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Does Genetic Variation Maintained by Environmental Heterogeneity Facilitate Adaptation to Novel Selection?

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ABSTRACT: Environmental heterogeneity helps maintain genetic variation in fitness. Therefore, one might predict that populations living in heterogeneous environments have higher adaptive potential than populations living in homogeneous environments. Such a prediction could be useful in guiding conservation priorities without requiring detailed genetic studies. However, this prediction will be true only if the additional genetic variation maintained by environmental heterogeneity can be used to respond to novel selection. Here we examine the effect of environmental heterogeneity on future adaptability using replicated experimental *Drosophila melanogaster* populations that had previously evolved for ~100 generations under one of four selective regimes: constant salt-enriched larvae medium, constant cadmium-enriched larvae medium, and two heterogeneous regimes that vary either temporally or spatially between the two media. Replicates of these experimental populations were subjected to a novel heat stress while being maintained in their original larval diet selection regimes. Adaptation to increased temperature was measured with respect to female productivity and male siring success after ~20 generations. For female productivity, there was evidence of adaptation overall and heterogeneous populations had a larger adaptive response than homogeneous populations. There was less evidence of adaptation overall for male siring success and no support for faster adaptation in heterogeneous populations.

Keywords: adaptation, climate change, genetic variation, environmental heterogeneity.

Introduction

The ability to adapt to climate change or any novel selection is expected to have a strong impact on the persistence and health of populations (Bradshaw and Holzapfel 2006). Consequently, an understanding of the forces shaping genetic variation in fitness within populations and how that variation relates to adaptive potential is central to evolutionary biology and conservation. Can we predict, over the short-term, which species or populations within a species are best able to adapt to novel selection pressures? Directly assessing adaptive potential by measuring the additive genetic variation in fitness, or fitness components, in the new (or predicted) selective environment is often empirically impractical. Surveys of variation of neutral DNA markers are relatively easy to perform but often provide a poor reflection of the genetic variation that is useful for novel selection (Hoffmann et al. 2003; Reed and Frankham 2003; Kelly et al. 2011; Mittell et al. 2015). Because environmental heterogeneity is thought to facilitate the maintenance of genetic variation, perhaps a population's adaptive potential can be predicted by assessing the degree of environmental heterogeneity it currently experiences. For example, some populations of phytophagous insects develop on a single type of host plant, whereas other populations of the same insect species may develop on multiple plant species. For these latter populations, does the genetic variation maintained by resource heterogeneity facilitate or dampen their response to climate-mediated selection?

If loci experience environmentally antagonistic selection (alternative alleles are favored by different environments), classic theory predicts that high levels of genetic variation in fitness can be maintained in spatially heterogeneous environments (less so in temporally heterogeneous ones) and that most variation will be purged in a homogeneous environment (Levene 1953; Dempster 1955; Felsenstein 1976). (Other modes of environmentally dependent gene action, such as conditional neutrality [Kawecki 1994; Fry 1996; Whitlock 1996], can lead to other predictions, but environmental antagonism is the most important with respect to maintaining high levels of genetic variation.) Empirical evidence tends to support the predicted effects of heterogeneity on genetic variation (e.g., McDonald and Ayala 1974; Mackay 1981; Bergland et al. 2014; Huang et al. 2015 and references therein), but it is unclear how this variation will affect the adaptive potential of a population facing novel selection along some separate phenotypic axis.

Variation maintained by environmental heterogeneity could be useful in adapting to novel selection if there is some pleiotropy relating the axis of novel selection to the genes under environmentally antagonistic selection. Alter-

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natively, if the loci relevant to heterogeneity have no effects relevant to the novel selection, we might expect no difference in the response between populations from heterogeneous and homogeneous environments. However, if the alleles favored by novel selection are closely linked to the antagonistically maintained variants, the spread of the adaptive alleles could be hindered by the indirect linked selection (Hill and Robertson 1966), as alleles beneficial to the novel selection have to spread onto the separate genetic backgrounds favored in each environment. Thus, populations from heterogeneous environments may adapt more slowly to novel selection.

We took advantage of a set of replicate experimental populations of *Drosophila melanogaster* that have been evolving for more than 100 generations under four larval diet regimes (each with five replicates): one constant regime always maintained in cadmium-enriched medium (Cad), one constant regime always maintained in salt-enriched medium (Salt), a temporally heterogeneous regime (Temp) where populations were maintained in either salt- or cadmium-enriched media in alternating generations, and a spatially heterogeneous regime (Spatial) where the population was split between the two media but surviving adults were mixed (in equal numbers from the two medium types) before producing offspring. We have previously reported how diet heterogeneity affects sequence diversity (Huang et al. 2014) as well as additive genetic variance (Huang et al. 2015) and inbreeding depression (Long et al. 2013) in fitness-related traits.

In this study, after ~110 generations of evolution, duplicates of each of the 20 populations were created and then maintained at an elevated temperature while keeping their original larval diet regimes. Female productivity and male siring success were assayed to compare the rate of adaptation across larval diet regimes. Two specific comparisons regarding adaptation under heat selection are made here: (i) homogeneous regimes (Cad, Salt) versus heterogeneous regimes (Temp, Spatial) and (ii) Temp versus Spatial. The latter comparison is motivated by theoretical predictions that genetic variation in fitness is more likely to be maintained with spatial heterogeneity than with temporal heterogeneity (Felsenstein 1976), a pattern generally supported by our previous empirical work (Huang et al. 2014, 2015). Our goal is to study a test case of how environmental heterogeneity affects adaptation, not to mimic the specific ecology of natural fly populations. The use of cadmium or salt is arbitrary but represents a case of environmental heterogeneity where there is clear differential selection (Huang et al. 2014). We chose high temperature as the novel selection, because climate change is a pressure affecting many organisms today.

