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Genetic variance in fitness and its cross-sex covariance predict adaptation during experimental evolution

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The additive genetic variation (V_A) of fitness in a population is of particular importance to quantify its adaptive potential and predict its response to rapid environmental change. Recent statistical advances in quantitative genetics and the use of new molecular tools have fostered great interest in estimating fitness V_A in wild populations. However, the value of V_A for fitness in predicting evolutionary changes over several generations remains mostly unknown. In our study, we addressed this question by combining classical quantitative genetics with experimental evolution in the model organism *Tribolium castaneum* (red flour beetle) in three new environmental conditions (Dry, Hot, Hot-Dry). We tested for potential constraints that might limit adaptation, including environmental and sex genetic antagonisms captured by negative genetic covariance between environments and female and male fitness, respectively. Observed fitness changes after 20 generations mainly matched our predictions. Given that body size is commonly used as a proxy for fitness, we also tested how this trait and its genetic variance (including nonadditive genetic variance) were impacted by environmental stress. In both traits, genetic 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.

KEY WORDS: **Evolvability, fundamental theorem of natural selection, heritability, nonadditive genetic effects, quantitative genetics.**

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. Species may adapt via plastic or genetic changes. Plastic changes, for example, physiological or behavioral adjustments, allow individuals to cope with stressful conditions (Charmantier et al. 2008). However, plastic responses are often costly and thus likely limited (Houle 1992; DeWitt et al. 1998; de Jong 2005; Valladares et al. 2007; Pfennig et al. 2010; Snell-Rood et al. 2010; Sokolova et al. 2012). It is also not clear whether they are sufficient to compensate negative effects of environmental changes (Duputié et al. 2015; Arnold et al. 2019). Instead, fast environmental shifts may require rapid adaptation by genetic evolution on short time scales. In this case, the standing genetic variation already present in the population is of particular importance (Kellermann et al. 2006; Bell and Gonzalez 2009). Adaptive evolution proceeds through different individual contributions to the next generation, that is, differences in their fitness. Importantly, this variation in individual fitness has to be at least partly due to some underlying genetic variation to change the genetic composition of a population. Usually, only variance due to additive genetic effects (V_A) is considered because additive effects are inherited from parents to offspring and determine the response to selection. V_A of relative fitness gives the expected increase of fitness in the next generation (Fisher 1930; Price 1972; Falconer and MacKay 1996). Thus, the existence of V_A in fitness is a prerequisite for adaptation and can be used as an overall estimate of a population's capacity to adapt, or its

evolvability (Houle 1992; Hansen et al. 2011; Shaw and Shaw 2014).

A common and intuitive expectation is that V_A in fitness should be low because selection depletes genetic variation in adapted populations. Accordingly, it was found that heritability (h^2, proportion of V_A in total variance V_P) was lower in traits more closely associated with fitness (Mousseau and Roff 1987; Kruuk et al. 2000; Merilä and Sheldon 2000; Teplitsky et al. 2009; Wheelwright et al. 2014). However, h^2 is not ideal for estimating V_A in fitness and the evolutionary potential of a population (Hansen et al. 2011; Wheelwright et al. 2014; Morrissey and Bonnet 2019) because low h^2 is often due to higher environmental variance (Schluter et al. 1991; Merilä and Sheldon 1999). Many populations may also not be at an evolutionary equilibrium (Shaw and Shaw 2014) in environments that have been recently changed by human impacts (Fugère and Hendry 2018). Estimates of V_A in fitness are scarce, especially in natural populations and tend to show large heterogeneity in the estimates of V_A for lifetime fitness (e.g., in vertebrate populations: Kruuk et al. 2000; Merilä and Sheldon 2000; McCleery et al. 2004; McFarlane et al. 2014). Although it is predicted that some species might not have the evolutionary potential to adapt to shifting environmental conditions (Etterson and Shaw 2001), some studies found substantial amounts of V_A in fitness in wild populations. Similarly, laboratory studies have reported significant V_A for fitness related traits in Drosophila *melanogaster* (Gardner et al. 2005; Fry 2008; Long et al. 2009).

Despite existing V_A in fitness traits, evolution to new conditions can be constrained by antagonistic pleiotropy if alleles influence several fitness components but with opposite effects. This leads to trade-offs because one component cannot be optimized without reducing the other, for example, fecundity and life length (Roff 2000). Another example of trade-offs is sexual antagonism (Foerster et al. 2007; Bonduriansky and Chenoweth 2009; Delcourt et al. 2009; Kirkpatrick 2009; Poissant et al. 2010; Calsbeek et al. 2015; Connallon and 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 revealed by negative genetic correlations between male and female fitness. Similar constraints may appear when adaptation to certain environmental conditions (e.g., elevated temperature) trades-off 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., resistance to one stress develops after exposure to another stress) were observed, and it is known that some stress responses rely on the same physiological mechanisms (Bubliy et al. 2012). Evolutionary adaptation to different stressors involving the same pathways may thus lead to correlated resistance to another stressor (Bubliy and Loeschcke 2005; Sikkink et al. 2015). However, examples of local adaptation suggest that selection may often favor different genotypes in different conditions (Hereford 2009). Immediately after exposure to an environmental change, genetic correlations between fitness in different conditions can inform us to what 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.

Changes in environmental conditions are also well known to affect genetic variances (Sgrò and Hoffmann 1998; Hoffmann and Merilä 1999; Rowiński and Rogell 2017), and covariances (Simons and Roff 1996; Sgrò and Hoffmann 2004; Wood and 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) because 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 and 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.

