Adaptive potential and evolution in new environmental conditions

Eva L. Koch[1,2], Sonja H. Sbilordo[1], and Frédéric Guillaume[1]

[1] Department of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland
[2] Department of Animal and Plant Science, University of Sheffield, Western Bank, Sheffield S10 2TN, United Kingdom

Corresponding author:
Frédéric Guillaume
phone: +41 44 635 66 23
frederic.guillaume@ieu.uzh.ch

Abstract

In presence of rapid environmental changes it is of particular importance to assess the potential of populations to adapt to new conditions. For rapid evolution to occur, standing genetic variation within a population is essential. Additive genetic variance (V_A) in fitness is therefore a key parameter for evaluating adaptive capacity. In this study we used *Tribolium castaneum* (red flour beetles) to investigate potential to adapt to three new environmental conditions (Dry, Hot, Hot-Dry). We estimated V_A in fitness (offspring number) and tested for potential constraints that might limit adaptation including negative genetic correlations between female and male fitness. We tested adaptation after 20 generations of experimental evolution. Based on V_A estimates for fitness we expected the highest relative fitness increase in the most stressful condition Hot-Dry and similar increases in single stress conditions Dry and Hot. High adaptive potential in females in Hot was reduced by a negative covariance with male fitness. We found that observed adaptation mainly matched our predictions. Although precise estimates of relative fitness increase over such a long period of time are not possible, we can make some qualitative predictions whether a population is likely to adapt. We further demonstrate how additive genetic effects of females and males on reproductive output can be analysed jointly to get estimates for fitness increase of a population. Given that body size is commonly used as a proxy for fitness, we also tested how this trait and its genetic variance (including non-additive genetic variance) were impacted by environmental stress. In both traits, fitness and body size, variances were sex and condition dependent, but they differed in their variance composition, cross-sex and cross-environment genetic covariances, as well as in the environmental impact on V_A.

Introduction

Environmental changes pose a substantial risk of extinction to many organisms (Thomas *et al.*, 2004; Parmesan, 2006). Predicting whether a population is able to persist is therefore of crucial importance in the face of ongoing environmental changes. To persist, species may adapt via plastic or genetic changes. Plastic changes, e.g. physiological or behavioural adjustments, allow individuals to cope with stressful conditions (Charmantier *et al.*, 2008). However, plastic responses are often costly and thus likely limited (DeWitt *et al.*, 1998; de Jong, 2005; Valladares *et al.*, 2007; Pfennig *et al.*, 2010; Snell-Rood *et al.*, 2010; Sokolova *et al.*, 2012). Adaptation to rapid environmental changes may then require rapid evolution fuelled by standing genetic variation (Kellermann *et al.*, 2006; Bell & Gonzalez, 2009, (Hoffmann & Sgró, 2011). Changes in genotype frequencies under climate change have been shown already for several species (Umina *et al.*, 2005; Balanya *et al.*, 2006; Karell *et al.*, 2011), although it is predicted that some species might not have the evolutionary potential to adapt to shifting environmental conditions (Etterson & Shaw, 2001). The additive genetic variance (V_A) of relative fitness quantifies the population's rate of evolution, i.e, the expected increase of mean fitness of the population (Fisher, 1930; Price, 1972; Falconer & MacKay, 1996). Usually, only variance due to additive genetic effects (V_A) is considered since they are inherited from parents to offspring and determine the response to selection. Thus, the existence of V_A in fitness is a prerequisite for adaptation and can be used as an overall estimate for a population's capacity to adapt, or its evolvability (Houle, 1992; Hansen *et al.*, 2011; Shaw & Shaw, 2014).

Estimates of V_A in fitness are scarce, especially in natural populations and tend to show large heterogeneity in the estimated values of V_A for lifetime fitness (e.g., in vertebrate populations: Kruuk *et al.*, 2000; Merilä & Sheldon, 2000; McCleery *et al.*, 2004; McFarlane *et al.*, 2014). More often, V_A of life-history traits closely linked to fitness is estimated (e.g., components of reproduction or longevity). For those traits, sizeable estimates have been provided, although often in the form of narrow-sense heritability (h^2, proportion of V_A). Many surveys of heritability in morphological, behavioural and life-history traits have reported lower estimates of h^2 for fitness related traits (Mousseau & Roff, 1987; Teplitsky *et al.*, 2009; Wheelwright *et al.*, 2014) as expected from the effect of stronger directional selection exhausting V_A at those traits. Nevertheless, some laboratory studies directly studying V_A of fitness have reported substantial V_A, for instance for lifetime fitness in females of *D. melanogaster* (Gardner *et al.*, 2005; Fry, 2008; Long *et al.*, 2009). This points at the fact, that lower h^2 values in fitness traits may be due to larger contribution of non-additive or environmental effects to V_P than in other traits (Crnokrak & Roff, 1995; Roff & Emerson, 2006; Chirgwin *et al.*, 2017). This is corroborated by the lack of correlation between h^2 and CV_A (V_A /mean^2), as shown in a large survey by Hansen et al. (2011).

Despite existing V_A in fitness traits, evolution to new conditions can be constrained by antagonistic pleiotropy when alleles influence several fitness components but with opposite effects. This leads to trade-offs since one component cannot be optimized without reducing the other, e.g. fecundity and life length (Roff, 2000). Another example of trade-off is sexual antagonism (Foerster *et al.*, 2007; Bonduriansky & Chenoweth, 2009; Delcourt *et al.*, 2009; Kirkpatrick, 2009; Poissant *et al.*, 2010; Calsbeek *et al.*, 2015; Connallon & Hall, 2016). Fitness optima might often differ between males and females. However, sharing a great part of their genome constrains independent evolution and limits adaptation when selection in the two sexes is opposite to the genetic correlation. Such a constraint should be evident by negative genetic correlations between male and female fitness. Similar constraints may appear when adaptation to certain environmental conditions (e.g., elevated temperature) trade-offs with adaptation to other conditions. Environmental changes often include simultaneous changes in several variables. For short-term stress exposure, effects like cross-tolerance and hardening, i.e. exposure to one stress increased resistance to another stress, were observed, and it is known that some responses rely on the same physiological mechanisms (Bubliy *et al.*, 2012). Evolutionary adaptation to different stressors involving the same pathways can lead to correlated resistance to another stressor (Bubliy & Loeschcke, 2005; Sikkink *et al.*, 2015).

However, examples of local adaptation (Hereford, 2009) suggest that selection may favour different genotypes in different conditions. Immediately after exposure to environmental change, genetic correlation between fitness in different conditions can inform us to which extent the genetic basis of fitness is shared between environments. If we observe negative genetic covariances between fitness in different conditions, it is likely that alleles providing fitness benefits in one condition become detrimental in another. Adaptation when both stress factors are experienced at the same time can then be limited. Thus, these estimates can inform us about the severity of stressor combination for long-term adaptation. Changes in environmental conditions are also well known to affect genetic variances (Sgrò & Hoffmann, 1998; Hoffmann & Merilä, 1999), and covariances (Simons & Roff, 1996; Sgrò & Hoffmann, 2004; Wood & Brodie, 2015), including cross-sex genetic covariance (Delcourt *et al.*, 2009; Poissant *et al.*, 2010; Punzalan *et al.*, 2014). This can have substantial implications for evolutionary potential (Wilson *et al.*, 2006; Husby *et al.*, 2011), since the environmental shift that imposes a risk of extinction can at the same time either increase V_A for fitness (Shaw and Shaw, 2014) or reduce the evolutionary potential to adapt to this new condition (Wood & Brodie, 2016). To fully understand the impact of environmental change on a population's persistence it is therefore essential to know how genetic variances change in different environments and to identify potential constraints.

Classical examples for co-occurring stressors are heat and drought. Their combined effects has been extensively studied in plants (e.g. Rizhsky, 2004), but less so in animals. Temperature is an important factor, especially for ectothermes, and understanding its effect in combination with drought as well as potential interaction on adaptive potential can be important in the context of climate change. In this study, we explore the potential for adaptation in the model organism *Tribolium castaneum* (red flour beetle) under four different conditions: a control condition, to which the population was adapted, and three stressful treatments where humidity was decreased (Dry), temperature was increased (Hot), or both (Hot-Dry). We evaluated the evolutionary potential to adapt to these conditions by estimating V_A in fitness for females and males as well as their genetic correlation. We further tested for genotype-by-environment interactions (G x E) for fitness in different conditions. They tell us whether the genetic basis for fitness is shared among environments and adaptation to one stress treatment can also result in higher resistance to another stress. It can also show whether there are trade-offs in responses to heat and drought, which may limit adaptive potential when they are experienced simultaneously. Although predictions based on V_A apply to changes after one generation, we were interested to test whether they can also inform us about evolution on longer time scales. Using experimental evolution, we tested for adaptation as well as correlated responses to stress treatments after 20 generations.

We were further interested to investigate the genetic architecture of body size, a trait closely associated with fitness and often used as a proxy for it (Kingsolver & Huey, 2008; Chown & Gaston, 2010):. Our data for size also allowed us to estimate the non-additive variance (V_D). In contrast to V_A, relatively little is known about V_D. One reason why it received less attention is that non-additive effects (dominance and epistasis) cannot be inherited. Furthermore, it is often not possible to get unbiased estimates since V_D is often confounded with maternal or common environmental effects. However, previous studies suggest that its proportion might be substantial (Crnokrak & Roff, 1995; Roff & Emerson, 2006; Wolak & Keller, 2014). Given the importance of non-additive effects in inbreeding it is desirable to obtain more data.

