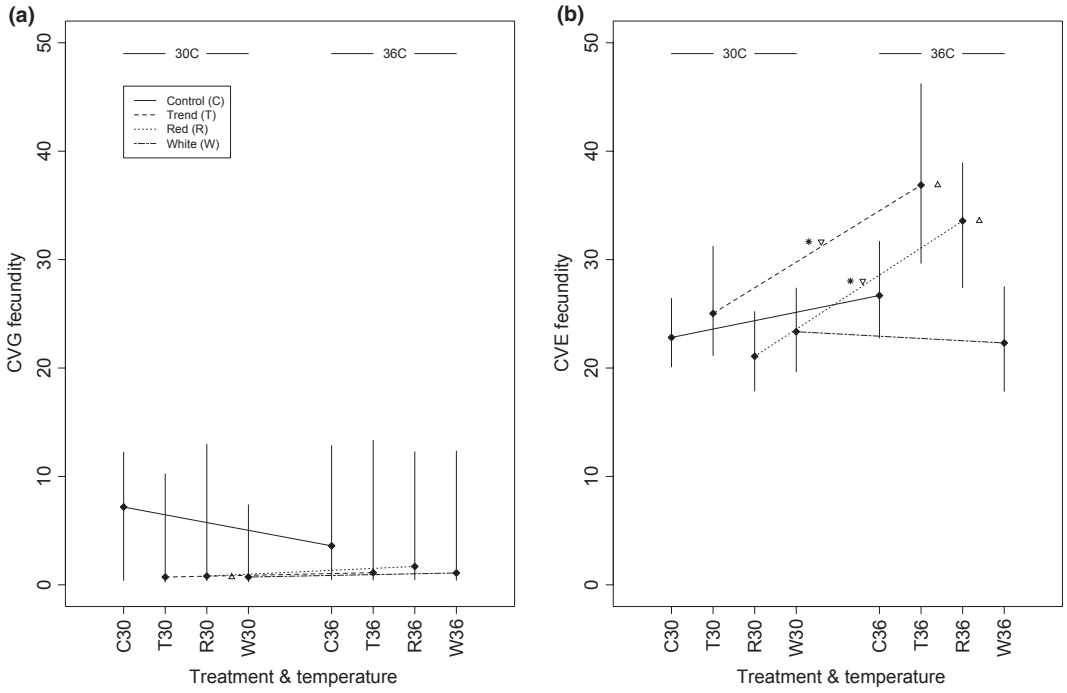


**Fig. 4** (a) $GV_G$ and (b) $CV_E$ for fecundity. Abbreviation as in Fig. 1; *Significant effect of environment within each treatment line. Triangles indicated significant differences of each treatment line to Control line; difference within temperature (Δ) and across temperatures (∇). Estimates of significance are shown in Appendix S1.

We expected an increase in $CV_G$ in the Trend lines, but this was not found. We found an increase in G × E in the Trend lines for developmental time (variance G × E and change in $r_G$).

In the Fluctuation lines, we expected an increase in plasticity, but found a decrease for hatching success and no difference to the Control for the other traits. We expected a decrease in $CV_G$ in the fluctuation lines, which was found for developmental time and for mass at emergence. Likewise, we expected an increase in $CV_E$; however, this was only found for Red lines for fecundity and mass at emergence (males only). A significantly decreased $CV_E$ was found in developmental time and mass at emergence (females). Finally, we found that variance G × E was reduced for developmental time (except females of the Red line), but so were genetic correlations across environments, indicating an increase in crossing G × E. For mass at emergence, the sign of the correlation changed from positive to negative in the Red lines. Expectations and results are summarized in Table 2.

*Phenotypic response in a changing environment*

The main phenotypic response was found in the Trend line. Selection in an environment with continuously increasing temperature resulted in fewer but bigger individuals hatching in both temperature environments. This suggests that selection leads to an increase in the size of individuals, which compensates for the reduced number of offspring hatched. Given the assumption that larger individuals have higher fecundity (e.g. Credland *et al.*, 1986), this response can be considered a strategy to cope with an environmental change in the form of a temperature increase. Although we found a significant increase in body mass, this did not result in higher fecundity. This finding could be explained by the fact that beetles were exposed to a competitive environment during their long-term selection (i.e. 200 individuals were transferred on fresh beans every generation), but in the final split-brood experiment, the traits were measured under noncompetitive conditions (i.e. only one mating pair on a sufficient amount of beans). Under competitive conditions, a large body size is advantageous, and thus beetles were selected to grow large. However, this advantage disappears under noncompetitive conditions, which could explain why we do not see an effect on fecundity. The Fluctuation lines did not show a significant phenotypic change, but their mean values were similar to those of the Trend line for both mass at emergence and hatching success. It might take populations longer to adapt to changes in the environment when it is fluctuating and thus 18 generations might not have been enough to detect signs of phenotypic adaptation. Importantly, we found significant changes in genetic and environmental variances as well as phenotypic plasticity and its variation.