Because of the interest in the evolutionary response to climate change, thermal adaptation has been extensively studied in experimental fly populations. The extent of adaptation differs among studies (Huey et al. 1991; Hoffmann et al. 2012; Condon et al. 2014; Schou et al. 2014; Kellermann et al. 2015; Kristensen et al. 2015) and depends on the amount and genetic architecture of standing variation as well as the type of thermal selection (e.g., constant increases, daily or seasonally variable selection, heat shock). Selection at high temperatures typically results in a correlated increase in tolerance to acute heat stress, but both positively and negatively correlated responses have been observed for performance at cold temperatures (Gilchrist et al. 1997; Hangartner and Hoffmann 2015; Tobler et al. 2015). Experimental populations maintained at higher temperatures have evolved differences in other life-history traits in some cases (e.g., starvation resistance, development time; Kellermann et al. 2015) but not others (Bubliy and Loeschcke 2005). In a few cases, quantitative trait loci or genes have been identified in thermal adaptation (e.g., McColl et al. 1996; Morgan and Mackay 2006; Hoffmann and Willi 2008), but these genetic factors almost certainly do not explain the entirety of observed responses; even less is known about the extent of pleiotropy of alleles involved in heat adaptation. We do not attempt to directly add to the growing knowledge of the mechanisms underlying thermal adaptation (though our results can be interpreted as providing indirect insights into pleiotropy). Rather, our goal is to ask a more general question about adapting populations, with thermal selection as our test case.

Methods

History of Selection Populations

A detailed history of these populations is available from Long et al. (2013) and Huang et al. (2014), and only a brief overview is given here. A large lab population ($N > 1{,}000$) adapted to a standard cornmeal diet was used to separately establish two other large populations, one maintained on salt-enriched food and another maintained on cadmium-enriched food. After ~1 or 2 years of adaptation (26 generations/year), flies from the two populations were crossed to establish 20 small replicate populations, which were divided among the four alternative diet selection regimes (Salt, Cad, Temp, and Spatial) described above. Each generation, 448 adults (with equal sex ratio) were used to produce the next generation. All these diet selection populations were maintained at the standard temperature of 25°C and are referred to below as control (*C*) populations, in contrast with the heat-selected (*H*) populations.

Creation of Heat-Selected Populations

In January 2014 (~110 generations after creation of the 20 control populations), a heat-selected population was

created from each of the control populations (fig. A1; figs. A1, A2 and tables A1–A3 are available online). The same set of parents (448 individuals) that was used to continue the maintenance of the control population was also used to establish the corresponding heat-selected population. Each heat-selected population was maintained at the same population size as the control (i.e., 448 individuals), with equal sex ratio. Each heat-selected population was maintained on the same larval diet selection regime as its corresponding control (Salt, Cad, Temp, or Spatial), following the same 14-day maintenance schedule. All heat-selected populations were maintained at 29°C; in addition, these populations were exposed to a 4-h 37°C heat shock on day 4, when the larvae were expected to be at the third instar stage (Ashburner et al. 2005). For the heat shock, the vials were submerged in a 37°C water bath, with the water surface ~1 cm higher than the medium in the vials. When covered by the lid, the air temperature within the water bath was ~33°C. All together there were 40 populations (20 heat-selected populations paired with 20 controls, divided equally among the four larval diet regimes).

Female Productivity and Male Fitness Assay

Female productivity, which integrates female fecundity and juvenile survival, was assayed in the high-temperature condition (i.e., maintained at 29°C and heat shocked) at several different generations using different diet mediums. The first assay occurred at generation 16 after the start of the thermal selection. In this assay, both the heat-selected and the control populations from the Cad, Temp, and Spatial regimes were assayed in cadmium medium. At generation 19, the populations from the Salt, Temp, and Spatial regimes were assayed in salt medium. At generation 22, all of the populations were assayed in regular benign medium. In addition, we assayed the productivity for the control populations from all regimes at standard 25°C in both cadmium and salt mediums.

The fitness assays made use of flies homozygous for *bw*, a recessive marker that confers brown rather than red eyes, as competitors for the focal flies. We used different *bw* competitors depending on the assay; cadmium-adapted *bw* and salt-adapted *bw* populations were used for the assay in cadmium-enriched medium and salt-enriched medium, respectively. The cadmium-adapted *bw* population was created by backcrossing stock *bw* flies for three generations to flies from a long-term cadmium-adapted population that was not used elsewhere in this experiment (the ancestral cadmium population of Huang et al. 2014); this population was maintained in cadmium for the course of the experiment. The salt-adapted *bw* population was created in the same way, using the ancestral salt population. For the assay in regular medium, flies mixed from the two *bw* medium–specific populations were used as competitors.

