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Ecological specialization in populations adapted to constant versus heterogeneous environments*

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Populations vary in their degree of ecological specialization. An intuitive, but often untested, hypothesis is that populations evolving under greater environmental heterogeneity will evolve to be less specialized. How important is environmental heterogeneity in explaining among-population variation in specialization? We assessed juvenile viability of 20 *Drosophila melanogaster* populations evolving under one of four regimes: (1) a salt-enriched environment, (2) a cadmium-enriched environment, (3) a temporally varying environment, and (4) a spatially varying environment. Juvenile viability was tested in both the original selective environments and a set of novel environments. In both the original and novel environments, populations from the constant cadmium regime had the lowest average viability and the highest variance in viability across environments but populations from the other three regimes were similar. Our results suggest that variation in specialization among these populations is most simply explained as a pleiotropic by-product of adaptation to specific environments rather than resulting from a history of exposure to environmental heterogeneity.

KEY WORDS: Drosophila, generalist, niche breadth, specialist, stress.

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Ecological specialization is the degree to which fitness is sensitive to the environment ("Grinnellian specialization" *sensu* Devictor et al. 2010). Specialization and its evolution affect how a population responds to diverse or changing environments with respect to population persistence, adaptation, and speciation (Futuyma and Moreno 1988; Schluter 2000; Hardy and Otto 2014; Sexton et al. 2017). Species or populations can vary in their degree of specialization and here we consider possible sources of this variation.

Environmental heterogeneity is presumed to be one of the most important factors in determining the degree of specialization (Levins 1968; Futuyma and Moreno 1988). Theoretical models have shown that populations experiencing more environmental heterogeneity typically evolve greater environmental tolerance, that is, less sensitivity to environmental changes (Lynch and Gabriel 1987; Ackermann and Doebeli 2004). Thus, ecological specialists tend to evolve in constant environments and generalists tend to evolve in heterogeneous environments. Specialists have greater variance in fitness across environments than generalists. Although environments can vary in space or time, temporal heterogeneity is thought to more readily select for generalists than spatial heterogeneity (Lynch and Gabriel 1987). In temporally changing environments, genotypes with the highest geometric mean fitness are favored, so genotypes with smallest variance in fitness between generations (i.e., generalists) outcompete more specialized genotypes that have variable fitness across environments but with similar arithmetic mean fitness (Lynch and Gabriel 1987; Gavrilets and Scheiner 1993).

Experimental evolution has been used to study how a history of selection under environmental heterogeneity affects the evolution of specialization (Kassen 2002). Most of these studies have used microbes. There are nice microbial examples in which temporal variation has led to the evolution of generalist genotypes (Bennett et al. 1992; Reboud and Bell 1997; Kassen and Bell 1998; Weaver et al. 1999). Spatial heterogeneity has also been shown to select for a more generalist population, in some cases achieved via the maintenance of multiple somewhat specialized genotypes (Reboud and Bell 1997; Barrett et al. 2005).

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There are fewer animal studies and these are difficult to interpret. In some studies, it is unclear whether adaptation occurred at all or whether there was any divergence between regimes with versus without heterogeneity (e.g., Riddle et al. 1986; Scheiner and Yampolsky 1998). Joshi and Thompson (1997) found that experimental populations of *Drosophila melanogaster* that evolved with exposure to both standard and ethanol-enriched larval media evolved to have high survival on both food types, whereas control populations that had evolved only on standard media had poor survival on ethanol-enriched food. However, in that experiment it is impossible to separate the effects of an evolutionary history with ethanol versus heterogeneity *per se* in driving the evolution of the more broadly tolerant larvae. Inferring a critical role for heterogeneity *per se* would be possible only if there had been a control population that evolved on just ethanol-enriched food and showed poor survival on standard media. This issue leads to consideration of an alternative hypothesis to environmental heterogeneity for among-population variation in specialization.