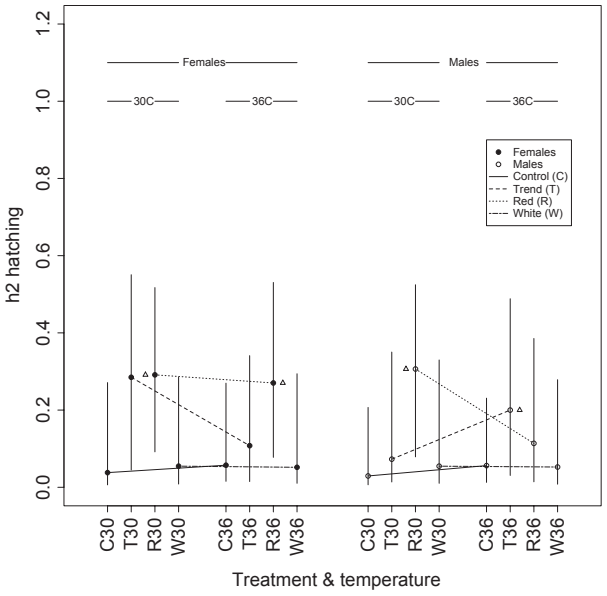


**Fig. 5** Heritability ($h^2$) for hatching. Abbreviation as in Fig. 1; Triangle (Δ) indicates significant differences of each treatment line to Control line within temperature. Estimates of significance are shown in Appendix S1.

### *Phenotypic plasticity and its variation*

The amount of plasticity after selection in a continuously increasing temperature environment (Trend) did not differ from the Control for developmental time or mass at emergence. This is surprising, as directional selection is thought to lead to an increase in phenotypic plasticity (Bonduriansky, 2007a,b; for review, see Stillwell *et al.*, 2010) and it contrasts with findings of a previous study (Teuschl *et al.*, 2007). Our results suggest that selection in an increasing temperature environment is maintaining rather than increasing the existing amount of plasticity. The high temporal autocorrelation in the Trend selection regime implies that the environment is predictable, which makes a plastic response a good strategy to respond to changes (Reed *et al.*, 2010; Jablonka *et al.*, 1995). If the environment is less predictable, a plastic response can be considered as partially adaptive or even maladaptive (Reed *et al.*, 2010). The results of this study show that these predictions partly hold true, because plasticity is indeed maintained for most traits of the Trend line. We suggest that although an increased plasticity under directional selection is beneficial, there might be a cost of increasing it (Relyea, 2002), and thus, a simple maintenance of plasticity appears to be the best overall solution. The potential for plasticity to evolve was affected by the selection in a continuously changing environment. The genetic correlation across environments ($r_G$) for mass at emergence and developmental time was low and indifferent from zero before selection, but was increased and differed from zero after selection. This suggests that selection in a continuously changing environment leads to an increased interdependency of trait expression across environments, which will partly limit the evolution of phenotypic plasticity. This might explain why we do not see the expected increase in plasticity.

In the Fluctuation lines, we detected a decreased or nonchanged plasticity after selection rather than the expected increase in plasticity. The Fluctuation lines might be exposed to greater/smaller changes and also to more extreme temperatures during their selection, which can be considered stressful for the populations. Also, the fluctuation selection regime is characterized by high environmental stochasticity and thus predictability of the environment is low. These factors have been shown to have consequences for the effectiveness of plastic responses to environmental change (Gavrilets & Scheiner, 1993; Scheiner, 1993; De Jong, 1999; De Jong & Gavrilets, 2000). Reed *et al.* (2010) showed that a plastic response of the phenotype can increase extinction risk and can be maladaptive when environmental stochasticity is high; therefore, we suggest that a plastic response in a highly fluctuating environment might not be beneficial. Interestingly, although we could not detect a change in plastic response due to fluctuating selection as such, we could detect changes in $G \times E$ and $r_G$, suggesting that the potential for the evolution of phenotypic plasticity changed due to selection. The Fluctuation lines showed an increased variation in phenotypic plasticity for developmental time (crossing reaction norms; crossing $G \times E$). Additionally, the low genetic correlation between environments suggests that selection in a randomly fluctuating environment can lead to a situation where trait expression in a higher-temperature environment is partly independent of trait expression in the environment that the trait had been adapted to. Thus, we can conclude that evolution of plasticity for developmental time in *C. maculatus* is possible. For mass at emergence, the genetic correlation across environments ($r_G$) was different from zero after selection in a fluctuating environment (positive $r_G$ for White and negative $r_G$ for Red), suggesting a partial dependency of trait expression across environments and potential limits to the evolution of phenotypic plasticity. However, we could also detect a significantly increased $CV_G$ at 36 °C in the White treatment line; this suggests that although there is some genetic dependency of traits (high $r_G$), the evolution of phenotypic plasticity may still be possible due to different amounts of genetic variation in the two environments (Cheverud *et al.*, 1985). The negative $r_G$ in the Red treatment lines suggests a reciprocal relationship or trade-off between values of character states across environments. Thus, selection in a fluctuating environment (Red) can lead to a shift in sign of $r_G$ and to a reversed relationship of character expression.