One generation before the assay, copies of the control and thermal populations were reared in the same food as the assay diet (cadmium-enriched, salt-enriched, or regular medium) with the same temperature as the actual assay (high temperature or 25°C). When the offspring emerged, the focal females were kept with males from the same vials for 1.5 days of mating at a density of 16 females and 16 males per vial with 20 μL of yeast solution (200 μg/μL; same adult density as during regular maintenance). After this time, 72 females were sampled per population as focal flies and divided into 12 competition vials, which were also kept at the assay temperature. In each vial, the six focal females competed with 10 *bw* competitor females for 20 μL of yeast solution for about 12 h. After yeast competition, the focal and *bw* flies were transferred to new vials with the appropriate assay medium to lay eggs for 18 h and produce offspring (day 0). The egg-laying vials were also incubated at 29°C. At day 4, these egg-laying vials were exposed to a 37°C heat shock for 4 h. On day 12, the number of wild-type offspring in each vial was counted as a measure of productivity. We did not count the number of *bw* offspring.

Male mating success was measured at generations 9 and 23. The focal males assayed at generation 9 were reared in either cadmium- or salt-enriched medium. The focal males assayed at generation 23 were reared in regular medium. Four or eight (divided into two groups of four) focal males were sampled from each of the rearing vials. The four focal males from the same productivity vial competed with the other 12 *bw* competitor males to mate with 16 *bw* females for 2 days at 29°C. The *bw* flies for the assay were collected within 2 days of emergence and were likely a mix of virgins and nonvirgins. We had ~15 male mating assay vials per population. After 2 days of mating competition, all flies were transferred to new vials with regular medium for 18 h of egg laying. For vials with a high number of eggs, some eggs were removed to reduce the density. The laying vials were incubated at 25°C to minimize selection on larval survival. After 14 days, when most flies emerged, the wild-type and *bw* adult offspring were scored for each laying vial to estimate the proportion of offspring sired by the focal males. For the assay at generation 9, male siring success for the heat-selected groups and the control groups were assayed at different weeks (1 week different). Therefore, there might be a block effect confounding the difference between the two groups. For the assay at generation 23, both heat-selected groups and control groups were assayed at the same time, allowing for a direct comparison. Furthermore, the additional 14 generations of evolution should give us a greater chance to detect thermal adaptation in male siring success. We present only the results from generation 23 in the main text; generation 9 results are given in the appen-

dix, available online, and show a similar pattern. All data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.kb830 (Huang et al. 2016).

Analysis

As a test for adaptation with respect to productivity, we first analyzed the productivity data for each diet selection regime in each assay environment separately using the lmer function in the lme4 package in R (R Development Core Team 2014):

$$W_F = H + \text{Pair} + \text{Pop},$$

where W_F is female productivity and H denotes whether the population is heat-selected or control. The random effect population (Pop) is nested within a higher level of random effect control-thermal population pair (Pair), as each heat-selected population was originally produced from a control population. Whether there is evidence of thermal adaptation is determined by the likelihood ratio test between the models with or without the heat-selection term (H).

To test whether the homogeneous regimes differ in adaptation when compared with the heterogeneous regimes, the productivity data across all three assay diets were analyzed:

$$W_F = R + L + H + A + (R \times H) + (R \times A) + (H \times A) + \text{Pair} + \text{Pop}.$$

R denotes whether the regime is homogeneous or heterogeneous; L is the larval diet regime (Cad or Salt, Temp or Spatial), which is nested within the homogeneous (Cad and Salt) or heterogeneous (Temp and Spatial) regime; and A is the assay diet (cadmium, salt, or regular medium). Note that A represents assay generation as well as assay diet, because the different diets were assayed in different generations. As we are interested in whether the effect of heat selection depends on the heterogeneity of the regime, the model above is compared with a reduced model without the interaction $R \times H$ using a likelihood ratio test. The model above is used because we are interested in the average effect of the $R \times H$ interaction over all three assays. However, when we tested for the three-way interaction $R \times H \times A$, it was significant ($P = .043$). Consequently, the data were further analyzed for each assay diet separately using a full model that contains one interaction term $R \times H$ and compared with a model without the interaction.

To compare the two heterogeneous larval regimes (Temp vs. Spatial), the data only from heterogeneous larval regimes were used in the model:

$$W_F = L + H + A + (L \times H) + (L \times A) + (H \times A) + \text{Pair} + \text{Pop}.$$

In this full model for the combined data set, L is the larval regime (Temp or Spatial). The model is compared with a reduced model without the interaction $L \times H$. Further, we tested the three-way interaction $L \times H \times A$. As it was significant ($P = .023$), separate tests for $L \times H$ in each assay diet were performed.

Male siring success (W_M) measured at generation 23 was modeled as a binomial trait via the glmer function in the lme4 package. We first tested for evidence of adaptation for each diet regime by comparing the models with or without the heat-selection term (H):

$$W_M = H + \text{Pair} + \text{Pop}.$$

Further tests of differential rates of adaptation (i.e., homogeneous vs. heterogeneous and Temp vs. Spatial) were performed using analogous models to those used for productivity. As needed, we performed pairwise comparisons among the four larval regimes using the glht function in the multcomp package in R.

Because of the potential for heterogeneity in variances among treatments, we supplemented the analyses above with permutation tests. Within each population pair, we randomly assigned replicate observations to temperature-selection regimes (C or H). We generated 2,000 permuted data sets and, from these, created an empirical null distribution of the regression coefficient for temperature-selection effect (H) or diet regime by temperature-selection interaction ($R \times H$) using the models above. The actual coefficient was compared to the null distribution to determine the P value. The results from the permutation analysis are generally consistent with the analyses based on the ANOVA and are not shown in the main text. Further details on the methods and results of the permutation tests can be found in the appendix.