Material and Methods

Animal rearing and stress treatments

We used the *Tribolium castaneum* Cro1 strain (Milutinović *et al.*, 2013), collected from a wild population in 2010 and adapted to lab standard conditions (33°C, 70% relative humidity) for more than 20 generations. Beetles were kept in 24h darkness on organic wheat flour mixed with 10% organic baker's yeast. We

sterilized the flour and yeast by heating them for 12h at 80°C before use. We measured fitness as well as size in control (=standard) conditions and three stress treatments with increased temperature or/and decreased humidity. The conditions in the treatments were: Hot: 37°C and 70% relative humidity, Dry: 33°C and 30% r. h., Hot-Dry: 37°C and 30% r. h.

In order to be able to estimate genetic variances, we applied a split-brood paternal half-sib breeding design. We produced 147 half-sib families by mating virgin males to three virgin females (Figure 1A). Mating took place in control conditions at the age of four weeks in 15 mL tubes with 1g of medium. After four days, in which the beetles could mate and lay eggs, the females were transferred to new vials. We repeated this three times, resulting in four vials per female containing medium and eggs. Vials were then randomly assigned to the four different conditions, resulting in full-sib families (F1) split across all conditions. After mating with the first female, each male was mated with a second, and subsequently, with a third virgin female. These females were also transferred to new vials after four days. As a result, we obtained paternal half-sib families, each consisting of three full-sib families. Half- as well as full-sib families were split across all conditions (Figure 1A). Male and female offspring (four females and two males per full-sib family and condition) were separated at the pupal stage and transferred to 10 mL tubes with 1 g of medium and remained there until they were used for the fitness assay eight weeks later.

Fitness Assay and producing double first cousins

To estimate fitness, we mated each virgin male with two unrelated virgin females from the same condition in 15mL tube with 1g medium. The male was removed after 24h and females transferred into two separate tubes. Females were removed from the tubes after one week of egg laying, and 9g medium was added to provide food for the developing offspring. After five weeks the number of adult offspring was counted. While we conducted the matings for the fitness assay, we followed a specific crossing design and always crossed two pairs of full-sib families (Figure 1B). Individuals resulting from these crosses (F2) were double first cousins.

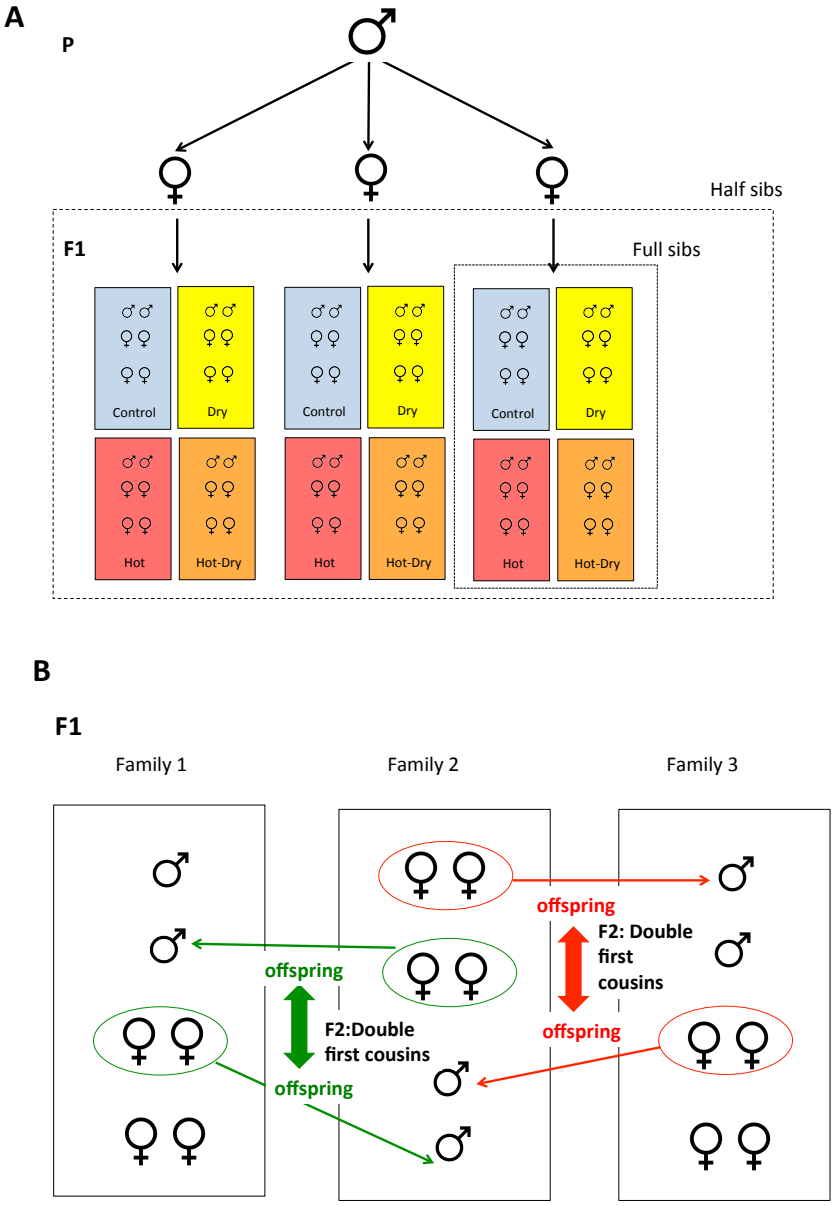

Figure 1: Crossing design. To produce paternal half-sib families, one male was mated subsequently with three females. Eggs of each female were randomly distributed across four different environmental conditions. Males and females of the next generation (F1) were used to estimate genetic variance in fitness **(A).** When we mated individuals of F1 to get offspring (used as our fitness estimate), we followed a specific crossing design. We always crossed two full-sib families (from different half-sib families) reciprocally. Offspring (F2) resulting from these two crosses are double first cousins **(B)**. F2 individuals were used to estimate additive and non-additive genetic variances in size.

Size

Size was measured in the F2, i.e. in the offspring of beetles that were used for the fitness assay. We used centroid size as proxy for total size since it can be measured more accurately than dry weight in very small insects and shows a high correlation with body mass (Wickman & Karlsson, 1989; Honěk, 1993). Dead beetles were fixed with a double-faced Scotch tape dorsally on a microscope slide. Sex was determined based on the sex patches of males at the inside of the femur. After sexing, all legs of the beetles were removed. Slides with single specimens were placed under a Wild M8 Heerbrugg M8 dissection microscope

with a transmitted light stand. Two further light sources from above were installed for better illumination. Images were captured with a 25x magnification using a Leica DFC495 digital camera connected to a PC running the Microscope imaging software LAS v4.6.2. File utility program "tpsUtil" was used to build tps files from the images and tpsdig2 for setting the landmarks (see http://life.bio.sunysb.edu/ee/rohlf/software.html for program information). To estimate beetle size, four Landmarks were set on the ventral part of the abdominal segment lV (see also https://cerambycids.com/ironcladid/IroncladID-morphology.html). The centroid size was calculated using the free software Past 3.14 (for information see: http://folk.uio.no/ohammer/past/).

Effects of condition on fitness and size

Statistical analysis was conducted in R version 3.4.2 (R Core Team, 2017). We used linear mixed models as implemented in the Rpackage *lme4* 1.1-17 (Bates *et al.*, 2015) to explore the effects of treatments on fitness and size. For fitness analysis we included treatment and batch (time of fitness assay) as fixed effects and mother identity as random effect to account for non-independence since some of our measured individuals were full-sibs. For analysing size we included batch (individuals that grow up at the same time), treatment and sex as well as their interaction as fixed effects and mother identity as random effect. Significance and confidence intervals were obtained using the Rpackages *lsmeans* 2.27-62 (Lenth, 2016)and *lmertest* 3.0-1(Kuznetsova *et al.*, 2017).

Adaptation

We started with six replicate lines per condition originating from the same ancestral population (same as before, Cro1) and let them adapt for 20 generations (Figure 2). Each line consisted of 120 individuals. Each new generation was set up by randomly selecting 120 pupae and placing them into a new vial with 70g medium. One selection line in Dry became extinct. Adult beetles of generation 20 from all selection lines were transferred to control conditions, in which they stayed for one week to mate and lay eggs. After removal of the adults, we waited until their offspring had reached the pupal stage and separated males and females. These individuals (generation 21) developed completely in control conditions. When they had reached the adult stage, each virgin male was mated with a virgin female of the same selection line and their offspring was transferred to all four conditions in the egg stage, resulting in full-sib families split across all conditions (Figure 2). As soon as these offspring (generation 22) had reached the pupal stage, males and females were separated. To compare fitness of different selection lines and test for adaptation, a virgin male and a virgin female of the same selection line in the same condition, but from different families were mated and the number of adult offspring produced within four days of mating and egg laying was used as an fitness estimate. To test for adaptation, we compared whether offspring number of selection lines in their native condition was significantly higher compared to non-adapted control lines. First, we analysed each condition separately. We used linear mixed models including selection regime and line type (normal/mixed) as fixed effects, lines and families nested within lines as random effects using the Rpackage *lme4* (Bates *et al.*, 2015). We were further interested to test for correlated responses, i.e. whether adaptation to a certain stress treatment could increase fitness in another. For this we run one analysis using the complete data set. Selection regime, conditions, line type and their interaction were used as fixed effects, lines, families nested within lines, and line-treatment interaction as random effects. Reported effect sizes and standard errors were obtained from the summary output of the model. p-values and confidence intervals were computed with the Rpackages *lmertest* (Kuznetsova *et al.*, 2017) and *lsmeans* (Lenth, 2016). Statistical analyses were conducted in R (R Core Team, 2017).