Consider two environment types, A and B, and populations that evolve with exposure to either a single or both environments. The alternative hypothesis focuses on populations that adapt to A only or B only and postulates that a higher degree of specialization will evolve in A than B. This difference in specialization arises because the genetic mechanism of adaptation to A happens to confer a genotype that is less tolerant to various, previously unselected, environmental stresses than is the genotype conferring adaptation to B. Thus, differences in specialization are a by-product of adaptation, not the target of direct selection. A population experiencing both environments A and B in its selective history will acquire both types of adaptations but neither to the full extent, assuming adaptations are costly. As a result, a heterogeneously selected population is hypothesized to have an intermediate degree of specialization, though it may be quite similar to one of the two types of constant-environment populations. This alternative hypothesis is not novel; others have observed that evolution in some constant environments can produce more generalist genotypes than evolution in other environments (Jinks and Connolly 1973; Falconer 1990; Bell and Reboud 1997; Bubliy and Loeschcke 2005).

We take advantage of a set of 20 *D. melanogaster* populations to investigate how different types of evolutionary histories shape the evolution of ecological specialization. Each of these populations had evolved in either a constant environment or in a heterogeneous environment for the preceding ~190 generations. Specifically, 20 populations come from four selection regimes: a cadmium-enriched larval environment (*Cad*), a salt-enriched larval environment (*Salt*), a temporally heterogeneous environment that alternates each generation between the two larval environments (*Temp*), and a spatially heterogeneous environment including discrete patches of each environment type (*Spatial*). These populations have been previously studied with respect to how these selection regimes affect inbreeding depression (Long et al. 2013), additive genetic variance (Huang et al. 2015), sequence variation (Huang et al. 2014), expression differences (Huang and Agrawal 2016), and evolvability (Huang et al. 2016). Previous studies showed that populations from each of the two constant-environment regimes are much better adapted to their home environment than are populations from the alternative constant-environment regime (Long et al. 2013; Huang et al. 2014, 2015, 2016). The populations from the heterogeneous regimes have intermediate fitness in both environments, although in some assays they do as well as populations from the adapted constant-environment regime.

The degree of specialization can be assessed in two different ways. It is often assessed with respect to a set of environments that have been previously experienced by some or all of the populations (Riddle et al. 1986; Joshi and Thompson 1997; Reboud and Bell 1997; Weaver et al. 1999). It can also be assessed in a set of novel environments, which offers a different perspective on what it means to be a generalist. Here, we do both by examining a single major fitness component, larval viability, in the two original environments as well as in a set of novel environments. We evaluate both the mean and variance in viability across environments; the variance serves as a measure of ecological specialization (Devictor et al. 2010). We consider the results with respect to the two hypotheses above: (1) ecological specialization will be greater in populations that have evolved without environmental heterogeneity or (2) differences in specialization are primarily a by-product of adaptation to different constant environments. The first hypothesis seems particularly applicable to predicting specialization with respect to the environments in which these populations have some evolutionary history. Populations from the heterogeneous selection regimes have been selected to cope with both salt and cadmium, so these populations should be more generalized in this context compared to populations from either constant selection regime. For novel environments, either of the hypotheses may hold.

Methods

SELECTIVE HISTORY OF EXPERIMENTAL POPULATIONS

The populations of *D. melanogaster* used here have been described in previous publications (Long et al. 2013; Huang et al. 2014, 2015, 2016) and only key details are presented here. The experimental populations were derived from a population originally collected from the Similkameen Valley, British Columbia in 2005. This population was maintained in a benign lab condition (cornmeal-yeast media; 25°C; 12L:12D photoperiod; 50% RH) at a population size of 2000–4000 flies; this population is referred to as *Grand Ancestor*. From the *Grand Ancestor*, another

large population (∼2000 flies) was created and maintained on a cadmium-enriched diet (selection began in July 2007); this population is referred to as *Ancestral Cad*. In August 2008, a subset of flies from the *Grand Ancestor* population was used to establish a third large population (∼2000 flies), which was maintained on a salt-enriched larval medium and is referred to as *Ancestral Salt*. The concentration of salt and cadmium in the two environments were gradually increased to 75 μg/mL and 29 mg/mL, respectively, before the establishment of the experimental evolution populations described below.

In October 2009, 448 virgin males and 448 virgin females were collected from each of the *Ancestral Cadmium* and the *Ancestral Salt* populations and were crossed to flies from the other population via mass mating. Offspring from the next generation were randomly assigned to one of the four selective regimes: a constant salt environment (hereafter *Salt*), a constant cadmium environment (*Cad*), a temporally heterogeneous regime (*Temp*) that alternates every generation between salt and cadmium environment, and a spatially heterogeneous regime (*Spatial*) that split the population between salt and cadmium environment within each generation. In the *Spatial* regime, every generation half the parents were taken from each environment and mixed, that is, soft selection. Each selective regime had five replicate populations that were maintained in 14 vials with 16 males and 16 females in each vial. All populations were maintained on a two-week nonoverlapping generation cycle. At generation 158, the maintenance scheme was switched from vials to bottles where each population was held in two bottles (∼250 flies per bottle) that were mixed each generation. The experimental populations had evolved independently in their respective selection regimes for ∼190 generations by the start of this study, which commenced in January 2017.