Results

Female Productivity Differences among Controls under Different Larval Diets at Standard and High Temperature

After evolving in different larval regimes for more than 120 generations, the control (C) homogeneous populations (Cad_C or Salt_C) have much higher female productivity when assayed at the standard temperature in the larval diet that matches their selective history compared to the alternative homogeneous control populations (fig. 1). The control heterogeneous populations have intermediate productivity between the two homogeneous populations, indicating that the heterogeneous populations are reasonably well adapted to both diets but not as well adapted as the homogeneous populations in their own selective diet. Similarly, the control heterogeneous populations tend to have lower mean fitness than the control homogeneous populations when assayed under heat stress (which is novel for

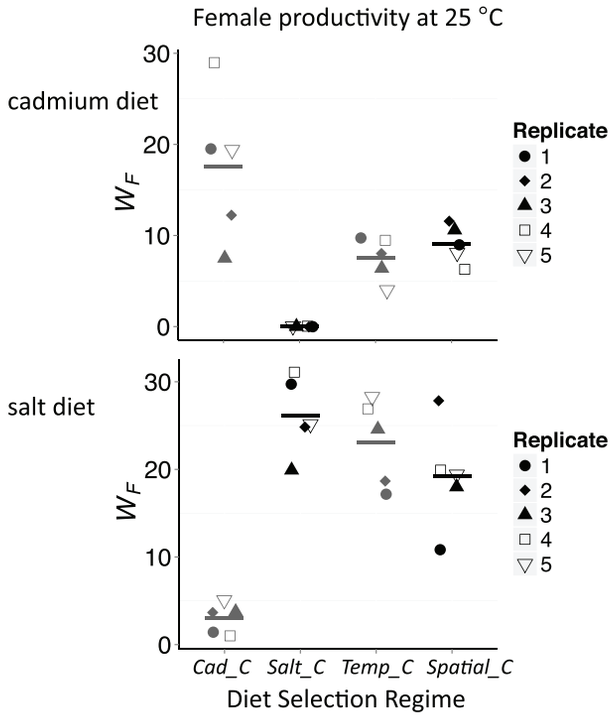

Figure 1: Mean female productivity (offspring produced by a group of six focal females per vial, W_F) of each control population at 25°C (standard temperature). The bars represent the grand means across five replicate populations within regimes. *Top*, assay in cadmium diet. *Bottom*, assay in salt diet.

all controls) in the homogeneous population's adapted diet (fig. 2*A*, control populations in cadmium assay medium: $F = 3.62$, df $= 2$, $P = .059$; Tukey test: Cad_*C* vs. Temp_*C*, $P_{adj} = .085$; Cad_*C* vs. Spatial_*C*, $P_{adj} = .095$; fig. 2*C*, control populations in salt assay medium: $F = 9.98$, df $= 2$, $P = .0028$; Tukey test: Salt_*C* vs. Temp_*C*, $P_{adj} = .0077$; Salt_*C* vs. Spatial_*C*, $P_{adj} = .0045$). The productivity results are qualitatively similar to the reported survival and female fecundity results on these populations by Long et al. (2013) and Huang et al. (2015) from earlier time points (approximately generations 20 and 50, respectively).

Evidence for Adaptation to Thermal Selection

At generation 16 of heat selection, we tested for adaptation to thermal selection, assaying the Cad, Temp, and Spatial populations in cadmium-enriched medium at high temperature. The heat-selected (*H*) populations had significantly higher productivity than control (*C*) populations for the Temp ($\chi^2 = 7.3$, df $= 1$, $P = .0068$) and Spatial ($\chi^2 = 6.2$, df $= 1$, $P = .013$) regimes. There was no significant difference between *H* and *C* populations for Cad ($\chi^2 = 0.97$, df $= 1$, $P = .32$), but the productivities of *H* populations tended to be greater than their controls (fig. 2). Similarly, at generation 19, we assayed the Salt, Temp, and Spatial populations in salt-enriched medium at high temperature for thermal adaptation. There were significant improvements in productivity for Temp ($\chi^2 = 11.5$, df $= 1$, $P = .0007$) and Spatial ($\chi^2 = 12$, df $= 1$, $P = .0005$) and a smaller but significant improvement in the Salt regime ($\chi^2 = 4.0$, df $= 1$, $P = .047$). To control for the different levels of adaptation to the chemical diets, at generation 22 we assayed productivity in regular medium at high temperature; all the regimes showed a difference between the heat-selected and the controls, though the evidence for adaptation in the Cad regime was marginally nonsignificant (Cad: $\chi^2 = 3.68$, df $= 1$, $P = .055$; Salt: $\chi^2 = 5.7$, df $= 1$, $P = .017$; Temp: $\chi^2 = 5.8$, df $= 1$, $P = .016$; Spatial: $\chi^2 = 5.4$, df $= 1$, $P = .02$).