The fundamental importance of V_A of fitness for predicting contemporary evolution (Shaw and Etterson 2012; Hendry et al. 2018) and recent statistical advances in quantitative genetics have fostered great interest in estimating the adaptive capacity of wild populations (Charmantier et al. 2014). Even more so with the recent progress in using genomic markers to infer genetic resemblance among individuals (Gienapp et al. 2017; Perrier et al. 2018). These advances open up new perspectives for applications of classical quantitative genetic and genomic tools in wild populations, addressing important questions regarding populations' persistence under environmental change (Waldvogel et al. 2020). However, so far relatively little information exists about the predictive value of V_A for fitness over several generations. In our study, we addressed this question by combining classical quantitative genetics with experimental evolution in the model organism *Tribolium castaneum*. We used a two-generation half-sib/full-sib breeding design to estimate genetic variances of fitness traits in four different conditions (Control, Dry, Hot, and Hot-Dry) representing two often co-occurring stressors, heat and drought. We measured offspring number as estimate of fitness in the F1 generation, and body size in the F2 generation, as an additional trait, often used as fitness proxy. We evaluated adaptation to heat, drought and their combination after

20 generations of experimental evolution. Thus, our experimental setup allowed us to explore many different facets of adaptation and to ask how the adaptive potential changes under stressful conditions, whether trade-offs between female and male fitness can constrain adaptation, and by testing for genotype-by-environment interactions (G × E) to which extent resistance to different stressors shares a common genetic basis. Having obtained such estimates in the founder populations, we could then gain deeper insights into the process of adaptation. Linking experimental evolution and classical quantitative genetics proved to be a powerful approach to evaluate the predictive power of genetic variance for fitness and obtain a better understanding of the observed adaptation after 20 generations in new environments.

Materials and Methods

ANIMAL REARING AND STRESS TREATMENTS

We used the *T. castaneum* Cro1 strain (Milutinović et al. 2013). This strain originated from a wild population that was sampled in 2010 and was established using multiple individuals from 165 random mating pairs (Milutinović et al. 2013). It was kept at high population size (>10,000) and adapted to lab standard conditions (33°C, 70% relative humidity) for more than 30 generations. Beetles were kept in 24 h darkness on organic wheat flour mixed with 10% organic baker's yeast. We sterilized the flour and yeast by heating them for 12 h 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.

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 (Fig. S1A). Half-sib as well as full-sib families were split across all conditions (Fig. S1A). 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 a 15 mL tube with 1 g medium. The male was removed after 24 h and females transferred into two separate tubes. Females were removed from the tubes after one week of egg laying, and 9 g medium was added to provide food for the developing offspring. After five weeks, the number of adult offspring was counted. Although we conducted the matings for the fitness assay, we followed a specific crossing design and always crossed two pairs of full-sib families (Fig. S1B). Individuals resulting from these crosses (F2) were double first cousins (DFCs).

SIZE

Body size was measured in the F2, that is, in the offspring of beetles that were used for the fitness assay. To estimate body size, we used the centroid size of the abdominal segment IV as proxy for total size because it can be measured more accurately than dry weight in very small insects and shows a high correlation with body mass (Wickman and Karlsson 1989; Honěk 1993). We decided to use the size of the abdominal segment because we could measure it with higher accuracy than the total size, which strongly depends on whether an individual is perfectly stretched. The size of other parts of the body shows a high correlation with this segment (Fig. S3). 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 25× 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 lV (https://figshare.com/articles/_Morphology_of_the_red_flour_beetle_Tribolium_castaneum_/759706/1). 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 nonindependence because some of our measured individuals were full-sibs. For analyzing 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 R packages *lsmeans* 2.27-62 (Lenth 2016) and *lmertest* 3.0-1 (Kuznetsova et al. 2017).

ADAPTATION

We used 10 replicate lines per condition originating from the same ancestral population (Cro1) and let them adapt for 20 generations (Fig. S2). Each new generation was set up by randomly selecting 120 pupae and placing them into a new vial with 70 g medium. One Dry treatment selection line 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 (Fig. S2). 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 a 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 analyzed each condition separately. We used linear mixed models including selection regime as fixed effect and 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, that is, whether adaptation to a certain stress treatment could increase fitness in another. For this we run one analysis using the complete dataset. Selection regime, conditions, and their interaction were used as fixed effects, lines, families nested within lines, and line-treatment interaction as random effects. Reported effect sizes and SEs were obtained from the summary output of the model. *P*-values and confidence intervals were computed with the R packages *lmertest* (Kuznetsova et al. 2017) and *lsmeans* (Lenth 2016). Statistical analyses were conducted in R (R Core Team 2017). Model diagnostic plots are shown in Figure S6 and the complete results in Table S2.

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 (Kruuk 2004). Nonadditive 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). All models reached convergence.

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 nonadditive 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 because we tested whether a certain variance is different from zero and variances cannot become negative (Wilson et al. 2010). We analyzed the data assuming a Gaussian distribution. This is appropriate for count data when the distribution converges to a normal distribution (de Villemereuil 2018). The distribution of offspring numbers is shown in Fig. S4. Model diagnostic plots and more details are given in Figure S5. To estimate 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 because they were very small and nonsignificant. Studies so far used bivariate models to investigate cross-sex genetic correlations (Brommer et al. 2007; Foerster et al. 2007; McFarlane et al. 2014; Punzalan et al. 2014; Wolak et al. 2018). In case of fitness, this means that the same observation (the number of adult offspring resulting from a mating) would be used twice, once in each fitness estimate of the respective female and male parent. Using univariate models including additive genetic effects of females and males avoids pseudoreplication. To test for genotype-by-sex interaction (G × 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.