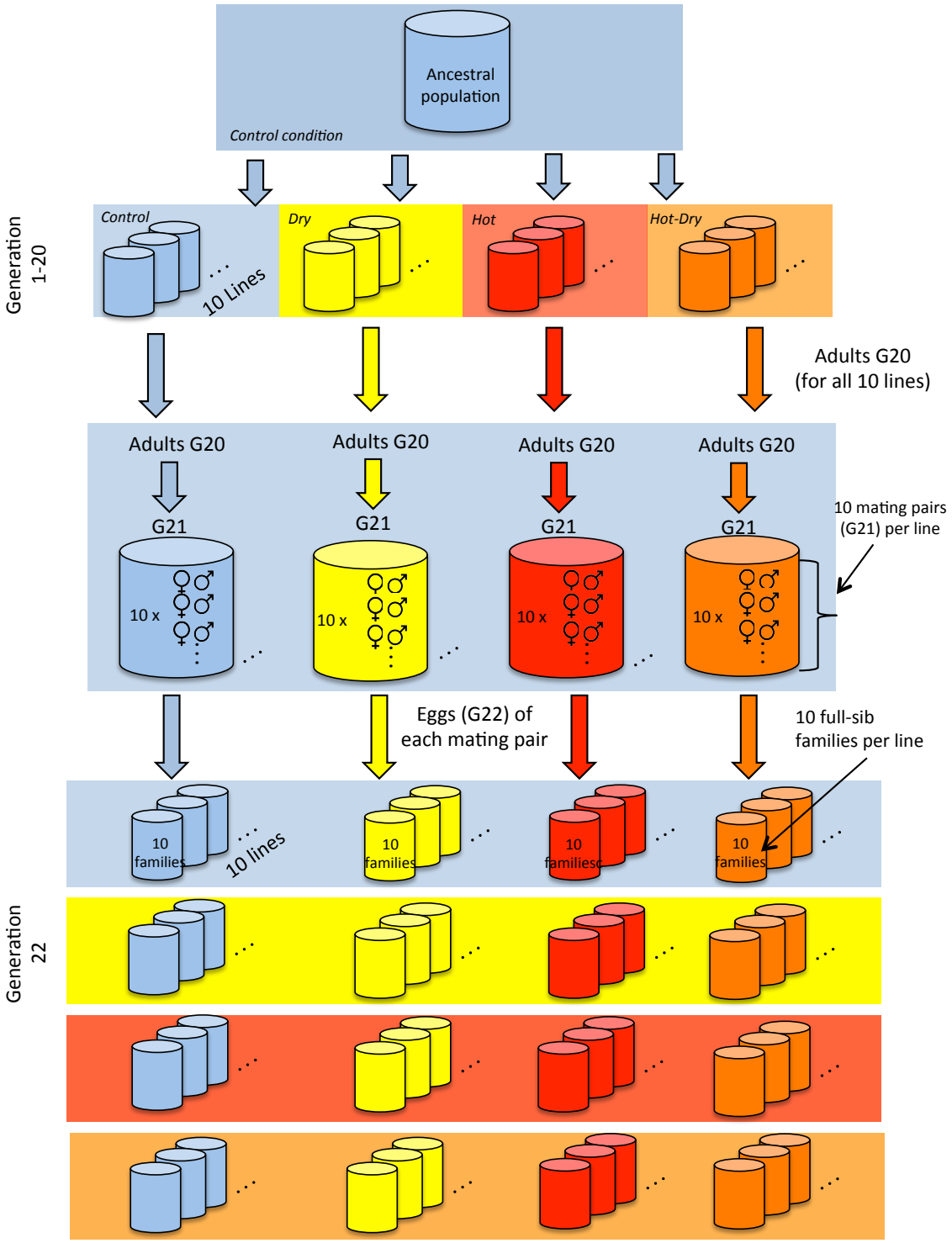

Figure 2: Schematic representation of the experimental evolution design. Lines were derived from a single outbred *Tribolium castaneum* population (collected in 2013, strain Cro1) that had lived under control conditions for ca. 30 generations. For each selection regime we used ten replicate lines. Lines stayed in control or treatment conditions for 20 generations. After one generation in control condition to remove potential maternal and epigenetic effects, individuals from all lines (generation 22) were transferred to control and treatment conditions in the egg stage. Fitness was measured in adult beetles by counting the number of adult offspring. Control conditions: 33°C, 70% relative humidity r.h.; Treatments: Dry: 33°C, 30% r.h.; Hot 37°C, 70% r.h.; Hot-Dry: 37°C, 30% r.h.

Quantitative genetic analyses

We estimated genetic variances, covariances and correlations using an animal model and restricted maximum likelihood estimation as implemented in Asreml version 3.0 (Gilmour *et al.*, 2009). The animal model is a linear mixed effect model that uses all known relationships from a pedigree as random effect to partition observed variance into additive genetic variance and other sources of variance (Morrissey *et al.*, 2012). Non-additive genetic relationship matrices were created with the Rpackage *nadiv* 2.16.0.0 (Wolak, 2012). All models were run in R by Asreml-R (Butler *et al.*, 2009).

Fitness

First, we ran a series of univariate models for each condition separately with offspring number as response variable, batch (samples where fitness assay was started on the same day) as fixed effect, additive genetic effects of females and additive genetic effects of males as random effects. Maternal identity (mother of female) was included as random effect to account for resemblance of full-sibs due to maternal, common environment or non-additive genetic effects. Significance of random effects was determined by likelihood ratio tests, testing whether excluding a certain random effect resulted in a significantly worse model. Reported P-values are one-tailed since we tested whether a certain variance is different from zero and variances cannot become negative. For estimating cross-sex genetic correlation we used the same univariate models but included a covariance between female and male additive genetic effects. Maternal effects were removed in subsequent analyses since they were very small and non-significant. Studies so far used bivariate models to investigate cross-sex genetic correlations (Brommer *et al.*, 2007; Foerster *et al.*, 2007; Punzalan *et al.*, 2014; Wolak *et al.*, 2018). In case of fitness this is problematic, because the same observation (number of adult offspring resulting from a mating) would be used twice. Using univariate models including additive genetic effects of females and males avoids pseudoreplication. Significance of genetic correlations was determined by two-tailed likelihood ratio tests comparing a model including correlations to a model with correlations set to zero. In order to test for genotype-by-sex interaction (G x S), which indicates that a genotype is differently expressed in males and females, we compared the model to a constrained model with genetic correlation fixed to one. To examine whether additive genetic effects of females and males were different, we tested whether the unconstrained model was significantly better than a model with V_A of females and V_A of males forced to be equal within each condition.

For estimating cross-condition genetic covariances and correlations we used bivariate models with fitness in two conditions as response, batch as fixed and additive genetic effects of females and males as random effects. Since each individual was only measured in one condition, residual covariance was set to zero. Significance of correlation was assessed by comparing to a model with correlation fixed to zero using two-tailed likelihood ratio tests. Comparison with a model with correlation fixed to one was used to test for G x E (one-tailed likelihood ratio tests). In all bivariate models, covariance between male effects was set to zero, because male effects were very small and not significant in some conditions and hence it was not meaningful to estimate covariances.

To allow comparisons across different conditions, we calculated h^2 and CV_A (square root of V_A divided by the phenotypic mean of the trait, multiplied by 100) of fitness for males and females in each condition. I_A (V_A divided by trait mean squared, multiplied by 100 (Houle, 1992)) was calculated as an estimate for the proportional change after one generation. Standard errors of CV_A and I_A were computed as described in Garcia-Gonzalez *et al.*, (2012).

To assess whether genetic variances were significantly affected by environmental change, we used a multivariate model with fitness in each conditions as separate response variable. Since fitness between conditions differed in mean and variance, we standardized data. We applied two different standardizations: 1) mean standardized (i.e. relative fitness) and 2) dividing by the standard deviation (sd) to have a variance of one. We then tested whether constraining the multivariate model and forcing V_A of standardized fitness to be equal in all conditions resulted in a significantly worse model. If the constrained model were worse, we can conclude that mean standardized V_A (I_A of absolute numbers), or sd standardized V_A (h^2 of absolute

numbers) are significantly influenced by the environment. Similarly, we tested whether genetic cross-condition correlations were different. We used a multivariate model and constrained all pairwise cross-condition correlations to be equal. We then tested if this model was significantly worse than an unconstrained model using a two-tailed likelihood-ratio test.

Size

Our crossing design during the fitness assay (reciprocal crossing of two pairs of full-sibs, see Figure 2) allowed us to estimate non-additive genetic variance (V_D) in the following generation (F2). In a paternal half-sib breeding design these effects contribute to resemblance among full-sibs and cannot be separated from maternal or common environmental effects. In contrast, double first cousins (DFC) share non-additive genetic effects without being confounded by maternal effects or a common environment. We partitioned the observed variance for size into V_A, V_M, V_D, and residual variance V_R while controlling for batch effects. Batches represent individuals that grew up at the same time, and thus accounts for variations in the medium or lab temperature. Similar to the analysis of fitness data, we first analysed each condition separately using univariate animal models with batch as fixed and maternal, additive, and non-additive genetic effects as random effects. To get sex specific estimates, we analysed male and female size separately. Genetic correlations between sexes and between conditions were assessed by bivariate models. Significance of correlations, G x E, environmental effects on I_A and h^2 of size were tested in the same way as we described before.

Results

Effect of treatment

Offspring number in the stress treatments was significantly lower than in control conditions ($F_{3,5130} = 805.45$, $p < 2.20E-16$). Effect of heat was stronger (mean offspring Hot = 33.01; [32.0, 34.0] 95% confidence interval) than effect of drought (mean offspring Dry = 43.87; [43.0, 44.7] 95% CI) and the lowest offspring number was found when both stressors were combined (Figure 3A). Treatments showed a significant effect on size ($F_{3,7911} = 138.57$, $p < 2.20E-16$) and size decreased in the stressful conditions. Similar to offspring number, the effect of drought was smaller. Combining both stressors did not result in an additional decrease in size. In contrast, we observed the lowest size in Hot and higher sizes in Hot-Dry (Figure 4A). The difference between males and females was significant ($F_{1,7709} = 710.76$, $p < 2.20E-16$) with females being larger. We also detected a significant sex-by-condition interaction ($F_{3,7709} = 7.27$, $p = 7.20E-05$) indicating that males were more sensitive to stressful conditions (Figure 4A).