Differences in Productivity Improvement by Heat Selection among Diet Regimes

The main goal of this study is to make two comparisons of the rate of fitness improvement (ΔW) under thermal selection: (i) between homogeneous populations and heterogeneous populations (Cad and Salt vs. Temp and Spatial) and (ii) between the two types of heterogeneous regimes (Temp vs. Spatial). Comparing homogeneous to heterogeneous regimes for female productivity, figure 2 reveals a consistent pattern across the three assays: the improvements in productivity are generally higher in heterogeneous diet regimes (Temp and Spatial) than in homogeneous regimes (Cad and/or Salt; fig. 2*B*, 2*D*, 2*F*). From the linear mixed model using data from all three assay environments, the overall interaction between the diet history (Homo or Heter) and heat selection (control or heat-selected) is significant ($\chi^2 = 6.7$, df $= 1$, $P = .01$), indicating that on average across the three assays, the heterogeneous populations have a larger response than the homogeneous populations. However, there is evidence that this interaction varies across assays (see "Methods"). When the data are analyzed for each assay individually, the interaction is significant in the second (salt) assay ($\chi^2 = 5.86$, df $= 1$, $P = .015$) but not the other two. However, the trends for all three assays are consistent ($\Delta W_{Homo} < \Delta W_{Heter}$), supporting the idea that the average fitness improvement across three assay diets is higher in populations in heterogeneous diet regimes than those in homogeneous regimes.

The alternative forms of environmental heterogeneity (Temp and Spatial) show little evidence of differing in the rate of adaptation. Using data from all three assays, the diet regime (Temp or Spatial) and heat selection interaction are

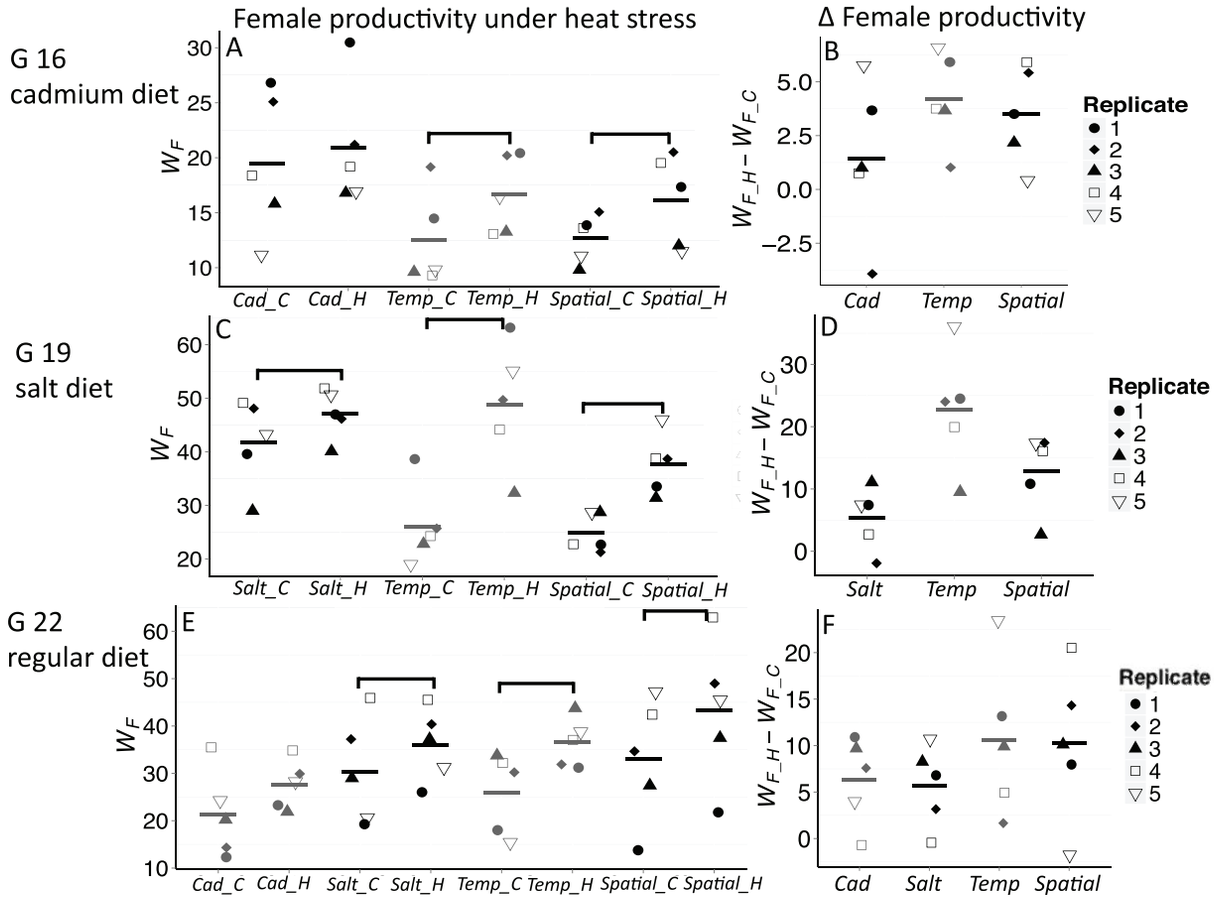

Figure 2: Mean female productivity at high temperature for each replicate population in each assay diet (left column; represented as different points for control [*C*] and heat-selected [*H*] populations). Horizontal solid lines linking the control and heat-selected populations within regimes indicate that productivity is significantly different between two groups based on a likelihood ratio test on the heat selection effect. The right column shows the difference in productivity between each control population and its paired heat-selected population at high temperature. The bars represent the grand means (left column) or the grand mean differences (right column) across five replicate populations within regimes. The top row shows a generation 16 assay in cadmium diet, the middle row shows a generation 19 assay in salt diet, and the bottom row shows generation 22 data from the standard cornmeal diet.

not significant ($\chi^2 = 1.59$, df $= 1$, $P = .21$). However, if the analysis is done separately for each assay, the interaction is significant in the second (salt) assay diet ($\chi^2 = 3.85$, df $= 1$, $P = .0497$) but not in the other two. The nonsignificant trend is that Temp populations have a larger average adaptive response than the Spatial populations, observed in all three assays.