To estimate cross-condition genetic covariances and correlations, we used bivariate models with fitness in two conditions as response, batch as fixed effect and additive genetic effects of females and males as random effects. Because each individual was only measured in one condition, residual covariance was set to zero. Genetic correlations between traits measured in different environments that are significantly less than unity, indicate a G × E interactions (Kruuk 2004; Charmantier and Garant 2005). Comparison with a model with correlation fixed to one

was used to test for G × E (Wilson et al. 2010). In all bivariate models, covariance between male effects was set to zero because male effects were very small and not significant in some conditions. The significance of genetic correlations (cross-sex and cross-condition) was determined by two-tailed likelihood-ratio tests comparing a model including correlations to a model with correlations set to zero.

To allow comparisons across different conditions, we calculated h^2 and CV_A (CV_A is the 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. SEs 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 condition as separate response variable. Because 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 SD to have a variance of 1. 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 models are 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 Fig. S2) allowed us to estimate nonadditive 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, DFCs share nonadditive genetic effects without being confounded by maternal effects or a common environment. We partitioned the observed variance for size into additive (V_A), maternal (V_M), nonadditive (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 analyzed each condition separately using univariate animal models with batch as fixed and maternal, additive, and nonadditive genetic effects as random effects. To get sex-specific estimates, we analyzed male and female sizes separately. Genetic correlations between sexes and between conditions were assessed by bivariate models. Significance of correlations, G × E, environmental effects on I_A and h^2 of size were tested in the same way as we described before.

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 the fitness increase after one generation. We applied the multivariate breeder's equation $\Delta z = G\beta$ (Lande 1979), where Δz is a vector of changes in the trait means, G the 2 × 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, $\beta^T = [1,1]$. Total change in fitness is then the sum of fitness increase in females and in males. Alternatively, we can 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 and 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 2). 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.

Results

EFFECT OF TREATMENT

The effect of the treatments on offspring number was tested previously in Koch and Guillaume (2020) and shown to be highly significant. Heat had a stronger effect on offspring number than drought, whereas offspring number was lowest in the combined stress treatment Hot-Dry (Koch and Guillaume 2020) (Fig. 1A). In addition, we show here that body size decreased significantly in the treatments ($F_{3,7710} = 76.30$, $P < 2.20 \times 10^{-16}$, Table S1). Similar to offspring number, the effect of drought was smallest. In contrast, we observed the lowest size in Hot but not with combined stressors in Hot-Dry (Fig. 2A). The difference between males and females was significant ($F_{1,7759} = 518.19$, $P < 2.20 \times 10^{-16}$) with females being larger. We also detected a significant sex-by-condition interaction ($F_{3,7759} = 7.27$, $P = 8.16 \times 10^{-4}$) indicating that males were more sensitive to stressful conditions (Fig. 2A).

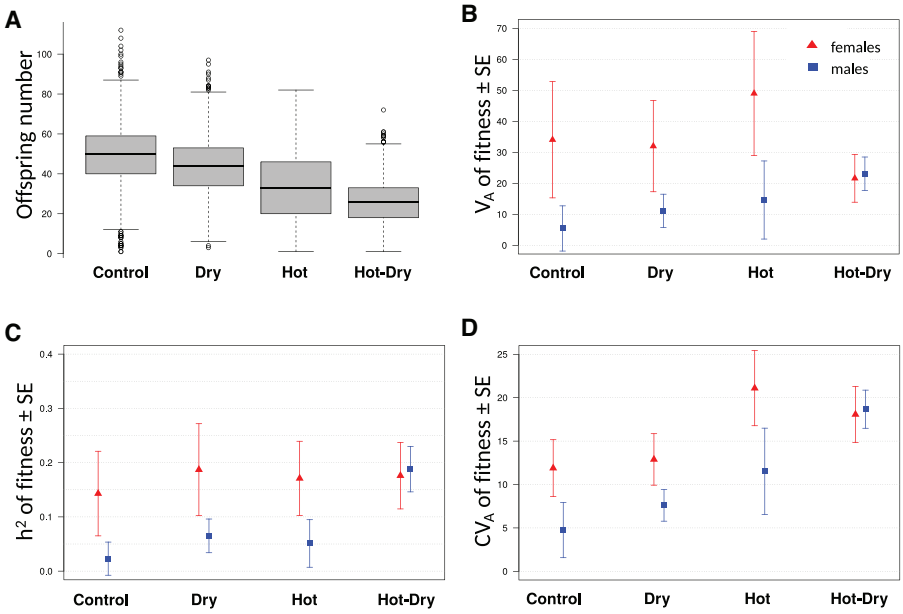

Figure 1. Fitness (=number of offspring reaching adulthood) (A) and estimates of its additive genetic variance (V_A) (B), heritability (h^2) (C), and coefficient of variation (CV_A), which is the square root of V_A divided by the mean offspring number, multiplied by 100 (D) in female and male flour beetles (*Tribolium castaneum*) in four different environmental conditions. Virgin females mated with one male. After mating they could lay eggs for one week and the resulting adult offspring was counted five weeks later. The conditions in the treatments were as follows: Control—33°C, 70% relative humidity; Dry—33°C, 30% r.h.; Hot—37°C, 70% r.h.; Hot-Dry—37°C, 30% r.h.