TEST ENVIRONMENTS

The two original selection environments (cadmium and salt) and 16 novel environments were chosen as test environments. The concentration of cadmium and salt was 76 μg/mL and 27 mg/mL, respectively (TableS1). The novel environments varied with respect to a range of abiotic factors including food quality, temperature, water availability, and chemical substance of the medium; further details on the composition of the novel environments can be found in Table 1 and TableS2. The novel environments chosen were based on previous work (Yun and Agrawal 2014) and preliminary studies.

VIABILITY ASSAY

Larva-to-adult viability (hereafter, "larval viability") was measured in the test environments conducted in several blocks from January to July 2017. For a given assay environment, all populations were tested in the same block, but different environments were tested in different blocks (i.e., block can be viewed as part of the assay environment). To reduce the potential impact of maternal effects, all experimental populations were reared on a common benign food media (cornmeal-yeast medium) for one generation before being tested in each environment. The resulting adults from each population were pooled into a large cage to mate and lay eggs (one cage per population with ∼2000 flies). To standardize the developmental stage of egg and larvae, three large grape juice lay-plates topped with live yeast paste were placed in each cage for ∼90 min to allow females to oviposit before being discarded. Immediately afterwards 10 small grape juice lay-plates with live yeast paste were placed in each cage for ∼8 hours. Plates with eggs were then incubated for ∼16 hours at 25°C before larvae collection. For every population in each test environment, 25 replicate vials were collected each with 30 first instar larvae; in several cases, there were less than 25 replicates. Larvae were collected from the lay-plates using a metal probe. The number of surviving adult flies in each vial were counted on the day when it was estimated that more than half of flies had emerged from the pupae; vials were then counted again a few days afterwards; vials were typically counted on days 11 and 14. All the vials for a given environmental assay were counted on the same days. We performed an additional viability assay in the original selective environments. The protocol was identical to the one used in the larval viability assay except 50 eggs were

Table 1. Sixteen novel larval environments (further details can be found in TableS2).

A	A standard sugar–yeast–agar recipe. 10 mL food per vial housed at 25°C.
B	A high-nutrition medium based on cornmeal, sugar, and yeast
C	A reduced volume of cornmeal-based food, only 2 mL/vial
D	Cornmeal medium housed at 17°C
E	Cornmeal medium housed at 31°C
F	25% less water in cornmeal medium
G	25% more water in cornmeal medium
H	Acetic acid (50 mL/L) added to cornmeal medium
I	Caffeine (0.75 g/L) added to cornmeal medium
J	Ethanol (100 mL 95% ethanol per 1 L of food) added to cornmeal medium
K	Urea (4 g/L) added to cornmeal medium
L	50% less water in cornmeal medium
M	50% more water in cornmeal medium
N	A poor-nutrition medium made with cornstarch
O	A cold shock at 3° for 4 hours is applied to second instar larvae
P	A heat shock at 39° for 4 hours is applied to second instar larvae

transferred into each vial and each population had ~15 replicate vials; we refer to this as egg-to-adult viability.

Data Analysis

The larval viability analyses are based on data from 1000 and 7951 replicates for the original and novel environments, respectively. (Repeating the analyses excluding outliers yields very similar results.) For each replicate, larval viability was estimated as the number of adults (summed across both scoring days) divided by 30. Each observation is not a true proportion because there is potential for some error in the number of larvae placed in each vial as well as in the counting of emerged adults (e.g., more than 30 adults are observed in a small fraction of vials). To avoid potential problems that may arise from assumptions about the structure of the data at the individual vial level, we treat "population" as the unit of replication in all analyses (e.g., by using the average across all replicates for a given population within an assay environment).