*Genetic variation in a changing environment*

After selection, we detected a decrease in $CV_G$ for the Fluctuation lines for both mass at emergence and developmental time. Thus, selection in an environment with increasing, fluctuating temperature leads to lower amounts of genetic variation, which is in line with expectations. We were able to attribute this response to the interaction of temperature increase with fluctuations, and exclude that it was due to an increase in temperature *per se*, because the Trend lines (continuous temperature increase only) responded similar to Control lines. This suggests that selection in a fluctuating environment does indeed lead to genetic canalization (Kawecki, 2000).

An alternative, but not mutually exclusive explanation to genetic canalization (Kawecki, 2000) is that the response of the Fluctuation lines was due to a change in the genetic background during selection in a fluctuating environment. A different set of genes may have been responsible for quantitative trait expression at 36 °C, and this set of genes could have been selected for during the selection treatment – resulting in low genetic variance. This suggestion is supported for developmental time by a low genetic correlation between environments in the fluctuation lines, suggesting that different genes are responsible for the expression of the same trait in different environments.

The observed lower genetic variance can also be explained by the characteristics of the selection regime. Fluctuating selection regimes (Red and White) had rapid and more severe changes in temperature, suggesting stronger selection in each generation, which may deplete genetic variation. Furthermore, the direction and strength of selection was periodically reversed during the experiment, and this reversal may lead to indirect canalization and decreased genetic variation (Kawecki, 2000). Alternatively, the fact that our fluctuating selection treatment applied directional selection that changed direction nearly every generation might have lead to net stabilizing selection over the course of the experiment. Stabilizing selection is known to reduce genetic variation (for a review, see Stillwell *et al.*, 2010).

$CV_G$ represents variability and the potential to respond to selection (Houle, 1992). Our results suggest that this potential is unchanged after a continuous increase in temperature, but decreased after fluctuating increases in temperature for developmental time. This has important implications for our ability to predict the evolutionary future for populations in a changing environment, and thus, more work (particularly of an empirical nature, e.g. Pélabon *et al.*, 2010 and this study) is needed to understand the overall impact of differences in response to differential changes in the environment. Also, one should be careful extrapolating results obtained from a single-generation experiment (such as the Control in this study), because the details of the selective past are affecting the outcome.

*Environmental sensitivity in a changing environment*

$CV_E$ measures within-family variation, and thus, it gives information about an individual's degree of environmental canalization (Stearns *et al.*, 1995). Included in the estimate of $CV_E$ is developmental stability (mechanisms buffering against stochastic perturbations of individual development, Waddington, 1957; Debat & David, 2002; Klingenberg, 2003), and it can contain effects of environmental heterogeneity, individual genotype, phenotypic plasticity, $G \times E$ and maternal effects (Falconer & Mackay, 1996). In our study, we control for environmental heterogeneity within families and temperature, and we assume that family members are closely related. Thus, we expect the effects of individual genotype, phenotypic plasticity and $G \times E$ within a family to be small. Maternal effects might also influence the estimate of $CV_E$, but are thought to be less important than developmental stability (Lajus & Alekseev, 2004). Furthermore, differences in $CV_E$ between temperatures are unlikely to be due to direct maternal effects, because members of each family (i.e. same mother) were split up after egg laying.

Generally, individuals showed an increase in $CV_E$ across temperatures, indicating that they were more sensitive and less environmentally canalized in the higher temperature (36 °C). For developmental time, an increase of $CV_E$ in 36 °C for the Control females but not males shows that females are the sex being more sensitive to a rapid change in environmental temperature. Hence, males are more environmentally canalized/buffered against environmental perturbations. According to the theory, canalization of development against environmental fluctuations is less efficient in novel and less optimal environments overall (Pal, 1998; Hoffmann & Parsons, 1991).