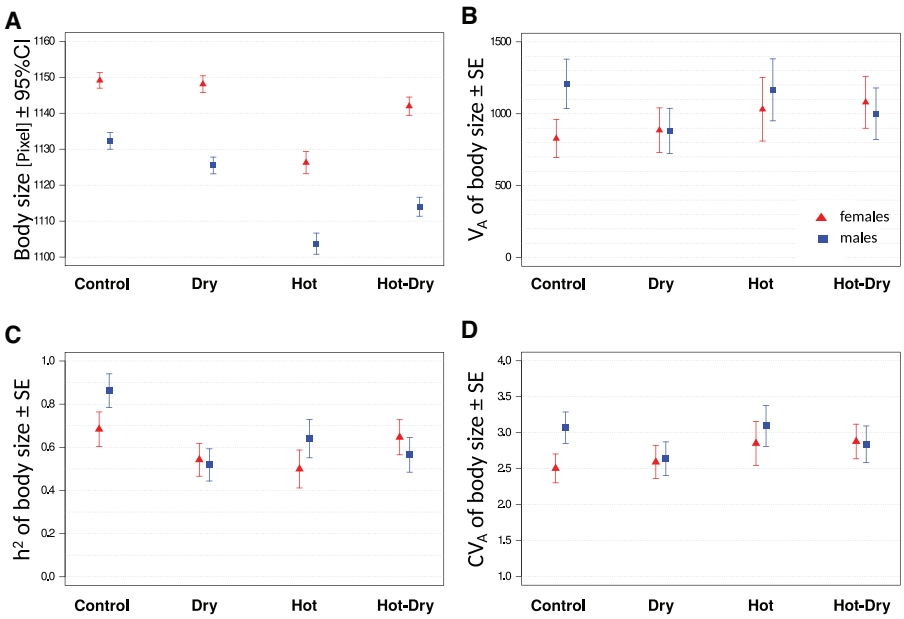

Figure 2. Body size proxy (centroid size of abdominal segment IV) (A) and estimates of its additive genetic variance (V_A) (B), heritability (h^2) (C), and coefficient of V_A (CV_A) (D) in female and male flour beetles (*Tribolium castaneum*) in four different environmental conditions. Conditions are as in Figure 1.

GENETIC VARIANCES AND COVARIANCES

Fitness

Overall, we found significant V_A of fitness, with significant differences between sexes and treatments, in most cases. V_M, the variance due to maternal, common environment, and nonadditive 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 ($P = 8.10 \times 10^{-3}$) and not

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), and I_A (V_A divided by trait mean squared, multiplied by 100) of offspring number and cross-sex additive genetic covariances (COV_A) and correlations (r_A) in different environmental conditions.

	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)**
I_A females (SE)	**1.59 (0.49)**	**2.05 (0.50)**	**4.32 (1.81)**	**3.20 (1.14)**
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)**
I_A males (SE)	0.30 (0.29)	**0.63 (0.27)**	1.25 (1.14)	**3.51 (0.81)**
V_M (SE)	5.18 (10.30)	4.90 (7.55)	2.26×10^{-7} (5.03×10^{-5})	2.76×10^{-5} (2.21×10^{-6})
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)**

***Note*: 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. The conditions in the treatments were as follows: Control: 33°C, 70% relative humidity; Dry: 33°C, 30% r.h.; Hot: 37°C, 70% r.h.; Hot-Dry: 37°C, 30% r.h.**

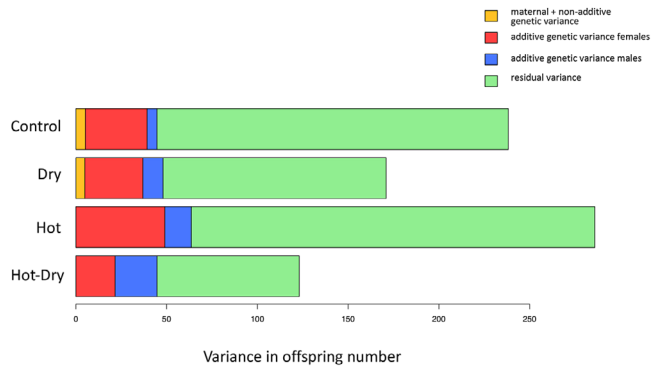

Figure 3. Variance components of offspring number of *Tribolium castaneum* in four different environmental conditions estimated using univariate animal models. Conditions are as in Figure 1.

significantly different from zero 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 (Fig. 3). V_A in females was similar in Control and Dry but increased in Hot and was reduced in Hot-Dry (Fig. 1B). Because residual variances changed simultaneously with V_A (Table 1), h^2 for females was similar in all stress conditions (Fig. 1C). When we compared genetic variances using standardized data, we found h^2 of males ($P = 2.75 \times 10^{-3}$), but not females ($P = 0.55$) to differ significantly between conditions. Similarly, I_A was affected by conditions in males ($P = 8.87 \times 10^{-5}$) 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 ($P = 2.46 \times 10^{-8}$) than an unconstrained model, thus showing that the total amount of heritable genetic variance (V_A of females and males) was different between treatments.

Genetic correlations between fitness in different conditions were always positive (Fig. 4) 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) (Fig. 4). We found significant G × E between Control and Dry, Control and Hot-Dry as well as Dry and Hot.