Results from the original and novel environments were analyzed separately and all analyses were performed in *R* 3.3.1 (R Core Team 2016). The first set of analyses pertains to the assays in the original environments. We examined heterogeneity in average viability by analyzing the mixed effects model *lmer*(*Mean_Viability* ~ *Regime* * *Assay_Environment* + (1|*Regime*:*Population*), where *Mean_Viability* is the average viability across all replicates for a given population within a given assay environment. The model above includes the effects of selection regime and the assay environment as well as their interaction; population is included as a random effect nested within selection regime. Because the interaction term was significant, we performed one-way analyses of variance (ANOVAs) in each environment separately; these ANOVAs do not include population as a random effect because, within a single environment, there is only one measure of each population's mean viability. We also analyzed the model *CrossEnv_Mean_Viability* ~ *Regime*, where *CrossEnv_Mean_Viability* is a population's viability averaged across the two environments. We used the *VarCorr* function to extract the between-environment variance in viability from the mixed effects model *lmer*(*Viability* ~ (1|*Assay_Environment*)), where assay environment is treated as a random effect and which was performed on each population separately using the viability data from all replicate vials. Using the five between-environment variance estimates per regime (i.e., one per replicate population), we asked whether regimes differed in the amount of between-environment variation by using a Kruskal–Wallis test. Post hoc comparisons were performed via Dunn's test using the function *dunn.test* with the *holm* option of adjusting *P*-values for multiple comparisons. A simpler analysis based on the each population's absolute difference in viability between environments yields similar results.

The second set of analyses pertains to the novel environments. We examined heterogeneity in average viability by analyzing the linear mixed effects model *lmer*(*Mean_Viability* ~ *Regime* + (1|*Assay_Environment* / *Selection_Regime*) + (1|*Regime*:*Population*), where selection regime is included as a fixed effect and the novel environments and populations were treated as random effects. The model specifies an interaction between assay environment and selection regime and populations are nested within selection regime. It is fit using REML. As in the analysis of the original environments, the analysis was performed using "population" as the level of replication. The interaction term was tested via a likelihood ratio test after re-fitting the model with maximum likelihood rather than REML for the purpose of assessing the interaction. Type III ANOVA was performed using Satterthwaite's method to assess heterogeneity among selection regimes. Post hoc comparisons were performed using the *glht* function to perform Tukey tests using the *holm* option for *P*-value adjustment. To examine the degree of specialization in the novel environments, we began by first obtaining an estimate of the among-environment variation for each population separately (following the same method as above for the between-environment variation). A Kruskal–Wallis test was used to test for heterogeneity among regimes in the among-environment variance.

In addition, we attempted to ask whether there was more among-population variance in novel environments that were more stressful (i.e., lower survival) by analyzing the linear model *Among_Pop_Var* ~ *Mean_Viability_Across_Pop* + *Assay_Env* + *Regime,* where *Among_Pop_Var* is the among-population variation in viability within a regime for a given environment and *Mean_Viability_Across_Pop* is the mean viability across populations within a regime for a given environment. The among-population variation for each environment was obtained by using *VarCorr* to extract this variance from the model *lmer*(*Viability* ~ (1|*Population*)) where population is a random effect. However, that analysis does not factor in the issue that there are some mathematical constraints on the relationship between the mean and variance for a trait that is bounded by 0 and 1; the variance must be low if the mean is close to 0 or 1. To create a null distribution, we estimated the regression coefficient from 1000 permuted data sets. For each permutation, we randomized the assay environment labels for each population within each regime; all replicates from a given population-assay combination were assigned the same new assay label.

Results

VIABILITY IN THE ORIGINAL ENVIRONMENTS

The viabilities in the original environments (i.e., cadmium and salt assays) are shown in Figure 1A. There was significant variation in viability associated with selection history ($F_{3,32} = 9.4$,