Differences in Male Siring Success under Heat Selection among Diet Regimes

At generation 23, the evidence of improvement in male mating success under heat selection is weaker than for productivity (fig. 3). Only the Cad regime had significant improvement at high temperature (Cad: $\chi^2 = 6.3$, df $= 1$, $P = .012$; Salt: $\chi^2 = 0.01$, df $= 1$, $P = .91$; Temp: $\chi^2 = 0.98$, df $= 1$, $P = .32$; Spatial: $\chi^2 = 1.5$, df $= 1$, $P = .22$). Furthermore, neither of our focal comparisons are significant (Homo vs. Heter: $\chi^2 = 0.39$, df $= 1$, $P = .53$; Temp vs. Spatial: $\chi^2 = 0.02$, df $= 1$, $P = .89$). Visual inspection of figure 3 reveals that the Cad regime had the largest improvement, while the Salt regime had the smallest. The two heterogeneous regimes are intermediate between the two homogeneous regimes, which is markedly different from the pattern for productivity.

To further examine the data after making our focal comparisons, we tested for differences among all regimes.

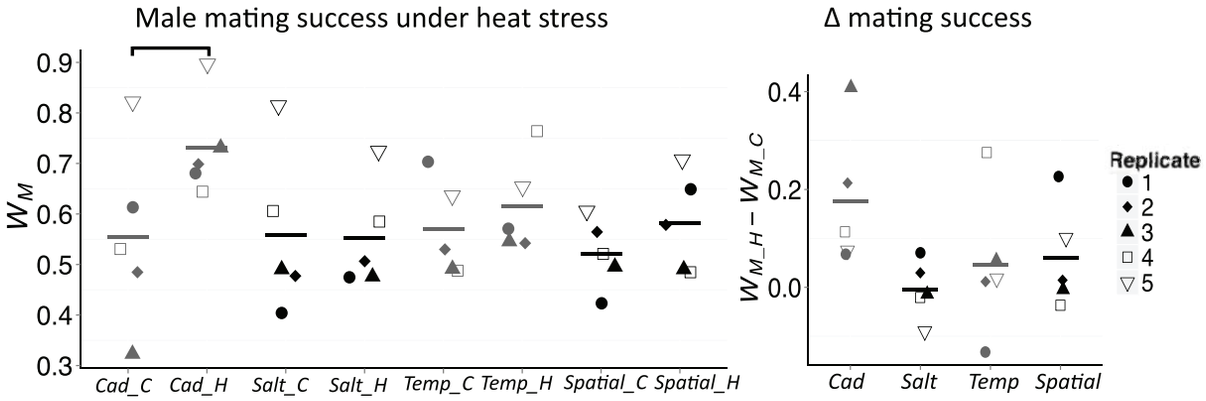

Figure 3: Mean male mating success measured as the proportion of offspring sired by focal males at high temperature for each replicate population (*left*). The graph on the right shows the difference in the proportion sired by focal males between each control population and its paired heat-selected population at high temperature. The bars represent the grand means across five replicate populations within regimes. The horizontal solid line linking the control and heat-selected populations indicates that the productivity is different between two groups based on a likelihood ratio test on the heat-selection effect.

Considering all four treatments simultaneously, the larval regime by heat selection interaction is intriguing though nonsignificant ($\chi^2 = 6.87$, df $= 3$, $P = .076$). However, in pairwise comparisons between diet regimes, there is evidence that the Cad had a significantly higher increase in male siring success than Salt ($z = -2.76$, $P = .03$). These patterns are qualitatively similar to earlier measurements on male mating success at generation 9 performed under slightly different conditions (fig. A2), with the Cad regime showing a larger improvement than the two heterogeneous regimes in the cadmium diet, while Salt had a smaller improvement than the heterogeneous regimes in the salt diet.

Discussion

Many studies exist showing evidence of rapid adaptation, but others have found that evolutionary response to a changing environment is constrained by low levels of genetic variation (Kellermann et al. 2009; Kelly et al. 2011; Kristensen et al. 2015) or by antagonistic genetic correlations among traits (Etterson and Shaw 2001; Sheldon et al. 2003; Colautti et al. 2010; but see a literature survey by Agrawal and Stinchcombe 2009). Our goal here was to test whether environmental heterogeneity can help predict whether a population is likely to have a high or low level of genetic variation available to respond to novel selection. We found that populations in heterogeneous regimes (Temp, Spatial) have larger adaptive responses in female productivity than populations in homogeneous regimes (Cad, Salt).

The result that productivity had a larger response to heat selection for populations in heterogeneous larval regimes (Temp, Spatial) than those in homogeneous regimes (Cad, Salt) is similar to the pattern of diversity levels for single-nucleotide polymorphisms (SNPs) under differential ecological selection: Cad, Salt < Temp < Spatial (Huang et al. 2014). This is consistent with the notion that genetic variation maintained by environmental heterogeneity can contribute to adaptation for high temperature. We note that the patterns of genetic variation at the start of the heat selection (at generation 110) may be different from the sequence diversity measured earlier (at generation 42). However, given that the main pattern appeared to follow the predictions of the environmentally antagonistic selection model, this pattern might be reasonably stable over time.