Genetic correlations in fitness between females and males (Fig. 5) were significant in Control ($P = 1.02 \times 10^{-6}$), Dry ($P = 1.34 \times 10^{-2}$), and Hot-Dry ($P = 4.47 \times 10^{-4}$) but not in Hot ($P = 0.10$). The correlation was highest in Control (Table 1, Fig. 5). 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: $P = 1.95 \times 10^{-3}$; Hot: $P = 5.78 \times 10^{-3}$; Hot-Dry: $P = 1.26 \times 10^{-4}$), indicating G × S, that is, genetic basis of fitness is different in the sexes. I_A, the expected evolutionary change as percentage

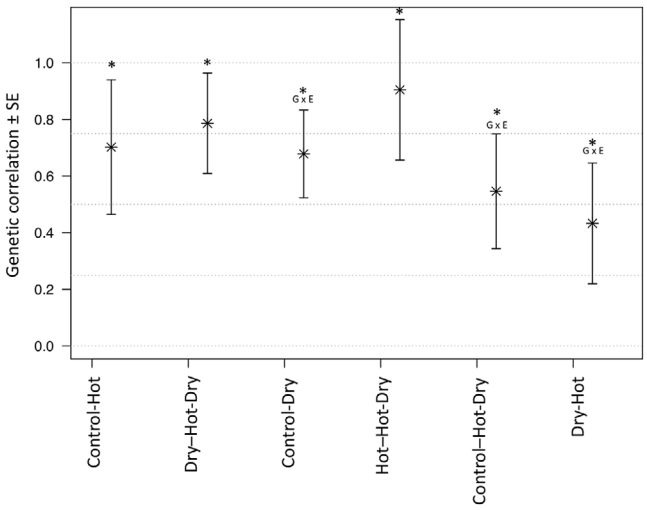

Figure 4. Pairwise cross-environment genetic correlations of fitness in *Tribolium castaneum* estimated using bivariate animal models. * shows correlations that are significantly different from zero. G × E (=genotype by environment interactions) indicate correlations significantly different from one. 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.

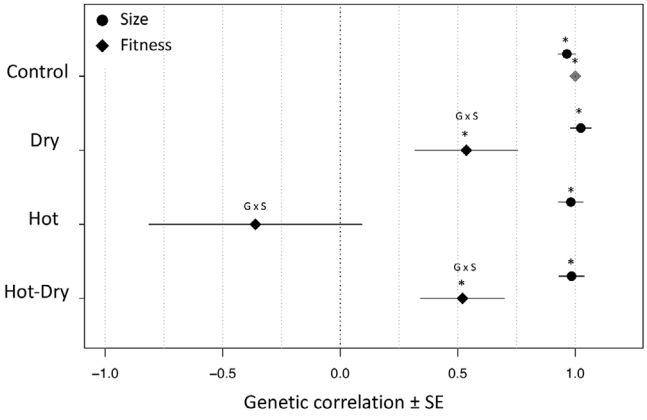

Figure 5. Cross-sex genetic correlations for size and fitness in *Tribolium castaneum* under different environmental conditions (conditions as in Fig. 1). * indicates a significant correlation. G × S (=genotype by sex interaction) indicates that correlation is significantly different from one. Estimate for genetic correlation of fitness in Control (gray diamond) 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.

of the mean, was the highest in Hot and the lowest in Control (Table 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 (Fig. 5, Table 1), the evolutionary response may be constrained and less than predicted. Taking female-male genetic correlations into account (see Methods) gave us an estimated increase of mean fitness of 3.90% in Dry, 3.89% in Hot, and 10.20% in Hot-Dry per generation. Assuming V_A remains constant, the total change of mean fitness after 19 generations (generation 20) would be 106.81% in Dry, 106.57% in Hot, and 532.54% in Hot-Dry.

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 nonadditive effects contributed little to observed variation (Fig. 6). In Dry and Hot, V_D was not significant, but variance estimates were high when it was included in the model (Table 2, Fig. 6). A model without nonadditive effects resulted in much higher estimates of V_A in Dry and Hot (Table S3). 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 lower although still not significant ($P = 0.13$ and 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 (Fig. S7), but no clear pattern emerged. We found significant G × E in female size between Control-Dry ($P = 2.78 \times 10^{-3}$). In male size, G × E was significant between Control-Dry ($P = 6.39 \times 10^{-3}$), Dry-Hot ($P = 5.39 \times 10^{-3}$), Dry–Hot-Dry ($P = 0.04$), and Hot–Hot-Dry ($P = 3.93 \times 10^{-3}$). Genetic correlations between female and male sizes were close to 1 (Table 2, Fig. 5) in all conditions, suggesting that size cannot evolve independently in both sexes. I_A of female and male sizes was not significantly different ($P = 0.08$) considering all conditions. Although differences in Control seemed to be substantial (Fig. 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 ($P = 0.13$; $P = 0.14$) nor male I_A ($P = 0.13$), but had a significant effect on male h^2 ($P = 0.02$).

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: $F_{1,16} = 10.43$, $P = 0.005$; Hot: $F_{1,18} = 4.80$, $P = 0.042$; Hot-Dry: $F_{1,18} = 14.78$, $P = 0.001$, Table S2). In all treatments, the native selection lines produced significantly more offspring than nonadapted control lines (Fig. 7). The largest difference and most significant fitness increase was observed in the most stressful condition of 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 (Fig. 7), showing that adaptation to stress treatments

Table 2. Body size 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) in female and male flour beetles and cross-sex additive genetic covariances (COV_A) and correlations (r_A) in different environmental conditions (conditions as in Table 1).