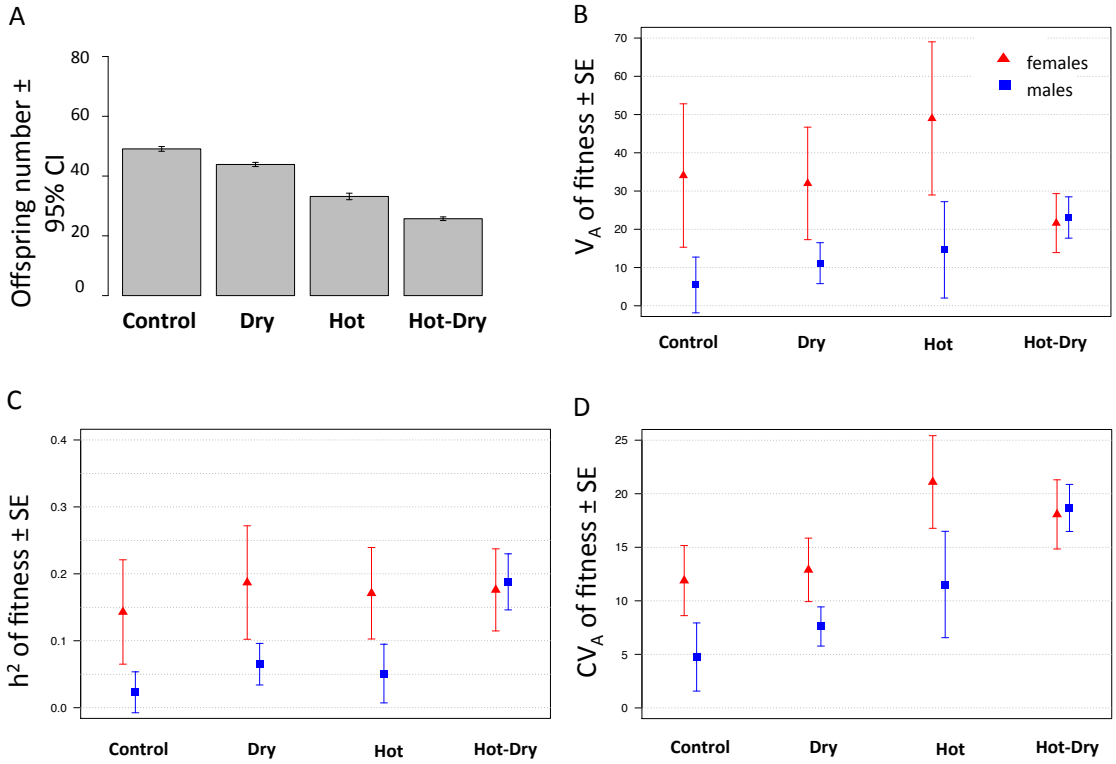

Figure 3: Fitness (= number of adult offspring) **(A)** and estimates for its additive genetic variance (V_A) **(B),** heritability (h^2) **(C),** and coefficient of V_A (CV_A), which is the square root of V_A divided by the phenotypic trait mean, multiplied by 100 **(D)** in female and male flour beetles (*Tribolium castaneum*) in four different environmental conditions.

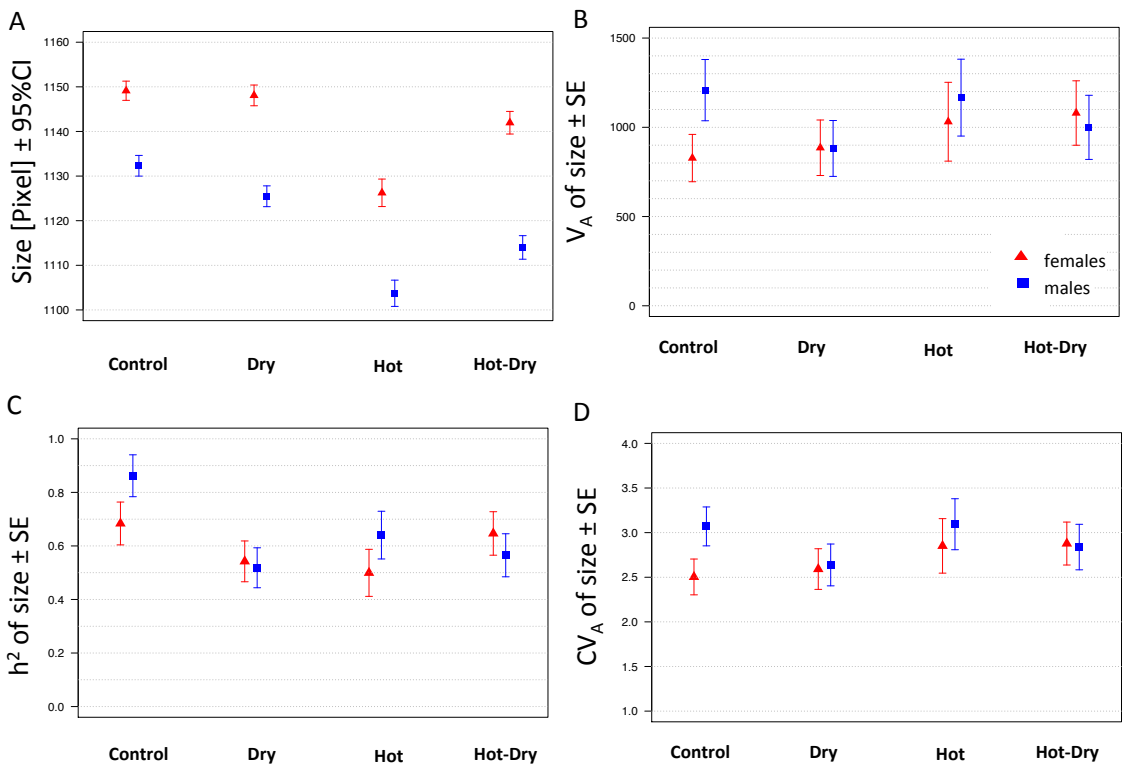

Figure 4: Size (centroid size of abdominal segment IV) **(A)** and estimates for its additive genetic variance (V_A) **(B),** heritability (h^2) **(C)**, and coefficient of V_A (CV_A) **(D)** in female and male flour beetles (*Tribolium castaneum*) in four different environmental conditions.

Genetic variances and covariances

Fitness

V_M, which consisted of variance due to maternal effects, common environment and non-additive effects, was not significant in any condition. We found significant V_A for female fitness in all conditions (Table 1). V_A for males was generally smaller (P = 8.10E-03) and not significant in Control and Hot. A remarkable exception was Hot-Dry, where male V_A was increased and of the same magnitude as female V_A (Figure 5). V_A in females was similar in Control and Dry but increased in Hot and was reduced in Hot-Dry (Figure 3B). Since residual variances changed simultaneously with V_A (Table 1), h^2 for females was similar in all stress conditions (Figure 3C). When we compared genetic variances using standardized data we found h^2 of males (P = 2.75E-03), but not females (P = 0.55) to differ significantly between conditions. Similarly, I_A was affected by conditions in males (P = 8.87E-05), but not in females (P = 0.07). When we tested a model constraining V_A of females and males to be the same in all conditions, we found it to be significantly worse (P = 2.46E-08) than an unconstrained model, thus showing that the total amount of heritable genetic variance (V_A of females and males) was different between treatments.

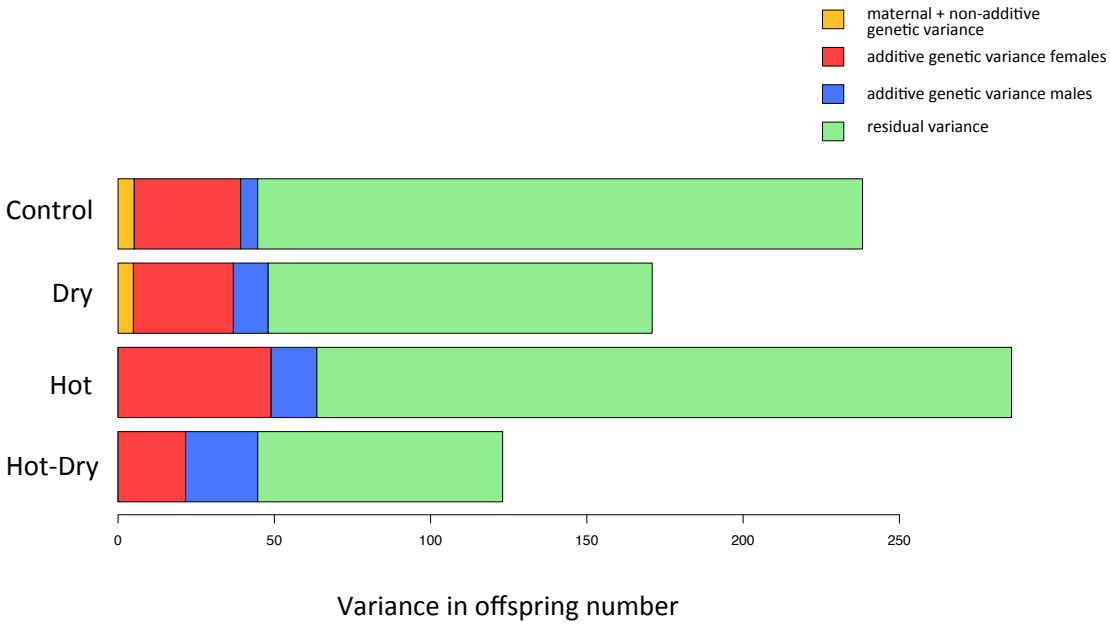

Figure 5: Variance components of offspring number of *Tribolium castaneum* in four different environmental conditions estimated using univariate animal models.