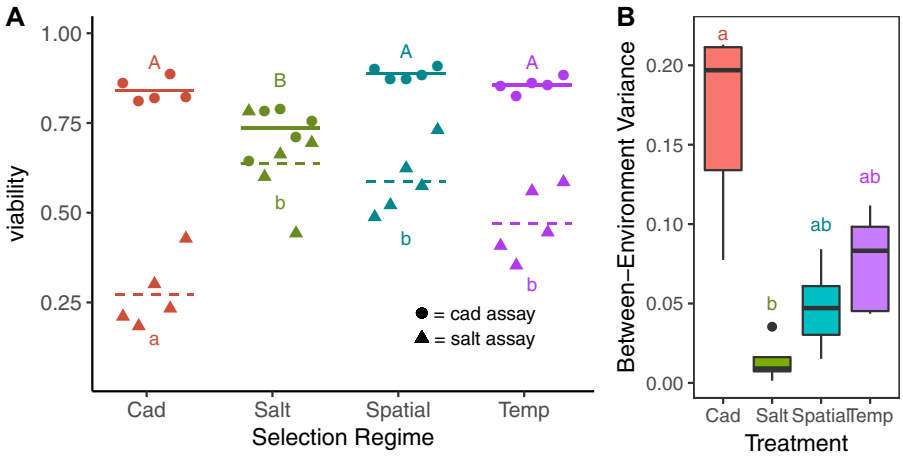

Figure 1. **Viability in the original environments. (A) Viability for each population in cadmium (circles) and salt (triangle) environments. Dashed and solid lines denote the mean viability for each regime in cadmium and salt environments, respectively. Regimes not marked with the same letter are significantly different; upper and lower case letters are used for analyses of cadmium and salt data, respectively. (B) Boxplots of the between-environment variance for each regime. Regimes not marked with the same letter are significantly different.**

$P = 0.00013$), environment ($F_{1,32} = 183.8$, $P = 8 \times 10^{-15}$), and their interaction ($F_{3,32} = 15.2$, $P = 2 \times 10^{-6}$). Analyzing each environment separately, there was significant variation among selection regimes (cadmium assay: $F_{3,16} = 16.3$, $P = 4 \times 10^{-5}$; salt assay: $F_{3,16} = 11.8$, $P = 0.00025$). Post hoc analysis indicates that *Cad* populations had significantly worse survival in the salt assay than the other populations; reciprocally, the *Salt* populations had significantly worse survival in the cadmium than the other populations. We also analyzed the cross-environment average viability of each population. There was significant heterogeneity among regimes ($F_{3,16} = 9.6$, $P = 0.0007$) with post hoc analyses showing *Cad* had lower average survival than each of the other regimes ($P < 0.05$ in all comparisons). The between-environment variance for each population is shown in Figure 1B. There was significant variation among regimes in the amount of between-environment variation (Kruskal–Wallis $\chi^2 = 13.9$, df $= 3$, $P = 0.003$). *Salt* had the lowest among-environment variance and *Cad* had the highest; *Salt* and *Cad* were significantly different from one another ($Z = 3.63$, $P_{adj} = 0.0008$). The data from the egg-to-adult viability assay yielded qualitatively similar results (Fig.S1).

VIABILITY IN THE NOVEL ENVIRONMENTS

Viability in the 16 novel environments is summarized in Figures 2 and 3 and FigureS2. There was significant variance in viability associated with selection regime ($F_{3,37.5} = 12.5$, $P = 8.4 \times 10^{-6}$) and its interaction with assay environment ($\chi^2 = 43.2$, df $= 1$, $P = 4.9 \times 10^{-11}$). Post hoc comparison shows populations from the *Cad* regime had significantly lower mean viability than the other three regimes (Fig. 2A, $P < 10^{-5}$ for all comparisons).

There is significant heterogeneity among regimes in the among-environment variance per population (Kruskal–Wallis $\chi^2 = 8.01$, df $= 3$, $P = 0.046$). On average, *Cad* populations had the highest among-environment variance, whereas the other three regimes were quite similar (Fig. 2B). In post hoc tests, the difference between *Cad* and *Salt* was significant ($Z = 2.51$, $P_{adj} = 0.036$) as was the difference between *Cad* and *Temp* ($Z = 2.35$, $P_{adj} = 0.047$). The lower mean and higher variance of the *Cad* regime is readily apparent in Figure 3, which shows the distribution of viabilities over the 16 novel environments, averaged over the five replicate populations per regime in each environment.

Finally, we attempted to examine the relationship between environmental stress and among-population variation as it is a popular idea that stress, particularly novel stress, reveals cryptic genetic variation (Hoffmann and Merila 1999). (The among-population variation is assumed to be reflective of genetic differentiation among populations.) A simple analysis of our data finds a negative relationship between mean viability of the environments and the among-population variance ($t = -2.231$, df $= 44$, $P = 0.031$). However, that analysis does not account for the bias toward observing a negative relationship in our data because of the constraints on a bounded trait such as viability. The observed slope is not significant when we compare it to a distribution of equivalent slopes from permutated data ($P > 0.05$).