	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)	6.63×10^{-4}	(1.73×10^{-4})	379.526	(782.07)	333.554	(1342.287)	8.670×10^{-4}	(2.21×10^{-4})
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)	4.94×10^{-3}	(7.90×10^{-4})
V_M (SE)	6.59×10^{-6}	(5.07×10^{-5})	4.88×10^{-4}	(2.18×10^{-4})	6.54×10^{-4}	(5.00×10^{-4})	3.14×10^{-4}	(5.02×10^{-5})
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)**

***Note*: 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.**

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: 12.10 ± 2.23, $P = 3.64 \times 10^{-7}$ in Hot; 12.18 ± 2.18, $P = 4.20 \times 10^{-7}$ 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 (Fig. 7). 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: 4.45 ± 2.10, $P = 0.04$; in Hot: 11.66 ± 2.17, $P = 1.20 \times 10^{-4}$). 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]).

The observed changes in fitness after experimental evolution were much lower than the predicted total changes. 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.

Discussion

Persistence of populations exposed to environmental changes strongly depends on their ability to adapt either by plastic or by 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 for the negative effects of environmental change. We found significant V_A in fitness in all conditions indicating potential to adapt by genetic changes with no evidence that environmental changes lowered the adaptive potential. In contrast, I_A of female and male fitness increased in the stress treatments, which should facilitate adaptation. Accordingly, we

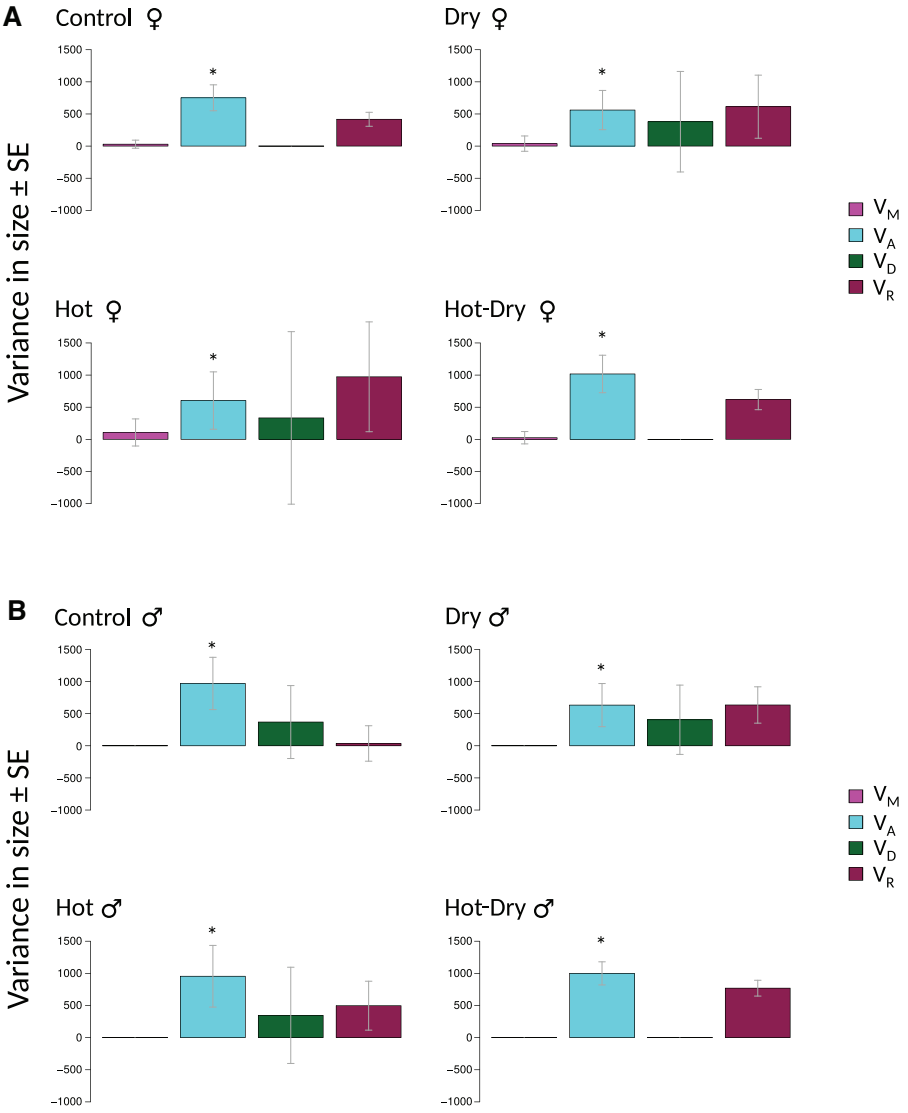

Figure 6. Variance components of size (centroid size of abdominal segment IV) in female (A) and male (B) flour beetles (*Tribolium castaneum*) under four environmental conditions (conditions as in Fig. 1):V_M: maternal variance,V_A: additive genetic variance,V_D: non-additive genetic variance,V_R: residual variance. * indicates a significant variance component.

found significant adaptation to all conditions after 20 generations of experimental evolution. We also did not find strong evidence of genetic constraints on adaptation between stressors or sexes as most genetic correlations were positive (but not in Hot, see Fig. 5). Taking account of both male and female variances and covariance in fitness allowed us to make better predictions of fitness increase. All in all, our study shows that although it is not possible to predict the precise fitness increase over this period of 20 generations, we can make qualitative predictions about adaptation based on V_A estimates. We can examine whether a population is likely to adapt and make relative comparisons between stress treatments, that is, identify those where we expect the largest relative increase in fitness. We thus show that existing quantitative genetic tools are informative over time scales beyond single generation responses. However, reliability of predictions may require a fully integrative approach including potential genetic covariances between the sexes as well as a careful choice of the traits used as fitness estimate.