Table 1: Genetic variances (V_A: additive genetic variance; V_M: maternal variance; V_R: residual variance), heritability (h^2), coefficient of additive genetic variance (CV_A) of offspring number and cross-sex additive genetic covariances (COV_A) and correlations (r_A) in different environmental conditions. Estimates for genetic variances were obtained from a univariate animal model including additive genetic effects of males and females as random effects. For COV_A and r_A univariate animal models were used with covariance between female and additive genetic effects. N gives the number of reproducing females used for the analysis. All significant results are in bold.

	Condition			
	Control	**Dry**	**Hot**	**Hot-Dry**
N	1514	1603	1005	1396
mean (SE)	49.09 (0.4)	43.88 (0.36)	33.18 (0.55)	25.73 (0.31)
V_A females (SE)	**34.06 (18.75)**	**32.00 (14.71)**	**48.99 (20.02)**	**21.62 (7.71)**
h^2 females (SE)	**0.14 (0.08)**	**0.19 (0.09)**	**0.17 (0.07)**	**0.18 (0.06)**
CV_A females (SE)	**11.89 (3.27)**	**12.89 (2.97)**	**21.10 (4.32)**	**18.07 (3.23)**
V_A males (SE)	5.44 (7.29)	**11.14 (5.35)**	14.62 (12.59)	**23.09 (5.40)**
h^2 male (SE)	0.02 (0.03)	**0.07 (0.03)**	0.05 (0.04)	**0.19 (0.04)**
CV_A males (SE)	4.75 (3.18)	**7.61 (1.83)**	11.53 (4.97)	**18.68 (2.20)**
V_M (SE)	5.18 (10.30)	4.90 (7.55)	2.26E-07 (5.03E-0.5)	2.76E-05 (2.21E-06)
V_R (SE)	193.39 (13.65)	122.88 (9.79)	222.27 (20.00)	78.35 (6.27)
V_P (SE)	238.22 (8.88)	170.91 (6.33)	235.88 (13.10)	123.05 (4.96)
$COV_{A\ female,male}$ (SE)	**25.73 (6.29)**	**11.75 (4.92)**	-9.24 (11.02)	**11.54 (4.21)**
$r_{A\ female,male}$ (SE)	**1.55 (0.66)**	**0.54 (0.22)**	-0.36 (0.45)	**0.52 (0.18)**

Genetic correlations between fitness in different conditions were always positive (Figure 6A) and significant. However, we found significant differences between the different cross-condition correlations (P = 0.05). Correlations were slightly lower when the conditions differed in temperature and humidity (Control–Hot-Dry, Dry-Hot) (Figure 6). We found significant G x E between Control and Dry, Control and Hot-Dry as well as Dry and Hot.

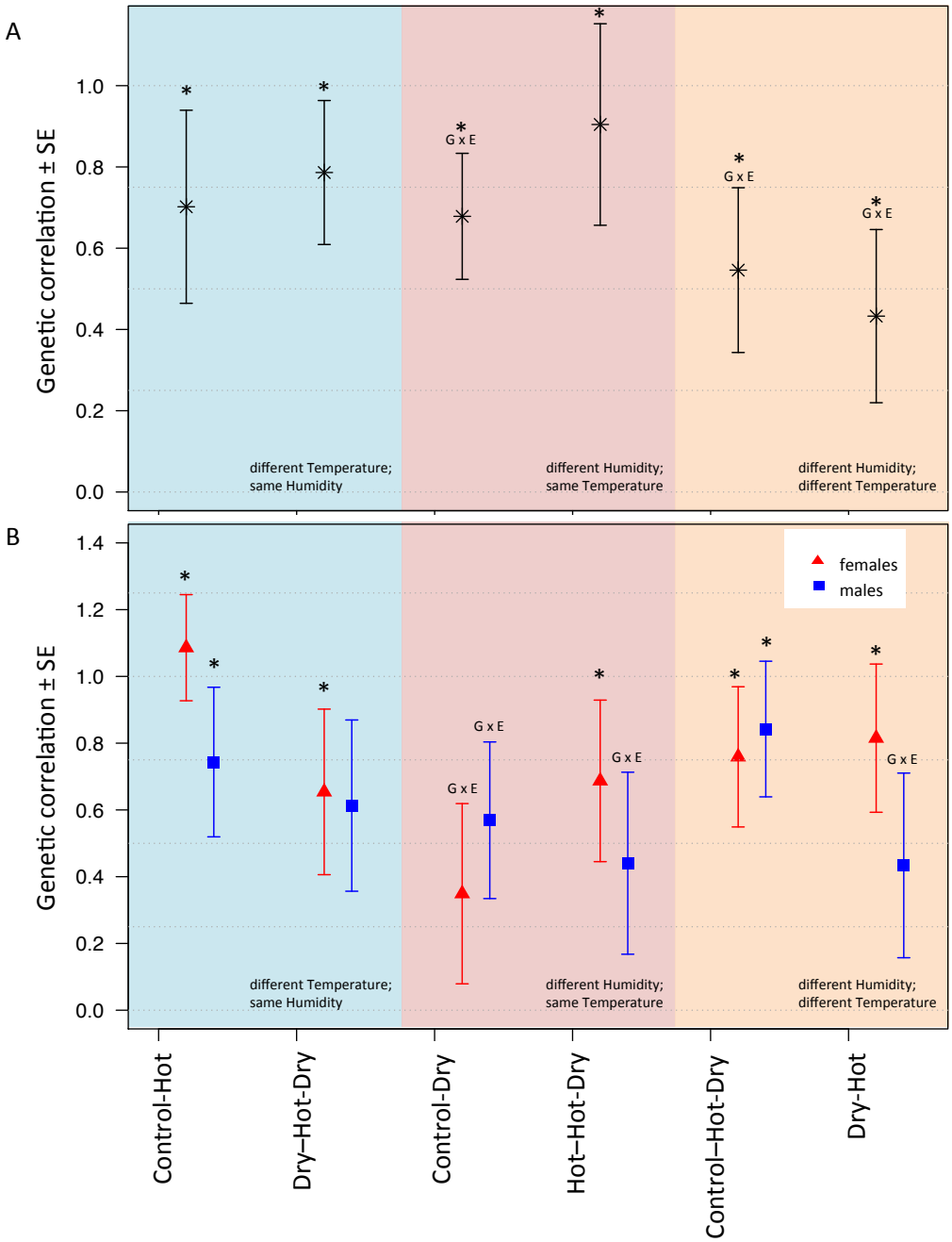

Figure 6: Pairwise cross-environment genetic correlations of fitness **(A)** and size **(B)** in *Tribolium castaneum* estimated using bivariate animal models. * show significant correlations, i.e. significantly different from zero. G x E (= genotype by environment interactions) indicate correlations significantly different from one. Background colours illustrate how the conditions differ. Blue: different temperature; red: different relative humidity; orange: different in temperature and humidity. Control: 33°C, 70% relative humidity (r.h.); Dry: 33°C, 30% r.h.; Hot: 37°C, 70% r.h.; Hot-Dry: 37°C, 30% r.h.

Genetic correlations in fitness between females and males (Figure 7) were significant in Control (P = 1.02E-06), Dry (P = 1.34E-02), and Hot-Dry (P=4.47E-04), but not in Hot (P=0.10). The correlation was highest in Control (Table 1, Figure 7). It decreased in the stress treatments and became even negative in Hot. In all conditions except Control, we found that the correlation was significantly different from one (Dry: P=1.95E-03; Hot: P= 5.78E-03; Hot-Dry: P=1.26E-04), indicating G x S, i.e. that genetic basis of fitness is different in the sexes. I_A, which gives the expected evolutionary change as percentage of the mean, was highest in Hot and smallest in Control (females/males ± SE: Control: 1.59 ± 0.49/0.30 ± 0.29; Dry: 2.05 ± 0.50/0.63 ± 0.27; Hot: 4.32 ± 1.81/1.25 ± 1.14; Hot-Dry: 3.20 ± 1.14/ 3.51 ± 8.1). Based on this, we should observe adaptation in all stress treatments with the largest relative fitness increase in Hot. However, given the negative correlation between male and female additive genetic effects (Figure 7, Table 1), the evolutionary response may be constrained and less than predicted. To demonstrate how responses are affected by covariances between the sexes we used estimated variances and covariances between male and female additive genetic effects of relative fitness to predict fitness increase after one generation. We applied the multivariate breeder's equation $\Delta z = G\beta$ (Lande & Arnold, 1983), where Δz is a vector of changes in the trait means, G the 2 x 2 genetic variance-covariance matrix for female and male relative fitness estimated in each condition, and β a vector of selection gradients. We used a vector of selection gradients that assumed equal selection on male and female fitness, β^T=[1,1]. Total change in fitness is then the sum of fitness increase in females and in males. Alternatively, we may consider male additive genetic effects as indirect genetic effects on female reproductive output. Joint effects of direct and indirect selection on evolutionary changes of a single trait can be calculated as described in Bijma & Wade (2008) (equation 14): $\sigma_T^2 = \sigma_{A_D}^2 + 2(n-1)\sigma_{A_{DI}}^2 + (n-1)^2\sigma_{A_I}^2$, where σ_T^2 is the total heritable variance, $\sigma_{A_D}^2$ additive genetic variance for direct effects (female V_A), $\sigma_{A_I}^2$ additive genetic variance for indirect effects (male V_A), and n gives the number of interacting individuals (here, it equals two). In case of relative fitness, this total heritable variance is equal to the predicted relative increase in fitness (Fisher, 1930). Thus, considering male and female fitness as two separate traits and applying the multivariate breeder's equation, or treating additive genetic effects of males as indirect genetic effects on female fitness is equivalent. This gave us an estimated increase for fitness of 3.90% in Dry, 3.89% in Hot, and 10.20% in Hot-Dry after one generation. Assuming V_A remains constant, the upper limit of fitness increase after 19 generations (generation 20) would be 106.81% in Dry, 106.57% in Hot and 532.54% in Hot-Dry.