The decreased $CV_E$ for developmental time and body mass (fluctuation lines only) after selection suggests that long-term selection in a changing environment leads to a higher degree of environmental canalization. Whether this response is due to the effect of fluctuation in the environment depends on the trait under observation. These results are unexpected, because especially fluctuating changes in the environment were thought to lead to a lower degree of environmental canalization, that is, higher $CV_E$ (Zhang & Hill, 2005). Our results suggest that environmental canalization might be an advantage when the environment is variable. Whether this increased environmental canalization in a fluctuating environment can be attributed to increased developmental stability remains to be tested.

*The relative importance of genetic and environmental variation*

The $CV_E$ was generally larger than the $CV_G$, especially for fecundity, where $CV_G$ was hard to detect because among-family variation was nonsignificant. This is not necessarily surprising because fecundity is a close to fitness-related trait and likely to be under stabilizing selection because of trade-off relationships with other traits. Thus, it should have both decreased genetic (Fisher, 1930; Robertson, 1955) and environmental variance. However, genetic variance is eroded to a larger extent than environmental variance (Zhang & Hill, 2005), resulting in low heritabilities and variation in the phenotype being mainly determined by environmental variation (Houle, 1992). Maintenance of environmental variation is thought to be due to phenotypic plasticity (Zhang, 2005) and/or the cost of reducing variability (Zhang & Hill, 2005). Our results are in line with previous findings (Zhang & Hill, 2005) and support the idea that even when genetic variation is decreased or exhausted, environmental variation can still be maintained and be responsible for phenotypic variation.

An interesting result is the similarity of Control and Trend lines versus Red and White (i.e. Fluctuation) lines regarding the relative contributions of genetic and environmental variation to the phenotype of mass at emergence. Control and Trend lines show a rather stable relative contribution of genetic and environmental variation to the phenotype over temperature, but the Fluctuation line phenotypes are determined by environmental variation to a greater/lesser extent depending on temperature. This suggests that under stabilizing or directional selection, genetic canalization and environmental canalization were affected in a similar way and thus may have been influenced by a common buffering mechanism (Stearns *et al.*, 1995; Klingenberg, 2003; Flatt, 2005). However, under fluctuation selection, this seems not to be the case, suggesting that different mechanism might be responsible for genetic versus environmental canalization or developmental stability (Réale & Roff, 2003; Pélabon *et al.*, 2010. Overall, this result highlights once more that fluctuation in the selective past is highly important for a population's response to environmental change.

## Conclusion

We show in this study that selection in a changing environment, and especially selection in a fluctuating environment, can have major consequences for adaptive trait evolution and the evolution of phenotypic plasticity. Some of our predictions regarding short-term responses to environmental change were supported: populations responded in a plastic way and genetic variance increased after exposure to a rapid shift in the environment. The third and major aim of our study was to investigate the role of environmental fluctuation in the evolutionary process of a population exposed to a changing climate. We found that a population's response to environmental change is dependent on whether it experienced fluctuations in its selective past. Selection in a fluctuating environment can lead to (i) a decreased genetic variance that indicates a reduced potential to respond to selection; (ii) an increased genetic variance that makes sex-specific evolution of plasticity possible; (iii) a reversed relationship of character expression across environments; (iv) a facilitation of independent trait evolution by decreasing genetic correlations across environments; and (v) a more environmentally canalized response of individuals. Thus, our results suggest that the potential to respond to selection, environmental sensitivity and the evolution of phenotypic plasticity is strongly dependent on the selective past and on whether selection involved fluctuations in the environment or not. Fluctuations in the environment are common and probably the most realistic and biologically relevant way in how our climate is changing and affecting populations (Steele, 1985; Inchausti & Halley, 2001). However, to date the vast majority of studies investigating fluctuations are theoretical modelling and simulation approaches (Halley, 1996; Ripa & Lundberg, 1996, Inchausti & Halley 2003, but see Pélabon *et al.*, 2010), and thus, more empirical work is needed. Future studies that aim to investigate a population's response to environmental change should take parameters such as genetic and environmental variation as well as plasticity and its variation into account. Most importantly, they would greatly benefit from considering the details of the selective past, because these have, as we show in this study, great impact on a populations response and thus also our ability to predict a population's response to a changing environment such as climate change.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Table S1** (A) Effect of environment within each treatment for mass at emergence, developmental time, hatching and fecundity presented for separate sexes with posterior mode and 95% credible intervals (95 % CI); and tested for significance (95% CI not overlapping zero). (B) Test for significance of random factors (family and replicate) via Deviance information criterion (DIC) of full and reduced models.

**Table S2** Effect of selection.

**Table S3** Genetic correlation ($r_G$) within treatments across environments ($G \times E$) for mass at emergence (pooled for sex) and developmental time.

**Appendix S1** Estimates of significance for Figs 2–5.

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