EVOLUTIONARY POTENTIAL AND ADAPTATION

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 conditions, V_A for males became

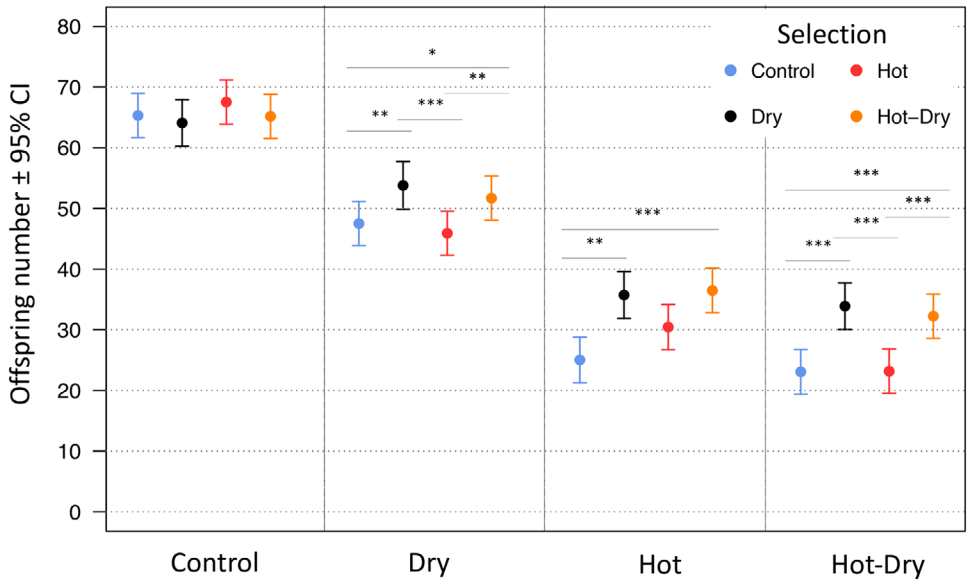

Figure 7. **Mean offspring number per female of lines from different selection regimes under different environmental conditions (conditions as in Fig. 1). Significant differences between selection regimes within the same condition (Tukey's HSD post hoc test,*P*-values adjusted for multiple comparisons) are indicated by **P*< 0.05, ***P*< 0.01, ****P*< 0.001.**

significant (but was still much smaller compared to females) and in Hot-Dry, V_A in both sexes were similar. It was observed that the condition of *Tribolium* males could have a significant influence on the 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 in male drought resistance would then translate into observed offspring variation and result in a higher male influence on female 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. In contrast to the between-sex differences in fitness V_A, we did not find clear differences in V_A between sexes for body size. The largest difference was in the Control treatment (Fig. 2), with a lower female V_A. It is likely that female size had been under strong positive selection because it is associated with fecundity (Honěk 1993), which might explain the observed reduction. Additionally, although we observed an increase in V_A for male fitness in the Dry treatment, V_A for male size was reduced in Dry. A drought effect was thus also detectable for size: When we explored the 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 than female size. G × E for male size occurred when conditions differed in humidity.

Positive cross-condition genetic correlations indicated an absence of an evolutionary trade-off between drought and heat adaptation. Consistently, we did not find any selection line performing worse than control lines in any condition after experimental evolution. It was previously shown that the treatments induce substantial changes in gene expression (Koch and Guillaume 2020). It might then be that selection in the stress treatments is on genes that are not expressed in control and thus allele frequency changes do not sufficiently affect fitness under control conditions. Because positive genetic correlations indicate a similar genetic basis for fitness under different conditions, adapting to one condition should result in a correlated response and increased fitness in other conditions. Accordingly, we found that drought adaptation improved heat resistance (Fig. 7). However, this correlated response was asymmetric because 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 temperature and humidity, which is the case in Control–Hot-Dry and Dry-Hot (Fig. 4) suggesting that the genetic basis for adaptation to heat and drought might be slightly different.

We found the highest positive cross-sex genetic correlation in fitness in Control with lower estimates in the treatments and a negative correlation in Hot. Several studies in the wild (Brommer et al. 2007; Foerster et al. 2007; Mokkonen et al. 2011) as well as in 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 cross-sex correlations are influenced by the environment and contrasting predictions have been made. For instance, it was argued that in a population far from its optimum after an environmental shift, females and males might experience similar directional selection leading to high a positive correlation in their fitness (Berger et al. 2014; Connallon and Hall 2016; Wolak et al. 2018). In contrast, genetic covariances between sexes have been predicted to be less negative in ancestral conditions (Delcourt et al. 2009), because selection on the long-term should favor alleles providing fitness benefits to both (Collet et al. 2016). This last prediction is consistent with our observations.

Taking the male V_A and the covariances between female and male fitness into account led us to predict the highest relative increase in fitness in Hot-Dry and smaller but similar increases in Dry and Hot. Ignoring male and cross-sex effects when studying the adaptive capacity of a population can thus lead to misleading predictions. The results of the fitness assay after 20 generations mainly matched our predictions but increases in fitness were much lower than our estimated upper limit. However, our predictions are based on the assumption of constant V_A and consequently exponentially increasing fitness (Falconer and MacKay 1996). Trade-offs between different fitness components are expected to occur because physiological limits, for example, 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. To measure the offspring number per female, each female was kept individually in egg-laying tubes. 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 influenced by evolution of control lines that were used as reference representing the ancestral nonevolved 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 in 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.