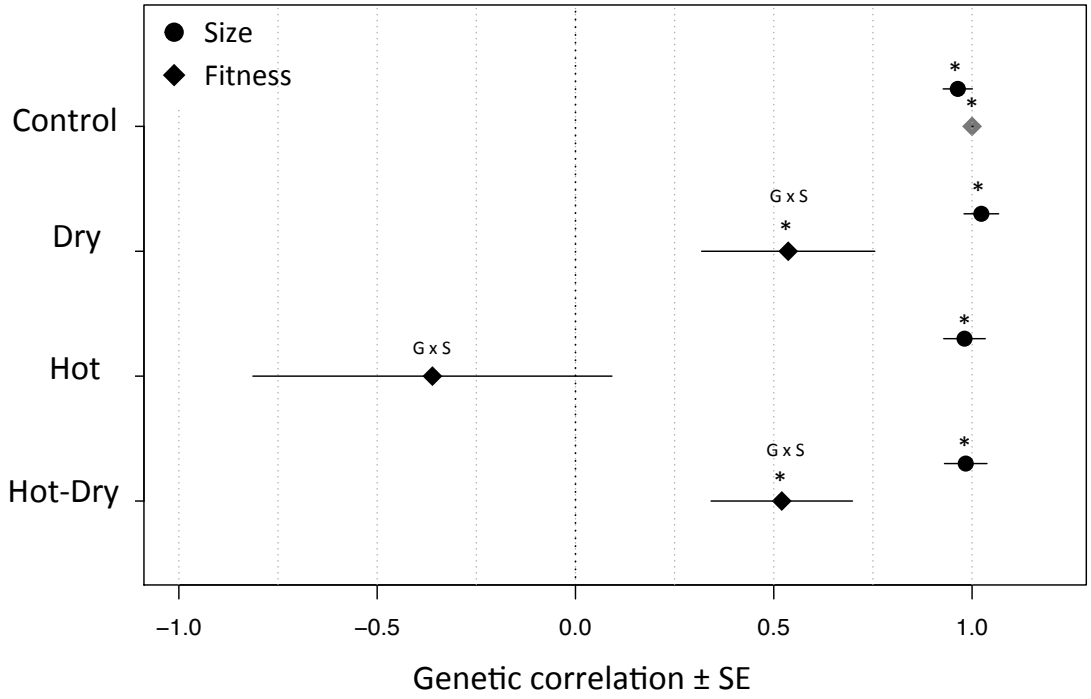

Figure 7: Cross-sex genetic correlations for size and fitness in *Tribolium castaneum* under different environmental conditions. * indicates a significant correlation. G x S (= genotype by sex interaction) indicates that correlation is significantly different from one. Estimate for genetic correlation of fitness in Control was bounded at one and no SE was available. Using models with unconstrained variances and covariances yielded an estimate of 1.55 ± 0.66. V_A of males was small in Control, which impedes precise estimation of cross-sex genetic correlation.

Size

V_A in size was highly significant in both sexes and in all conditions (P < 0.001). The results for V_D were less clear. In Control and Hot-Dry estimates for V_D were extremely small and estimates bounded at zero, indicating that non-additive effects contributed little to observed variation (Figure 8). In Dry and Hot, V_D was not significant, but variance estimates were high when it was included in the model (Table 2, Figure 8). A model without non-additive effects resulted in much higher estimates of V_A in Dry and Hot (Supplemental Table S1.1). In most cases, p-values for V_D were far from significance (P > 0.2), but for females in Dry and in Hot p-values were P = 0.13 and P = 0.10. When we combined males and females and added sex as fixed effect in the model, we obtained a P-value of 0.075 in Hot. Genetic correlations for size across conditions were always positive (Figure 7), but no clear pattern emerged. We found significant G x E in female size between Control-Dry (P = 2.78E-03). In male size, G x E was significant between Control-Dry (P = 6.39E-03), Dry-Hot (P = 5.39E-03), Dry–Hot-Dry (P = 0.04), and Hot–Hot-Dry (P = 3.93E-03). Genetic correlations between female and male size were close to one (Table 2, Figure 7) in all conditions, suggesting that size cannot evolve independently in both sexes. I_A of female and male size was not significantly different (P = 0.08) considering all conditions. Although differences in Control seemed to be substantial (Figure 4B-D), they were not significant (h^2: P=0.26; I_A: P=0.06). We found that environmental change did not influence I_A and h^2 of female size (P = 0.13; P = 0.14) and male I_A (P = 0.13), but had a significant effect on male h^2 (P = 0.02).

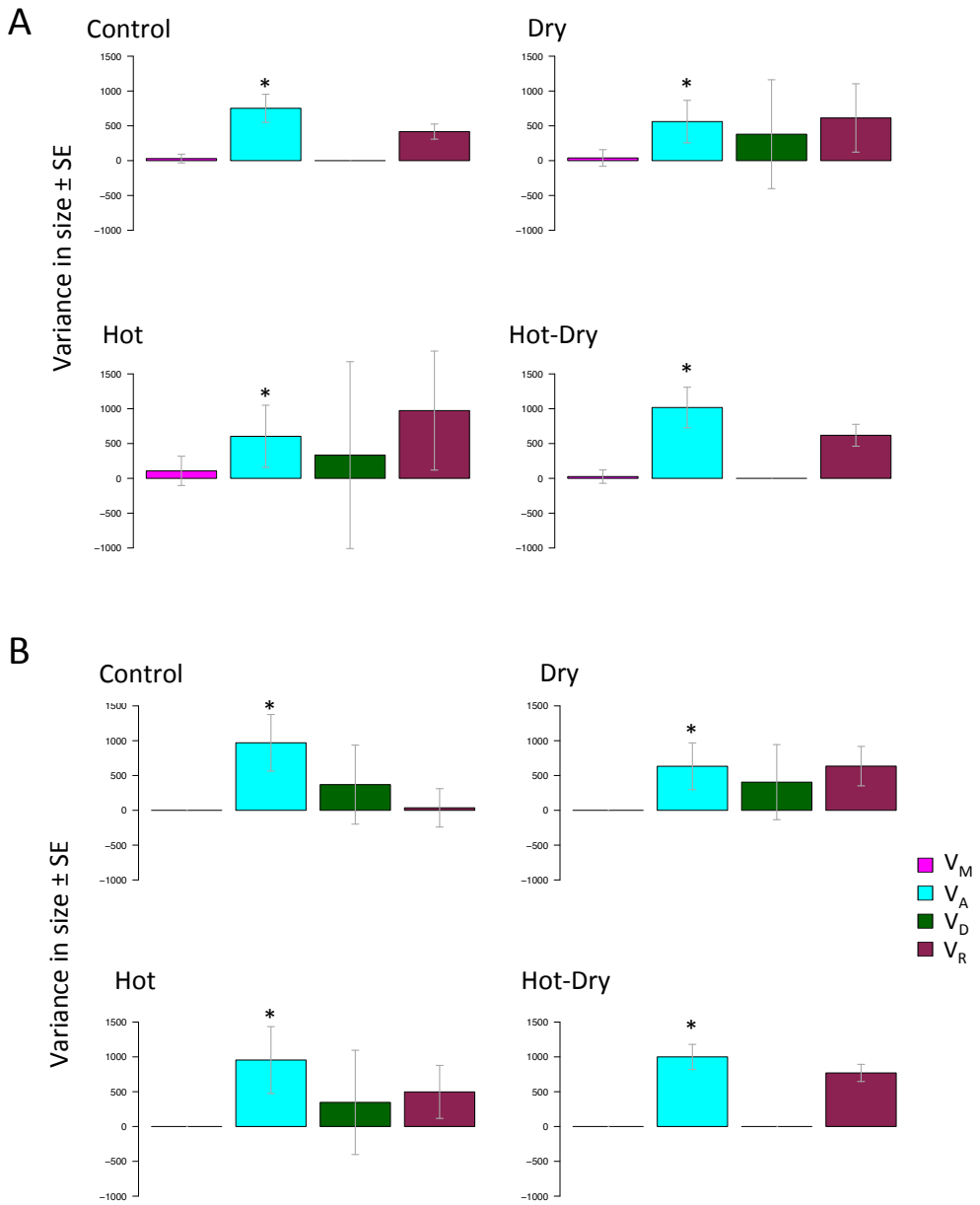

Figure 8: Variance components of size in female **(A)** and male **(B)** flour beetles (*Tribolium castaneum*) under four environmental conditions: V_M: maternal variance, V_A: additive genetic variance, V_D: non-additive genetic variance, V_R: residual variance. * indicates a significant variance component.

Table 2: Genetic variances (V_A: additive genetic variance; V_D: dominance variance; V_M: maternal variance; V_R: residual variance), heritability (h^2), coefficient of additive genetic variance (CV_A) of size in female and male flour beetles and cross-sex additive genetic covariances (COV_A) and correlations (r_A) in different environmental conditions. Estimates for

genetic variances were obtained from univariate animal models for each sex separately. For COV_A and r_A bivariate animal models were used. All significant results are in bold.