Discussion

Why are some populations able to thrive under a broader range of conditions than others? We exploited 20 experimental fly populations that had evolved for ~190 generations in alternative selective regimes to ask how these alternative histories affected viability across different environments. In both the original environments as well as in a set of novel environments, we found that one of the constant selection regimes, *Cad*, had much higher

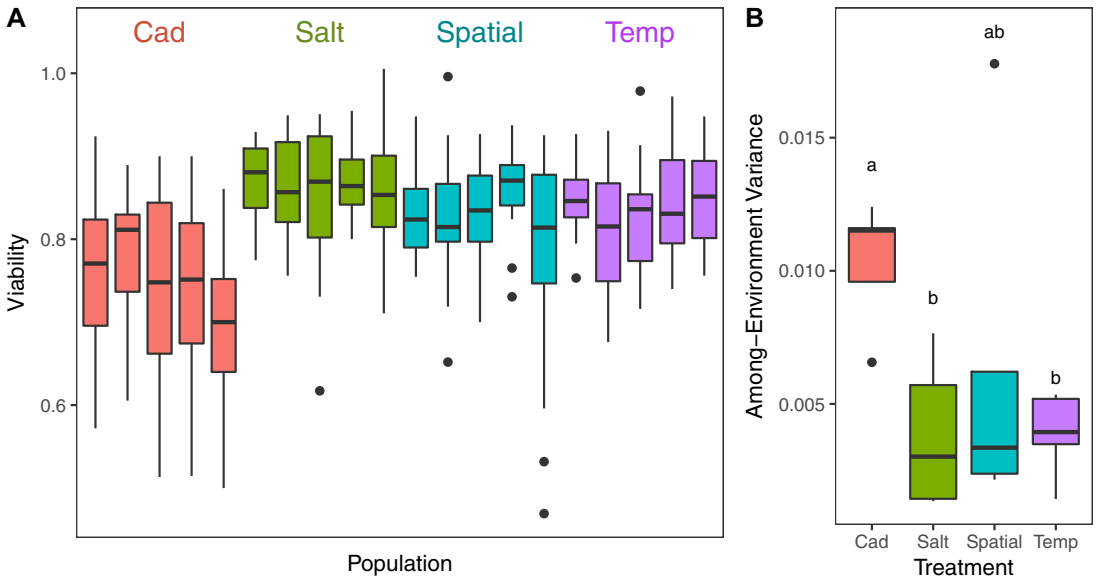

Figure 2. Viability in the novel environments. (A) Boxplots for each of the 20 populations (five per regime); each boxplot represents the distribution of viability estimates over the 16 novel environments for a single population (i.e., each boxplot is based on the 16 estimates of mean viability, one for each environment). (B) Boxplots of the among-environment variance for each regime. Regimes with different letters are significantly different.