If the sequence diversity estimates of Huang et al. (2014) reflect the genetic variation just prior to heat selection, the productivity results suggest that the genetic variation maintained by cadmium or salt heterogeneity is related to heat adaptation. Our previous work suggests that many genes are under differential selection between cadmium and salt environments, spanning multiple functional classes (Huang et al. 2014). Previous studies on other experimental fly populations identified many candidate genes for heat adaptation (Orozco-terWengel et al. 2012; Tobler et al. 2014). Those genes share an enriched gene ontology (GO) category ("metabolic process") with the set of genes responding to cadmium and salt differential selection. However, given the severe limitations of the "evolve and resequencing" approach (Tobler et al. 2014), there is a high degree of uncertainty as

to which genes are truly the targets of selection in either cadmium or salt differentiation (Huang et al. 2014) or thermal adaptation (Orozco-terWengel et al. 2012; Tobler et al. 2014). Moreover, the genetic basis of the thermal response observed in that work may differ from what occurred in our populations. Further, our scan of physiological and functional studies did not uncover any genes that are directly involved in heat tolerance as well as cadmium or salt tolerance, though one study shows that cadmium stress and heat stress induce similar transcriptomic responses in adult flies (Brown et al. 2014). Planned resequencing of the populations used in our study may help establish a link between thermal adaptation and sites under antagonistic environmental selection.

In the introduction, we discussed reasons why genetic variation maintained by environmental heterogeneity may be irrelevant to adaptation or even hinder it, contrary to what we observed. Here we speculate that life-history theory (Kozlowski and Wiegert 1986; Roff 1992) may provide a reason to expect that variation maintained by environmental heterogeneity might commonly be useful in adapting to other selective pressures. Each constant environment may favor its own specific optimal life-history strategy represented by a specific pattern of resource allocation to different life-history traits. In a homogeneous environment, most genetic variation in resource allocation is purged. In a heterogeneous environment, variation in some allocation pathways may be maintained. When a population experiences a novel selection pressure, the optimal life-history strategy changes. Those populations harboring more variation in allocation pathways have more potential to evolve toward the newly favored optimum. Depending on the structure of the allocation network and where the variation maintained by environmental heterogeneity lies within this network, evolutionary shifts toward a wide variety of alternative life histories may be possible. This type of argument will not apply if either the novel selection or the axis of environmental heterogeneity involves only genes with highly specific functions rather than involving some genes affecting allocation to life-history traits. As many genes are potentially involved in cadmium and salt differentiation (Huang et al. 2014) and high-temperature adaptation (Orozco-terWengel et al. 2012), it is possible that some of those genes directly or indirectly influence resource allocation. It is plausible that the shared enriched GO category metabolic process (Orozco-terWengel et al. 2012; Huang et al. 2014) includes genes that affect a wide variety of life-history traits.

Alternatively, the faster adaptation observed in heterogeneous populations may result from stronger selection on the heat-adapted alleles in heterogeneous regimes than in homogeneous regimes. Both theory (e.g., Orr 2005) and empirical data (Barrick et al. 2009; Kryazhimskiy et al. 2014) suggest that beneficial alleles experience stronger selection on average in populations that are further from their optima. Is it possible that the heterogeneous populations are further from the optimum than the homogeneous populations? Our data show that the control heterogeneous populations have lower mean fitness at high temperature than the control homogeneous populations when assayed in the homogeneous population's home environment (fig. 2*A*, 2*C*). However, it is not obvious that this type of data should be used as evidence that heterogeneous populations are further from their optimum in the classic sense. The optimal phenotype(s) in the face of heterogeneity may be quite different than one may predict based on habitat specialists.

Though populations further from the optimum are expected to show larger increases in fitness, they are not expected to obtain higher fitness than those that begin closer to the optimum. When we compare all populations at the high temperature but in the absence of either cadmium or salt (fig. 2*E*), the heat-selected heterogeneous populations are as good or better than the homogeneous ones. This observation should be treated with caution, because it involves a fitness assay in an ancestral media that none of the populations has experienced for more than 100 generations. Nonetheless, it is difficult to reconcile the trend toward higher productivity of heterogeneous populations with the notion that their faster rate of adaptation is a result of starting further from their optimum. Rather, we believe the more likely explanation is due to a difference between homogeneous and heterogeneous populations in the amount of genetic variance available for adaptation.

For reasons similar to those that motivated our contrast between homogeneous and heterogeneous populations, we also compared adaptation in Temp and Spatial populations. In our past work, we found that the Spatial regime maintained more variation than the Temp regime in both genome-wide and environmentally divergent SNPs as well as quantitative traits related to fitness (Huang et al. 2014, 2015). However, in our study, Temp and Spatial regimes were not significantly different in the rate of adaptation with respect to productivity. The inconsistency between the amount of genetic variance and adaptation might be because the extra variation maintained by spatial heterogeneity is unrelated to thermal performance.

The discussion above focused on the results for productivity, but the results for siring success were very different. Among the four regimes, only the Cad regime had significant improvement for siring success. The Salt regime had no sign of improvement at all, and the heterogeneous regimes showed only a minor and nonsignificant improvement (figs. 3, A2). This is different from that of female productivity, in which the Cad regime had the smallest (and a nonsignificant) improvement while other regimes all showed significant improvement within 22 generations (fig. 2).