		Condition							
		Control		Dry		Hot		Hot-Dry	
Females	**N**	1020		1181		837		960	
	mean (SE)	1149.13	(1.10)	1148.08	(1.18)	1126.25	(1.57)	1141.96	(1.29)
	V_A (SE)	**751.79**	**(201.63)**	**560.98**	**(305.17)**	**603.78**	**(446.35)**	**1017.45**	**(291.52)**
	V_D (SE)	6.63E-04	(1.73E-04)	379.526	(782.07)	333.554	1342.287	8.670E-04	(2.21E-04)
	V_M (SE)	29.61	(63.13)	38.57	(119.09)	107.14	(210.86)	25.58	(96.18)
	V_R (SE)	417.11	(108.70)	613.14	(490.95)	973.23	(854.49)	618.81	(157.41)
	V_P (SE)	1198.52	(72.27)	1592.19	(82.03)	2017.74	(117.05)	1661.84	(100.31)
	h^2 (SE)	**0.63**	**(0.14)**	**0.35**	**(0.18)**	**0.30**	**(0.21)**	**0.61**	**(0.15**
	CV_A (SE)	**2.39**	**(0.32)**	**2.06**	**(0.56)**	**2.18**	**(0.81)**	**2.79**	**(0.40)**
Males	**N**	1008		1183		834		962	
	mean (SE)	1132.31	(1.18)	1125.47	(1.19)	1103.73	(1.50)	1114.01	(1.34)
	V_A (SE)	**969.79**	**(406.67)**	**632.66**	**(335.68)**	**954.83**	**(479.70)**	**999.70**	**(179.66)**
	V_D (SE)	369.18	(567.38)	404.65	(539.19)	346.49	(748.68)	4.94E-03	(7.90E-04)
	V_M (SE)	6.59E-06	(5.07E-05)	4.88E-04	(2.18E-04)	6.54E-04	(5.00E-04)	3.14E-04	(5.02E-05)
	V_R (SE)	35.85	(275.83)	634.64	(282.89)	496.90	(380.49)	769.42	(123.08)
	V_P (SE)	1374.81	(293.93)	1672.04	(87.39)	798.22	(118.96)	1769	(98.50)
	h^2 (SE)	**0.71**	**(0.25)**	**0.38**	**(0.19)**	**0.53**	**(0.25)**	**0.57**	**(0.08)**
	CV_A (SE)	**2.75**	**(0.58)**	**2.23**	**(0.59)**	**2.80**	**(0.70)**	**2.84**	**(0.26)**
	$COV_{A\ female,male}$ (SE)	**955.25**	**(120.93)**	**1002.06**	**(128.10)**	**1140.11**	**(175.33)**	**976.20**	**(137.10)**
	$r_{A\ female,male}$ (SE)	**0.96**	**(0.04)**	**1.02**	**(0.04)**	**0.98**	**(0.05)**	**0.98**	**(0.05)**

Adaptation after 20 generations and asymmetric correlated responses

We found significant adaptation to all conditions after 20 generations, shown by significant effects of selection regime on offspring number (Dry: $F_{1,14} = 9.20$, $p = 0.009$; Hot: $F_{1,16} = 4.78$, $p = 0.044$; Hot-Dry: $F_{1,16} = 23.51$, $p = 1.786E-04$). In all treatments, the native selection lines produced significantly more offspring than non-adapted control lines (Figure 9). The largest difference and most significant fitness increase was observed in the most stressful condition Hot-Dry. In contrast to the three stress treatments, we did not find any differences between lines from different selection regimes in the ancestral control condition (Figure 9), showing that adaptation to stress treatments did not come at a cost of reduced fitness in control conditions. Interestingly, drought selection resulted in higher heat resistance. Dry lines showed a significantly higher offspring number in Hot and Hot-Dry (estimated increase in offspring number relative to Control lines: 14.22 ± 2.98, $p = 6.68E-06$ in Hot; 12.68 ± 2.92, $p = 3.70E-05$ in Hot-Dry). In Hot, they performed even better than the native Hot-lines (mean offspring number in Hot [95% CI]: Control-lines: 24.59 [20.71, 28.47]; Hot-lines: 29.94 [26.11, 33.77]; Dry-lines: 35.69 [31.77, 39.61]; Hot-Dry-lines: 36.91 [33.14,40.68]). We did not observe such a correlated response in the Hot-lines. Their offspring number in Dry was not different from those of

Control-Lines. Lines in Hot-Dry that adapted to a combination of heat and drought showed an increased fitness in both single stressor treatments (in Dry: 5.31 ± 2.76, p = 0.06; in Hot: 11.37 ± 2.83, p = 1.20E-04). Despite high genetic correlation in fitness between Hot and Hot-Dry estimated in the first generation, we found no correlated response of Hot-lines to Hot-Dry conditions. Their offspring number was not different from non-adapted Control-Lines (Hot-lines: 23.14 [19.43, 26.84 CI]; Control-lines: 23.03 [19.30, 26.76 CI]).

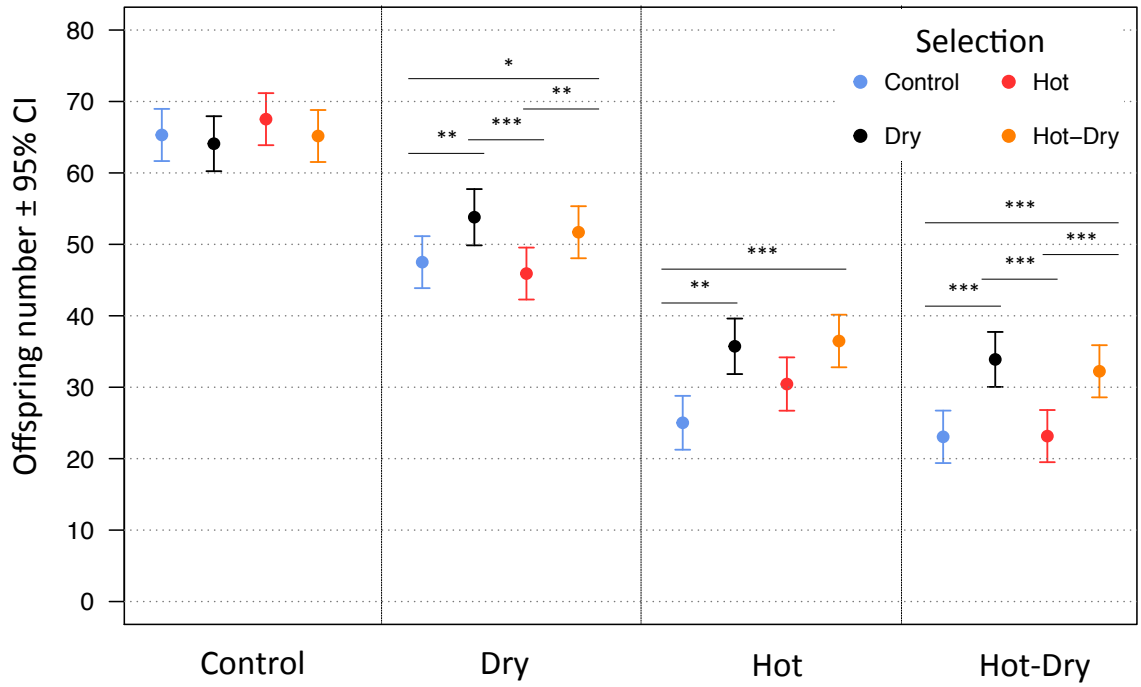

Figure 9: Mean offspring number per female of lines from different selection regimes under different environmental conditions. Significant differences between selection regimes within the same condition (Tukey's HSD post hoc test, p-values adjusted for multiple comparisons) are indicated by *P<0.05, **P<0.01, ***P<0.001.

The observed changes in fitness after experimental evolution were much lower than the estimated upper limit. Observed fitness increases relative to Control-lines were: Dry: 16.43 ± 3.24 %, Hot: 22.37 ± 4.34 % and 46.29 ± 2.17 % in Hot-Dry. Nonetheless, in agreement with our predictions we observed the strongest increase in Hot-Dry and similar increases in Dry and Hot. Although it is not possible to predict the precise fitness increase over the long time period of 20 generations, we can make some qualitative predictions about long-term adaptation based on V_A estimates. We can examine whether a population is likely to adapt and make relative comparisons between stress treatments, i.e. identify those where we expect the largest relative increase in fitness.

Genetic correlation between fitness and size

Unfortunately, it was not possible to get unbiased estimates for genetic correlations between these two traits. In principle it is possible to estimate genetic correlations between fitness and size with our data set. However, we could not rule out a strong confounding effect with density. Size was measured in the F2 (Figure 1B), the offspring of beetles used for the fitness assay. Consequently, offspring of females with a high fitness (i.e. high offspring number) grew up at a higher density since we used identical tubes with the same amount of flour for all females in the fitness assay. Genetic correlations were not significant in any condition. With the exception of Control we found positive genetic correlations (Supplemental Table S1.2).

Discussion

Evolutionary potential and adaptation

Consequences of environmental change on persistence of populations strongly depend on their ability to adapt either by plastic or genetic changes. The stress conditions we applied had a strong impact on fitness. Offspring number in Hot-Dry was reduced to ca. 50% of control level thus clearly showing that plasticity was not able to fully compensate negative effects of environmental change. We found significant V_A in fitness in all conditions indicating potential to adapt by genetic changes. We could not find evidence that environmental changes lowered adaptive potential. I_A of female and male fitness showed an increase in the stress treatments, which should facilitate adaptation. In accordance with that we found significant adaptation to all conditions after 20 generations of experimental evolution.