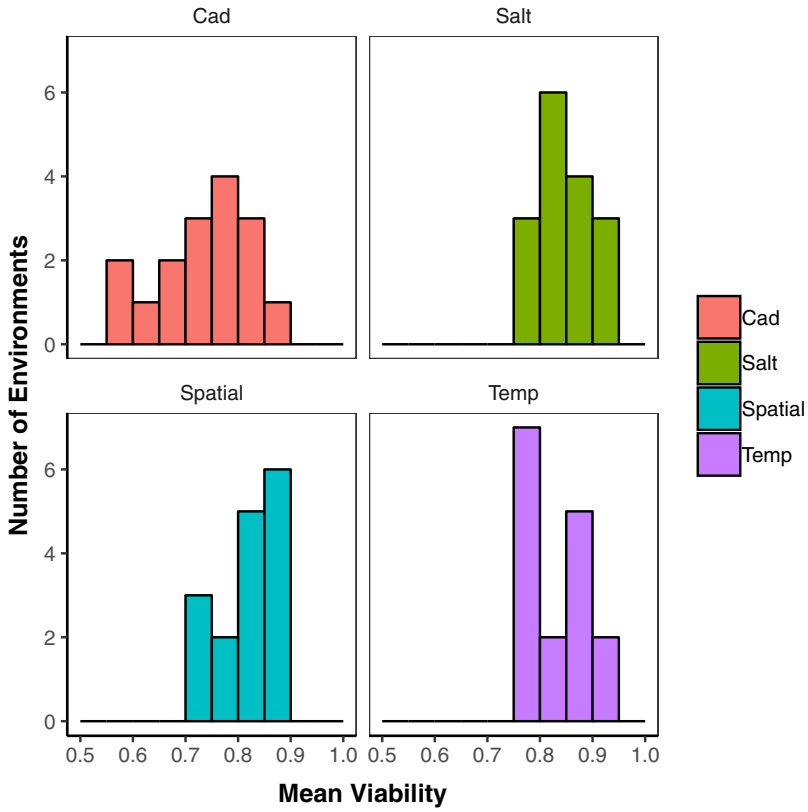

Figure 3. Distribution of mean viability for each selection regime across the 16 novel environments. For each regime, the mean viability per environment is calculated as the average across the five replicate populations.

among-environment variance than the other constant selection regime, *Salt*. We interpret this as indicating *Cad* populations are more ecologically specialized than *Salt* populations. The degree of specialization of the two heterogeneous selection regimes, *Spatial* and *Temp*, was not particularly low; it was similar to or higher than that of the *Salt* regime.

It seems counterintuitive that the heterogeneous regimes do not have the most generalist response—defined as having the lowest between-environment variance—with respect to the original environments because these populations have been selected to survive in both environments, whereas the constant selection regime populations have not. A closer inspection of the data helps to explain why this is so. The results within each environment are sensible from an adaptation perspective (Fig. 1). In the salt assay, *Salt* performs best, *Cad* performs worst, and the heterogeneous regimes are intermediate. In the cadmium assay, *Salt* performs worst, and the remaining regimes all perform similarly well. However, the salt assay is much harsher than the cadmium assay; all regimes, including *Salt*, have lower survival in the salt assay. The *Salt* populations do the worst in the cadmium assay and best in the salt assay, so they exhibit the least change between the two environments and thereby have the lowest between-environment variance, that is, the least specialized response. Although we have presented the results based on absolute viability, the results are qualitatively similar if we examine viability relative to the viability of the most fit population in each environment (Fig.S3).

We evaluated specialization beyond the ecological dimensions encompassed by our selection regimes by assaying viability in novel environments that varied in a range of ecological dimensions (e.g., temperature, osmotic, chemical, nutrient). In all 16 of the novel environments, the point estimate of mean viability for *Salt* populations was greater than for *Cad* populations. This suggests that adaptation to salt engenders a more general form of ecological tolerance, or that adaptation to cadmium carries pleiotropic costs that outweigh the benefits in the absence of cadmium (i.e., adaptation to cadmium is a more "specialized" form of adaptation). An alternative interpretation is that the 16 novel environments are inherently more "similar" to the salt than the cadmium environment, creating the illusion of *Cad* being more specialized. We cannot exclude this possibility as it is difficult to identify an appropriate and meaningful way to quantify the "similarity" of environments that vary qualitatively across a range of ecological dimensions. In the analysis of viability in the novel environments, the interaction between environment and regime was significant. The fact that different regimes vary in their response to these environments suggests that these environments are not merely variations along a common theme. This is consistent with previous studies in *Drosophila* that found positive correlations in selection responses to different stressors but that such correlations are neither universal nor consistently strong when present (Hoffmann and Parsons 1993; Bubliy and Loeschcke 2005). Further inspection of our data does not support the idea that the novel environments are more similar to the salt than the cadmium environment with respect to how they affect variation among populations (Fig.S4).

Theoretical and empirical studies have used the among-environment variance in performance or fitness as a measure of specialization (Lynch and Gabriel 1987; Ackermann and Doebeli 2004; Devictor et al. 2010; see Barrett et al. 2005 for a related measure). This measure is appealing but can be misleading. For example, consider a case where one population has moderate fitness in all environments (i.e., low variance), whereas another population has similarly moderate fitness in some of the environments but high fitness in other environments. It would be odd to refer to the latter population as a "specialist" if it performs as well or better than the "generalist" in all environments. However, this issue in not a major concern in our data; no regime is consistently best or worst across all environments. The *Cad* regime comes the closest to this. It has the lowest viability in a majority of the environments, though not all. Moreover, the *Cad* regime has the greatest among-environment variance and so would be characterized as the most specialized regime, which seems a fair description of it in the current context.