VARIANCE COMPONENTS OF FITNESS AND BODY SIZE

Both traits differed markedly in the proportion of different variance components and in the environmental effect on genetic variances. h^2 was much higher in size than in fitness. This is a common finding in many studies (Roff and Mousseau 1987; Kruuk et al. 2000; McCleery et al. 2004; Teplitsky et al. 2009). It had been initially interpreted as evidence for strong selection depleting V_A in fitness related traits. However, a high V_A can be concealed in h^2 by a simultaneous increase of the total variance (Houle 1992; Merilä and 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 and Sheldon 1999) because it represents a larger 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 contributing trait may increase the influence of environmental variation, leading to a higher total variance in fitness (Price and Schulter 1991) and thus a lower h^2. According to those previous considerations, we found much higher estimates of mean-scaled V_A (CV_A, I_A) in offspring number than in body size and a lower h^2 in fitness. The lower h^2 was mainly due to a higher proportion of environmental variance (V_R) when compared to body size. Contrary to body size, we could not directly estimate V_D for fitness because we could not disentangle V_D from V_M and common environmental effects in the F1 with our half-sib/full-sib breeding design. However, if V_D were present, then it should be included in V_M, because it contributes to full-sib resemblance via a shared mother. Comparative studies investigating 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 and Roff 1995; Wolak and Keller 2014). An increased proportion of V_D is expected in populations under strong selection because V_D is not affected by natural selection (Crnokrak and Roff 1995; Merilä and Sheldon 1999; Roff and Emerson 2006; Sztepanacz and Blows 2015), or with increased inbreeding (Falconer and Makay 1996). In our experiment, V_M for fitness was much lower than V_A, even close to zero in Hot and Hot-Dry (Fig. 3), suggesting that V_D contributed much less to total genetic variance than V_A.

We could directly estimate V_D for body size in the F2 cross and can thus provide data for this rarely estimated variance component. Although our V_D estimates for size were always associated with large SE, they suggest that V_D is present and environment dependent. The highest estimates of V_D were found in Dry and Hot for both male and female sizes, whereas it remained close to zero in Control and Hot-Dry. This environmental dependence of V_D has not been studied in detail before, although the environmental dependence of inbreeding depression was

previously described (Bijlsma et al. 1999; Armbruster and Reed 2005; Fox and Reed 2011). Both V_D and inbreeding depression are expected to increase with inbreeding, for instance in shrinking populations when allele and genotype frequencies change because of increased drift (Falconer and Makay 1996). This could have important implications because environmental changes may emphasize the effects of inbreeding on survival and thus on population size too, directly affecting genetic variances.

The large uncertainty around our estimates of V_D may come from the DFC breeding design used in the F2, despite the large sample size we had (827 family pairs with DFC offspring). Although the DFC design was proposed to estimate V_D (Fairbairn and Roff 2006), it may have limited statistical power because the probability that DFC share alleles identical by descent at a given locus is only 6.25%, resulting in large SE, in contrast to full-sibs where this probability is 25%. Therefore, comparing maternal half-sibs and full-sibs might be a much more powerful approach to estimate V_D, while it allows us to disentangle nonadditive from maternal effects at the same time.

Although we could estimate genetic correlations between fitness and size with our dataset (Table S4), it was not possible to get unbiased estimates of those correlations. With our design, we could not rule out a strong confounding effect of population density because body size was measured in the F2, the offspring of beetles used for the fitness assay (Fig. S1). Consequently, offspring of females with a high fitness (i.e., high offspring number) grew at a higher density because we used identical tubes with the same amount of flour for all females in the fitness assay.

Conclusions

Our study showed an increased adaptive potential in stressful conditions and a corresponding adaptation to those conditions after 20 generations. Although precise predictions of relative increase in fitness were not possible over this time period, we could make correct qualitative predictions. 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 of 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 the adaptive potential. Comparing genetic variances of fitness and size showed that they differed in their variance composition and in their cross-sex and cross-environment genetic covariances. Environmental effects on genetic variances were also not consistent between the two traits. We thus advise caution if studies interested in fitness V_A and the adaptive capacity of a population use body size as a proxy. Overall, we found that genetic variance in fitness is a key estimate of a population's adaptive capacity for time scales over 20 generations. As such, it may help predict the adaptive response of populations exposed to new environmental conditions and help identify the populations most at risk of extinction. However, the reliability of such predictions will depend on the fitness estimate chosen and on the full integration of the multifaceted aspects of adaptation. Inclusion of genetic covariances between female and male fitness and genotype by environment interactions is thus important.

AUTHOR CONTRIBUTIONS

ELK and FG designed experiment. ELK and SHS conducted the experiment (crossing and fitness assay). SHS performed size measurements. ELK analyzed the data. FG and ELK wrote the manuscript. SHS contributed to and commented on manuscript.

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DATA ARCHIVING

Data are available from Zenodo (http://doi.org/10.5281/zenodo.4048074).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Material

Figure S1: Experimental design: Crossing design to produce paternal half-sib families and double-first cousins

Figure S2: Experimental Evolution design

Figure S3: Correlation abdominal segment IV Size with Prosternum Size

Figure S4: Distribution offspring number

Figure S5: Model diagnostic plots for analysis of genetic variance of offspring number

Figure S6: Model diagnostic plots for analysis of offspring number in control and selection lines after experimental evolution

Figure S7: Pairwise cross-environment genetic correlations of size

Table S1: Effect of condition on size. Results of linear mixed models

Table S2: Effect of selection regime on offspring number under different conditions after experimental evolution. Results of linear mixed models

Table S3: Genetic variances of size in female and male flour beetles and cross-sex additive genetic covariances and correlations in different environmental conditions when non-additive genetic effects were not included in the model

Table S4: Additive genetic correlations between fitness and size of females and males