V_A of female fitness was higher than V_A of male fitness in all conditions except Hot-Dry. Egg production is likely to be costly and reduced when females are stressed due to a resource allocation trade-off. It is therefore not surprising that variation in reproductive output could be mainly explained by genetic differences among females, whereas genetic variation among males had only a minor influence. However, this changed when humidity was reduced. In Dry V_A for males became significant (but was still much smaller compared to females) and in Hot-Dry V_A in both sexes showed the same amount. It was observed that condition of *Tribolium* males could have a significant influence on reproductive output of their mating partner. Starvation of males reduced insemination success (Lewis *et al.*, 2012), the number of eggs laid, and the proportion of unfertilized eggs (Sbilordo *et al.*, 2011). It seems plausible that desiccation might have similar effects. Producing ejaculate may be costly and decreased under dry conditions to reduce water loss. Genetic differences how well males are able to cope with drought would then be translated into observed offspring variation resulting in a higher male influence on reproductive output. Although male V_A was small in ancestral conditions, evolution of males seemed to play an important role in adapting to Hot-Dry. The predicted fitness increase was much higher when male V_A was taken into account. This matched our result after experimental evolution showing a much higher relative fitness increase in Hot-Dry compared to single stress treatments. Ignoring male effects when studying adaptive capacity of a population can lead to misleading predictions.

Positive cross-condition genetic correlations indicate an absence of an evolutionary trade-off between drought and heat adaptation. Consistent with that, we did not find any selection line performing worse than control lines in any condition after experimental evolution. In contrast, positive correlations indicate a similar genetic basis for fitness under different conditions. Adapting to one condition should result in a correlated response and increase fitness in other conditions. We found that drought adaptation improved heat resistance (Figure 9). This correlated response was asymmetric since we did not observe an effect of heat adaptation on drought resistance. A possible explanation is that selection in dry and hot conditions shifted allele frequencies of genes with different pleiotropic effects (Bohren *et al.*, 1966). Alternatively, the pleiotropic degree of a gene might be environment dependent (Barrett *et al.*, 2009). Interestingly, we found that genetic correlations in generation one were lower when the conditions differed in both environmental variables, temperature and humidity, which is the case in Control–Hot-Dry and Dry-Hot (Figure 7) suggesting that genetic basis for adaptation to heat and drought might be slightly different.

Several studies in wild (Brommer *et al.*, 2007; Foerster *et al.*, 2007; Mokkonen *et al.*, 2011) as well as laboratory populations (Delcourt *et al.*, 2009; Punzalan *et al.*, 2014) reported negative genetic correlations between female and male fitness. It is not well understood how they are influenced by the environment and contrasting predictions have been made. It was argued that in a population far from the optimum after an environmental shift females and males might experience similar directional selection leading to high positive correlation in their fitness (Berger *et al.*, 2014; Connallon & Hall, 2016; Wolak *et al.*, 2018). Contrary to that,

genetic covariances between sexes were predicted to be less negative in ancestral conditions (Delcourt *et al.*, 2009), because selection on the long-term should favour alleles providing fitness benefits to both (Collet *et al.*, 2016). Consistent with that prediction we found the highest cross-sex genetic correlation in fitness in Control and lower estimates in the treatments with a negative correlation in Hot. Taken the covariances between female and male fitness into account led us to predict the highest relative increase in fitness in Hot-Dry and similar increases in Dry and Hot. The results of the fitness assay after 20 generation mainly matched these expectations but increases in fitness were much lower than the estimated upper limit. However, these predictions are based on the assumption of constant V_A and consequently exponentially increasing fitness (Falconer & MacKay, 1996). Trade-offs between different fitness components are expected to occur, physiological limits, e.g. for egg laying rate, exist and should prevent an infinite fitness increase. It is also important to note that the conditions during the fitness assay were not exactly the same as during evolution. In order to be able to get offspring numbers of each individual, female beetles were kept separately with one female per tube. In contrast, all individuals of the same line were kept in one vial during experimental evolution. Effects of density and competition that might have influenced adaptation were not captured in our fitness assay. Our results after 19 generations might be further compromised by evolution of control lines that were used as reference representing the ancestral non-evolved stage. Significant V_A under control conditions in addition to high and positive genetic correlations between fitness in control and stress conditions suggest a fitness increase in control lines over time and correlated responses in the treatments. This might have led to an underestimation of the true fitness increase of adapted selection lines. Interestingly, we found no difference between lines from different selection regimes in Control. A reason might be that genes responsible for fitness in the treatments are not expressed under control conditions. Evolutionary changes in these genes that occurred during adaptation to the treatments would not show any effect on fitness under control conditions leading to cryptic genetic variation. In control lines these genes were not exposed to selection and consequently control lines showed a lower fitness compared to adapted selection line when exposed to the treatments.

Genetic variances of fitness and size and impact of environmental change

Both traits differed considerably in the proportion of different variance components as well as in the environmental effect on genetic variances. h^2 was much higher in size than in fitness. This was a common finding in many studies (Roff & Mousseau, 1987; Kruuk *et al.*, 2000; McCleery *et al.*, 2004; Teplitsky *et al.*, 2009) and had been initially interpreted as evidence for strong selection depleting V_A. However, often high V_A exists but is concealed at the h^2 level by a simultaneous increase in the total variance (Houle, 1992; Merilä & Sheldon, 1999; Hansen *et al.*, 2011; Wheelwright *et al.*, 2014). Given the highly polygenic nature of fitness it was even argued that fitness might show higher V_A (Merilä & Sheldon, 1999) since it represents a large mutational target. Furthermore, fitness is a composite character with a high number of morphological, physiological and behavioral traits contributing to it, each of them with some underlying genetic variance. However, each of the traits contributing to fitness might be influenced by environmental factors leading to a high total variance in fitness (Price & Schulter, 1991) and thus a lower h^2. Our results fit well to these previous observations. When V_A was scaled by the mean (CV_A, I_A) we found much higher estimates in offspring number compared to size. Lower h^2 was mainly due to a higher proportion of V_R. Related to the expectation of low V_A in fitness traits was the prediction of an increased proportion of V_D since it is not affected by natural selection (Crnokrak & Roff, 1995; Merilä & Sheldon, 1999; Roff & Emerson, 2006; Sztepanacz & Blows, 2015). Unfortunately, it was not possible to disentangle V_D from V_M and common environmental effects in our half-sib breeding design that we used for the fitness assay. However, if V_D is present, it should be included in V_M, because it contributes to full-sib resemblance and these share the same mother. V_M was low or zero in all conditions (Figure 5), but higher in Control and Dry. Our *Tribolium* lines should be adapted to Control since they had lived there for ca. 20 generations and the species is known to be well adapted to drought (Sokoloff, 1972; Park & Beeman, 2008; King & Denholm, 2014). This observation

might thus be consistent with the idea of selection depleting V_A and increasing V_D. Our V_D estimates for size were always associated with large SE. However, our results suggest that V_D might be present and that the amount is environment dependent. In male as well as female size we obtained the highest estimates in Dry and Hot. This might have important implications since it means that effects of inbreeding could be increased by environmental changes. Environmental changes might affect inbreeding depression not only by reducing survival and hence population size, but could also directly affect genetic variances. Environmental dependence of inbreeding depression was described before (Bijlsma *et al.*, 1999; Armbruster & Reed, 2005; Fox & Reed, 2011), but environment dependence of V_D has not been studied in detail. Comparative studies of the relative amount of V_A and V_D suggested that proportion of V_D can be substantial and of the same magnitude as V_A (Crnokrak & Roff, 1995; Wolak & Keller, 2014). However, estimates of V_D were surrounded by a high degree of uncertainty and might be biased by a confounding with maternal and common environmental effects. Although we used a breeding design suitable for estimating V_D and had a high sample size (number of family pairs where offspring were DFC: 827), we were not able to obtain significant estimates. The probability that DFC share alleles identical by descent at a given locus is only 6.25 %, resulting in large uncertainty, and consequently large SE when estimating V_D. Although a DFC breeding design was proposed as experimental setup for detecting non-additive genetic effects (Fairbairn & Roff, 2006), it might have limited statistical power. Comparing maternal half-sibs and full-sibs might be a much more powerful approach for detecting of V_D while controlling for maternal effects.

In contrast to the described differences between female and male fitness V_A, we did not find a clear pattern for body size and differences between sexes were not significant. We observed the largest difference Control. It is likely that female size had been under strong positive selection since it is associated with fecundity (Honěk, 1993). This might have led to the observed reduction in V_A in the ancestral control conditions relative to male V_A (but was only marginally significant, P=0.06). V_A of male fitness was significantly affected by the different environmental conditions. Effects on female fitness V_A were close to significance (P=0.07). Results for size seem to be similar: Mean and variance scaled estimates for females (h^2 and CV_A/I_A) remained constant, but h^2 of male size differed significantly between conditions. However, we observed an increase in V_A of fitness in the treatments, while V_A of size is reduced. Interestingly, the drought effect we observed in fitness was also detectable in size: When we explored effects of temperature and humidity on size we found a significant (although small) sex-treatment-interaction indicating that male size was more sensitive to drought. G x E for male size occurred when conditions differed in humidity.

Conclusions

Our study showed increased adaptive potential in stressful conditions and that this estimated potential was indeed associated with observed adaptation after 20 generations. Although precise predictions of relative increase in fitness were not possible over such a long time period, we could make qualitative prediction. We expected and observed the highest relative fitness increase in the most stressful hot-dry condition and similar increases in single stress treatments. The apparently high adaptive potential for female beetles in Hot was limited by a negative genetic correlation with male fitness. We further found that genetic effects of males on fitness can be large and can increase adaptive potential. Comparing genetic variances of fitness and size showed that they differed in their variance composition as well as cross-sex and cross-environment genetic covariances. The impact of the environment on genetic variance varied between the two traits. We advice caution if studies interested in fitness V_A and adaptive capacity of a population use size as a proxy. Our study also provides estimates of non-additive genetic variances in size. Dependent on the environment, its proportion might be substantial. Focusing on V_A only might therefore lead to biased estimates.

Acknowledgements

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