Neither the results from the original or novel environments are consistent with the hypothesis that evolving in a heterogeneous environment results in greater ecological generalization. Rather, these results are consistent with a simple alternative. The process of adapting to the *Salt* regime inadvertently creates genotypes that are more ecologically robust than those created by adaptation to the *Cad* regime. This difference in ecological tolerance is presumably a by-product of the different genetic mechanisms underlying adaptation to these constant environments as neither constant environment should directly select for ecological robustness to alternative environments. We infer that the genetic factors that are responsible for adaptation to salt confer benefits for survival against a variety of other stresses, whereas those genes that confer adaptation to cadmium (e.g., metallothioneins; Egli et al. 2006) do not. Populations from the heterogeneous selection regimes are expected to share adaptations with populations from each constant selection regime, although their adaptation to either environment will be less extensive if the adaptations are costly. Because populations from the heterogeneous regimes have somewhat intermediate genotypes with respect to adaptation to salt and cadmium, these populations might be expected to have an intermediate niche breadth, or at least not greatly exceed that of either constant regime.

Although we are aware of few other animal studies that have attempted related tests, it is worth noting the study of Hallsson and Bjorklund (2012) who allowed populations of seed beetles (*Callosobruchus maculatus*) to adapt to increasing temperature for 18

generations either with or without fluctuations around the temperature trend line. Their data hint at evolved differences among selection lines in sensitivity to temperature but they did not formally test for such differences. To the extent that such differences are apparent in their figures, the major differences occur between the selected lines (increased temperature) and control (original temperature), rather than between selected lines with versus without temperature fluctuations. This might indicate that the alleles that confer adaptation to higher temperature pleiotropically affect sensitivity to temperature, a result analogous to ours. At the macroscale, thermal tolerance breadths of terrestrial invertebrates are larger at higher latitudes (Sunday et al. 2010), which presumably implies that tolerance breadth is related to mean temperature. However, the relationship between thermal tolerance breadth and latitude is reasonably interpreted as a result from greater temperature variation at higher latitudes rather than as a pleiotropic by-product of adapting to cooler average temperatures.

Specialization measured at the population level, as it was here, depends on specialization of individual genotypes as well as variation among genotypes, that is, a population's mean viability can appear insensitive to the environment either because it is composed of a single genotype with modest viability across all environments or because it is composed of many genotypes each specialized to a subset of the environments (Roughgarden 1972; Taper and Chase 1985). In their experimental evolution study on *Pseudomonas*, Barrett et al. (2005) found that populations that evolved in complex (i.e., heterogeneous) environments were more generalist at the population level than populations involved in simple (i.e., homogeneous) environments, in part, because the former were composed of a mix of moderately specialized genotypes. Although it is worth acknowledging this possibility, we have no reason to believe that this type of phenomenon drives the patterns we observe. A collection of specialists would be a more likely explanation if the heterogeneous regimes were the least sensitive to the environment as heterogeneity, particularly spatial heterogeneity, can maintain such variation (Felsenstein 1976).

Two additional caveats should be considered. Our results are based on a single major fitness component; an analysis of total fitness could lead to a different pattern. For example, perhaps *Salt* populations may be more specialized than they appear if fecundity was also considered. Second, these populations represent the outcome of relatively short-term evolution. Perhaps, more broadly generalist genotypes would have evolved in heterogeneous selection regimes over a longer time. Notwithstanding these caveats, our current data are most compatible with the idea that differences among populations in specialization are a pleiotropic by-product of the alleles conferring adaptation to specific environments. Although there is no doubt that environmental heterogeneity can favor the evolution of generalist genotypes, our study illustrates that environmental heterogeneity is not the only, or necessarily the dominant factor, in creating among-population variation in ecological specialization in all cases.

AUTHOR CONTRIBUTIONS

AFA and YH conceived the idea. AFA and AW designed the experiment. AW and AS performed the experiment. AW and AFA analyzed the data and wrote the paper.

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DATA ARCHIVING

Data are available from Dryad at doi: http://10.5061/dryad.gm3vr7s

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Supporting Information

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

Figure S1. Egg-to-adult viability in the original environments.
Figure S2. Viability in the 16 novel environments.
Figure S3. Boxplots of the between-environment variance for the original environments.
for each treatment.
Figure S4. Heatmaps of viability in all 18 (two original and 16 novel) environments.
Table S1. Details for assays in the two original environments.
Table S2. Details for assays in the 16 novel environments.