It has been suggested that sexual selection can reinforce natural selection if males with more adapted genotypes also

have higher mating success (Rowe and Houle 1996; Lorch et al. 2003; Candolin and Heuschele 2008; Whitlock and Agrawal 2009). From this line of thinking emerges the prediction that populations showing large improvements in nonsexual fitness (e.g., productivity) should also show large improvements in male sexual fitness (Dolgin et al. 2006). The contrasting patterns between productivity and siring success observed here are not consistent with the idea that adaptation involves a positively pleiotropic genetic basis.

Our results may indicate that different loci affect sexual and nonsexual fitness. It is somewhat surprising that juveniles with genotypes adapted to elevated temperature would not develop into high-condition adults able to achieve higher mating success (Rowe and Houle 1996). Perhaps the increase in productivity is due to alleles that improve survival to the heat shock experienced by juveniles but that have no carryover effect on adult condition. Alternatively, our results may indicate that positive pleiotropy at some loci is balanced by negative pleiotropy at others. Adaptation in a short-term experiment such as this is expected to result largely from standing variation (Falconer and Mackay 1996); genes with antagonistic effects between fitness components (as well as between fitness in different environments) are expected to contribute disproportionately to standing variation (Houle 1991). Dolgin et al. (2006) did observe significant improvement in male mating success for experimental fly populations adapted to different temperatures, but they used populations that both were of larger size (thousands rather than hundreds of flies per population) and had adapted for much longer (~10 years rather than less than 1 year). On the other hand, two other experiments using experimental fly populations with shorter histories of divergent selection also found no evidence of adaptation with respect to male mating success (Correia et al. 2010; Arbuthnott and Rundle 2014).

Given the challenge in quantifying useful genetic variation for adaptation in populations of conservation concern, the productivity results from this experiment represent a case study showing that the level of environmental heterogeneity experienced by populations could be an indicator of their adaptive potential. However, not all fitness components will necessarily respond in the same manner, as illustrated by siring success in this study. Moreover, we have outlined reasons why in some cases environmental heterogeneity may have no effect or even hinder future adaptation. Further experiments in other systems will be required to evaluate how broadly applicable environmental heterogeneity is as a predictor of evolutionary potential. Nonetheless, our study illustrates that the selective history of a population can have a large influence on how it adapts (i.e., which fitness components evolve) as well as the overall level of adaptation. Further insight into the mechanisms of thermal adaptation for these populations will require a combination of phenotypic measurement and population genomic approaches (Stinchcombe and Hoekstra 2007; Porcelli et al. 2015).

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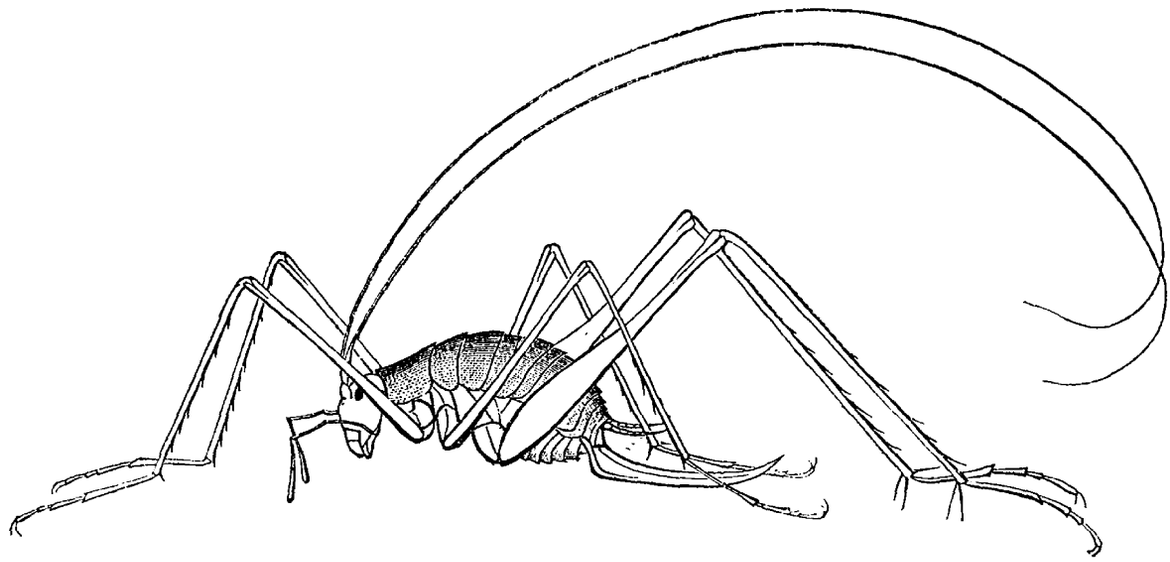

"Two wingless grasshoppers (sometimes called crickets) like the common species found under stones (*R. maculata* Harris), have been found in our caves; one is the *Raphidophora subterranean* [illustrated] described by Mr. Scudder, and very abundant in Mammoth Cave. The other species is *R. stygia* Scudder, from Hickman's cave, near Hickman's landing, upon the Kentucky river." From "The Mammoth Cave and Its Inhabitants" by A. S. Packard Jr. (*The American Naturalist*, 1871, 5:739–761).